The eternal molecule

As a prelude to the many celebrations around the world saluting the 50th anniversary of the discovery of the DNA double helix, *Nature* presents a collection of overviews that celebrate the historical, scientific and cultural impacts of a revelatory molecular structure.

Few molecules captivate like DNA. It enthrals scientists, inspires artists, and challenges society. It is, in every sense, a modern icon. A defining moment for DNA research was the discovery of its structure half a century ago. On 25 April 1953, in an article in *Nature*, James Watson and Francis Crick described the entwined embrace of two strands of deoxyribonucleic acid. In doing so, they provided the foundation for understanding molecular damage and repair, replication and inheritance of genetic material, and the diversity and evolution of species.

The broad influence of the double helix is reflected in this collection of articles. Experts from a diverse range of disciplines discuss the impact of the discovery on biology, culture, and applications ranging from medicine to nanotechnology. To help the reader fully appreciate how far the double helix has travelled, we also include the original landmark paper by Watson and Crick and the two accompanying papers by Maurice Wilkins, who shared the Nobel Prize with Watson and Crick in 1962, and by co-discoverer Rosalind Franklin, and their co-authors (pages 83–87).

Transforming science

Given the immense significance of the double helix, it is difficult to imagine a world that wasn't transfixed by its discovery. Yet, as Robert Olby recalls on page 88, the proposed structure initially received a lukewarm reception. Maclyn McCarty, who, together with Oswald Avery and Colin MacLeod, had previously showed DNA to be the substance of inheritance, shares his personal perspective (page 92).

In science, where a lifetime's work can often be encapsulated in a few shining moments, the greatest controversies are sometimes over the sharing of credit. The discovery of the double helix is no exception. The premature death and posthumous treatment of Rosalind Franklin, whose X-ray images of DNA fibres revealed telltale clues of a double helical structure, propelled her portrayal as a feminist icon. But, as discussed here by her biographer Brenda Maddox (page 93), Franklin is better remembered as a committed and exacting scientist who saw no boundaries between everyday life and science.

Most of our readers will have grown up with the double helix, and yet it is still startling to consider how quickly DNA biology has progressed in just a lifetime. Bruce Alberts reviews how the elegant pairing of the two strands of the double helix revealed the mechanism for replicating the essential units of inheritance (page 117). Errol Friedberg considers the vulnerability of the DNA molecule to damage and the multitude of ways in which cells repair the damage (page 122). And Gary Felsenfeld and Mark Groudine describe how the gargantuan DNA molecule is packaged inside the minuscule cells of the body, and how an additional layer of information is encrypted within the proteins intimately associated with DNA (page 134). It is perhaps salutary also to recognize what is still to be learnt about the physiological states in which DNA exists, as discussed by Philip Ball (page 107).

As reviewed by Leroy Hood and David Galas (page 130), DNA science generated the tools that spawned the biotechnology revolution. It enabled the cloning of individual genes, the sequencing of whole genomes and, with the application of computer science, transformed the nature and interactions of molecules into an information science. Carlos Bustamante and co-authors consider how we are still learning much about the distinct structural and physical properties of the molecule (page 109). And according to Nadrian Seeman, DNA may develop new applications as a material for nanoscale engineering (page 113).

Influencing society

Beyond scientific and technological forums, the double helix has imprinted on society's views of history, medicine and art. As discussed by Svante Pääbo (page 95), the records of evolution have been recalibrated with information traced through DNA sequence. On page 98, Aravinda Chakravarti and Peter Little revisit the 'nature versus nurture' debate and our developing view of the interplay between genetic and environmental factors in human disease. And DNA science will transform clinical medicine according to John Bell (page 100), providing a new taxonomy for human disease and triggering a change to health care practice. On page 126, Gustav Nossal reviews how an understanding of DNA processes, such as recombination, have transformed the field of immunology.

As a visual icon, and as a profound influence on our nature, the DNA molecule has permeated the imagery and art of our time, and is described by Martin Kemp (page 102) as the *Mona Lisa* of this scientific age. Given that broad impact, and revolutions that are yet to come, it is perhaps appropriate to leave the last word to an artist. Written in 1917, the poem *Heredity* by Thomas Hardy (see inset) seems to foreshadow both the essence and the fascination of the molecule that we celebrate here. □

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Heredity

I am the family face;
Flesh perishes, I live on,
Projecting trait and trace
Through time to times anon,
And leaping from place to place
Over oblivion.

The years-heired feature that can
In curve and voice and eye
Despise the human span
Of durance — that is I;
The eternal thing in man,
That heeds no call to die.

Thomas Hardy
(First published in *Moments of Vision and Miscellaneous Verses*, Macmillan, 1917)

J. Clayton et al. (eds.), *50 Years of DNA*

No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

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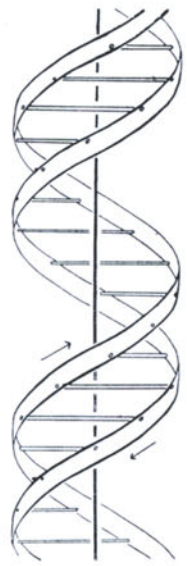
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the *z*-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical *z*-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

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Molecular Structure of Deoxypentose Nucleic Acids

While the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[1]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[1-3], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid ('structure *B*' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the inter-nucleotide repeat along the fibre axis. The ~ 34 A. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

It may be shown[5] (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the nth layer line being proportional to the square of J_n, the nth order Bessel function. A straight line may be drawn approximately through

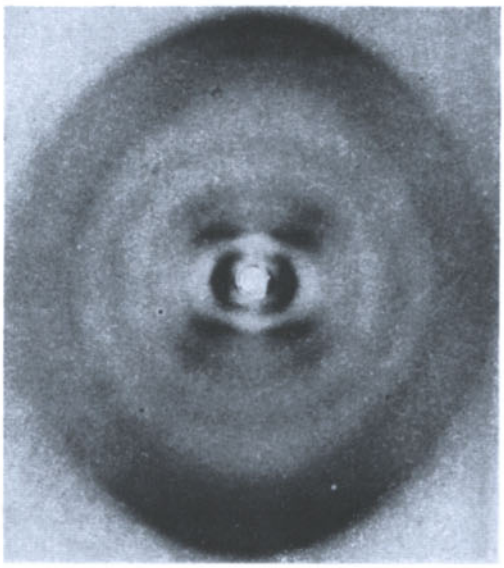

Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats n times along the helix there will be a meridional reflexion (J_0^2) on the nth layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the nth layer line, corresponding to *C* in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

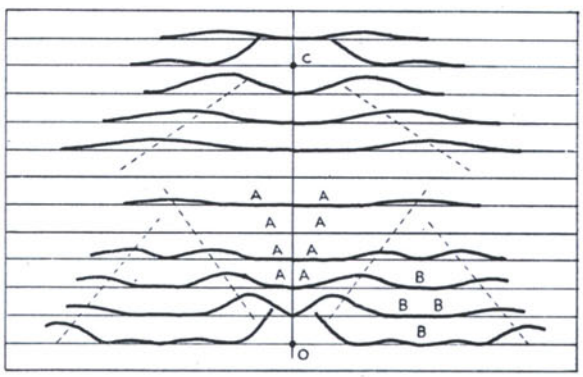

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The squares of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 A. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About *C* on the tenth layer line similar functions are plotted for an outer diameter of 12 A.

No. 4356 April 25, 1953 NATURE 739

most maxima and, in general, owing to phase difference, cancellation of all other maxima. Such a system of helices (corresponding to a spiral staircase with the core removed) diffracts mainly over a limited angular range, behaving, in fact, like a periodic arrangement of flat plates inclined at a fixed angle to the axis. Third, if the nucleotide is extended as an arc of a circle in a plane at right-angles to the helix axis, and with centre at the axis, the intensity of the system of Bessel function layer-line streaks emanating from the origin is modified owing to the phase differences of radiation from the helices drawn through each point on the nucleotide. The form factor is that of the series of points in which the helices intersect a plane drawn through the helix axis. This part of the diffraction pattern is then repeated as a whole with origin at C (Fig. 2). Hence this aspect of nucleotide shape affects the central and peripheral regions of each layer line differently.

Interpretation of the X-Ray Photograph

It must first be decided whether the structure consists of essentially one helix giving an intensity distribution along the layer lines corresponding to $J_1, J_2, J_3 \ldots$, or two similar co-axial helices of twice the above size and relatively displaced along the axis a distance equal to half the pitch giving $J_2, J_4, J_6 \ldots$, or three helices, etc. Examination of the width of the layer-line streaks suggests the intensities correspond more closely to J_1^2, J_2^2, J_3^2 than to $J_2^2, J_4^2, J_6^2 \ldots$ Hence the dominant helix has a pitch of ~ 34 A., and, from the angle of the helix, its diameter is found to be ~ 20 A. The strong equatorial reflexion at ~ 17 A. suggests that the helices have a maximum diameter of ~ 20 A. and are hexagonally packed with little interpenetration. Apart from the width of the Bessel function streaks, the possibility of the helices having twice the above dimensions is also made unlikely by the absence of an equatorial reflexion at ~ 34 A. To obtain a reasonable number of nucleotides per unit volume in the fibre, two or three intertwined coaxial helices are required, there being ten nucleotides on one turn of each helix.

The absence of reflexions on or near the meridian (an empty region AAA on Fig. 2) is a direct consequence of the helical structure. On the photograph there is also a relatively empty region on and near the equator, corresponding to region BBB on Fig. 2. As discussed above, this absence of secondary Bessel function maxima can be produced by a radial distribution of the nucleotide shape. To make the layer-line streaks sufficiently narrow, it is necessary to place a large fraction of the nucleotide mass at ~ 20 A. diameter. In Fig. 2 the squares of Bessel functions are plotted for half the mass at 20 A. diameter, and the rest distributed along a radius, the mass at a given radius being proportional to the radius.

On the zero layer line there appears to be a marked J_{10}^2, and on the first, second and third layer lines, $J_9^2 + J_{11}^2$, $J_8^2 + J_{12}^2$, etc., respectively. This means that, in projection on a plane at right-angles to the fibre axis, the outer part of the nucleotide is relatively concentrated, giving rise to high-density regions spaced c. 6 A. apart around the circumference of a circle of 20 A. diameter. On the fifth layer line two J_5 functions overlap and produce a strong reflexion. On the sixth, seventh and eighth layer lines the maxima correspond to a helix of diameter ~ 12 A. Apparently it is only the central region of the helix structure which is well divided by the 3·4-A. spacing, the outer parts of the nucleotide overlapping to form a continuous helix. This suggests the presence of nitrogen bases arranged like a pile of pennies[1] in the central regions of the helical system.

There is a marked absence of reflexions on layer lines beyond the tenth. Disorientation in the specimen will cause more extension along the layer lines of the Bessel function streaks on the eleventh, twelfth and thirteenth layer lines than on the ninth, eighth and seventh. For this reason the reflexions on the higher-order layer lines will be less readily visible. The form factor of the nucleotide is also probably causing diminution of intensity in this region. Tilting of the nitrogen bases could have such an effect.

Reflexions on the equator are rather inadequate for determination of the radial distribution of density in the helical system. There are, however, indications that a high-density shell, as suggested above, occurs at diameter ~ 20 A.

The material is apparently not completely paracrystalline, as sharp spots appear in the central region of the second layer line, indicating a partial degree of order of the helical units relative to one another in the direction of the helix axis. Photographs similar to Fig. 1 have been obtained from sodium nucleate from calf and pig thymus, wheat germ, herring sperm, human tissue and T_2 bacteriophage. The most marked correspondence with Fig. 2 is shown by the exceptional photograph obtained by our colleagues, R. E. Franklin and R. G. Gosling, from calf thymus deoxypentose nucleate (see following communication).

It must be stressed that some of the above discussion is not without ambiguity, but in general there appears to be reasonable agreement between the experimental data and the kind of model described by Watson and Crick (see also preceding communication).

It is interesting to note that if there are ten phosphate groups arranged on each helix of diameter 20 A. and pitch 34 A., the phosphate ester backbone chain is in an almost fully extended state. Hence, when sodium nucleate fibres are stretched[3], the helix is evidently extended in length like a spiral spring in tension.

Structure *in vivo*

The biological significance of a two-chain nucleic acid unit has been noted (see preceding communication). The evidence that the helical structure discussed above does, in fact, exist in intact biological systems is briefly as follows:

Sperm heads. It may be shown that the intensity of the X-ray spectra from crystalline sperm heads is determined by the helical form-function in Fig. 2. Centrifuged trout semen give the same pattern as the dried and rehydrated or washed sperm heads used previously[6]. The sperm head fibre diagram is also given by extracted or synthetic[1] nucleoprotamine or extracted calf thymus nucleohistone.

Bacteriophage. Centrifuged wet pellets of T_2 phage photographed with X-rays while sealed in a cell with mica windows give a diffraction pattern containing the main features of paracrystalline sodium nucleate as distinct from that of crystalline nucleoprotein. This confirms current ideas of phage structure.

Transforming principle (in collaboration with H. Ephrussi-Taylor). Active deoxypentose nucleate allowed to dry at ~ 60 per cent humidity has the same crystalline structure as certain samples[3] of sodium thymonucleate.

We wish to thank Prof. J. T. Randall for encouragement; Profs. E. Chargaff, R. Signer, J. A. V. Butler and Drs. J. D. Watson, J. D. Smith, L. Hamilton, J. C. White and G. R. Wyatt for supplying material without which this work would have been impossible; also Drs. J. D. Watson and Mr. F. H. C. Crick for stimulation, and our colleagues R. E. Franklin, R. G. Gosling, G. L. Brown and W. E. Seeds for discussion. One of us (H. R. W.) wishes to acknowledge the award of a University of Wales Fellowship.

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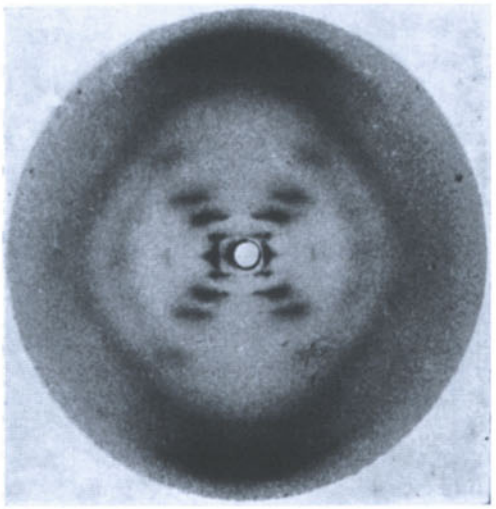
Molecular Configuration in Sodium Thymonucleate

Sodium thymonucleate fibres give two distinct types of X-ray diagram. The first corresponds to a crystalline form, structure *A*, obtained at about 75 per cent relative humidity; a study of this is described in detail elsewhere[1]. At higher humidities a different structure, structure *B*, showing a lower degree of order, appears and persists over a wide range of ambient humidity. The change from *A* to *B* is reversible. The water content of structure *B* fibres which undergo this reversible change may vary from 40–50 per cent to several hundred per cent of the dry weight. Moreover, some fibres never show structure *A*, and in these structure *B* can be obtained with an even lower water content.

The X-ray diagram of structure *B* (see photograph) shows in striking manner the features characteristic of helical structures, first worked out in this laboratory by Stokes (unpublished) and by Crick, Cochran and Vand[2]. Stokes and Wilkins were the first to propose such structures for nucleic acid as a result of direct studies of nucleic acid fibres, although a helical structure had been previously suggested by Furberg (thesis, London, 1949) on the basis of X-ray studies of nucleosides and nucleotides.

While the X-ray evidence cannot, at present, be taken as direct proof that the structure is helical, other considerations discussed below make the existence of a helical structure highly probable.

Structure *B* is derived from the crystalline structure *A* when the sodium thymonucleate fibres take up quantities of water in excess of about 40 per cent of their weight. The change is accompanied by an increase of about 30 per cent in the length of the fibre, and by a substantial re-arrangement of the molecule. It therefore seems reasonable to suppose that in structure *B* the structural units of sodium thymonucleate (molecules on groups of molecules) are relatively free from the influence of neighbouring



Sodium deoxyribose nucleate from calf thymus. Structure *B*

molecules, each unit being shielded by a sheath of water. Each unit is then free to take up its least-energy configuration independently of its neighbours and, in view of the nature of the long-chain molecules involved, it is highly likely that the general form will be helical[3]. If we adopt the hypothesis of a helical structure, it is immediately possible, from the X-ray diagram of structure *B*, to make certain deductions as to the nature and dimensions of the helix.

The innermost maxima on the first, second, third and fifth layer lines lie approximately on straight lines radiating from the origin. For a smooth single-strand helix the structure factor on the *n*th layer line is given by:

$$F_n = J_n(2\pi rR) \exp i\, n(\psi + \tfrac{1}{2}\pi),$$

where $J_n(u)$ is the *n*th-order Bessel function of u, r is the radius of the helix, and R and ψ are the radial and azimuthal co-ordinates in reciprocal space[2]; this expression leads to an approximately linear array of intensity maxima of the type observed, corresponding to the first maxima in the functions J_1, J_2, J_3, etc.

If, instead of a smooth helix, we consider a series of residues equally spaced along the helix, the transform in the general case treated by Crick, Cochran and Vand is more complicated. But if there is a whole number, m, of residues per turn, the form of the transform is as for a smooth helix with the addition, only, of the same pattern repeated with its origin at heights mc^*, $2mc^*$ . . . etc. (c is the fibre-axis period).

In the present case the fibre-axis period is 34 A. and the very strong reflexion at 3·4 A. lies on the tenth layer line. Moreover, lines of maxima radiating from the 3·4-A. reflexion as from the origin are visible on the fifth and lower layer lines, having a J_5 maximum coincident with that of the origin series on the fifth layer line. (The strong outer streaks which apparently radiate from the 3·4-A. maximum are not, however, so easily explained.) This suggests strongly that there are exactly 10 residues per turn of the helix. If this is so, then from a measurement of R_n the position of the first maximum on the *n*th layer line (for n $5\leqslant$), the radius of the helix, can be obtained. In the present instance, measurements of R_1, R_2, R_3 and R_5 all lead to values of r of about 10 A.

No. 4356 April 25, 1953 NATURE 741

Since this linear array of maxima is one of the strongest features of the X-ray diagram, we must conclude that a crystallographically important part of the molecule lies on a helix of this diameter. This can only be the phosphate groups or phosphorus atoms.

If ten phosphorus atoms lie on one turn of a helix of radius 10 A., the distance between neighbouring phosphorus atoms in a molecule is 7·1 A. This corresponds to the P . . . P distance in a fully extended molecule, and therefore provides a further indication that the phosphates lie on the outside of the structural unit.

Thus, our conclusions differ from those of Pauling and Corey[4], who proposed for the nucleic acids a helical structure in which the phosphate groups form a dense core.

We must now consider briefly the equatorial reflexions. For a single helix the series of equatorial maxima should correspond to the maxima in $J_0(2\pi rR)$. The maxima on our photograph do not, however, fit this function for the value of r deduced above. There is a very strong reflexion at about 24 A. and then only a faint sharp reflexion at 9·0 A. and two diffuse bands around 5·5 A. and 4·0 A. This lack of agreement is, however, to be expected, for we know that the helix so far considered can only be the most important member of a series of coaxial helices of different radii ; the non-phosphate parts of the molecule will lie on inner co-axial helices, and it can be shown that, whereas these will not appreciably influence the innermost maxima on the layer lines, they may have the effect of destroying or shifting both the equatorial maxima and the outer maxima on other layer lines.

Thus, if the structure is helical, we find that the phosphate groups or phosphorus atoms lie on a helix of diameter about 20 A., and the sugar and base groups must accordingly be turned inwards towards the helical axis.

Considerations of density show, however, that a cylindrical repeat unit of height 34 A. and diameter 20 A. must contain many more than ten nucleotides.

Since structure B often exists in fibres with low water content, it seems that the density of the helical unit cannot differ greatly from that of dry sodium thymonucleate, 1·63 gm./cm.^{3} [1,5], the water in fibres of high water-content being situated outside the structural unit. On this basis we find that a cylinder of radius 10 A. and height 34 A. would contain thirty-two nucleotides. However, there might possibly be some slight inter-penetration of the cylindrical units in the dry state making their effective radius rather less. It is therefore difficult to decide, on the basis of density measurements alone, whether one repeating unit contains ten nucleotides on each of two or on each of three co-axial molecules. (If the effective radius were 8 A. the cylinder would contain twenty nucleotides.) Two other arguments, however, make it highly probable that there are only two co-axial molecules.

First, a study of the Patterson function of structure A, using superposition methods, has indicated[6] that there are only two chains passing through a primitive unit cell in this structure. Since the $A \rightleftharpoons B$ transformation is readily reversible, it seems very unlikely that the molecules would be grouped in threes in structure B. Secondly, from measurements on the X-ray diagram of structure B it can readily be shown that, whether the number of chains per unit is two or three, the chains are not equally spaced along the fibre axis. For example, three equally spaced chains would mean that the nth layer line depended on J_{3n}, and would lead to a helix of diameter about 60 A. This is many times larger than the primitive unit cell in structure A, and absurdly large in relation to the dimensions of nucleotides. Three unequally spaced chains, on the other hand, would be crystallographically non-equivalent, and this, again, seems unlikely. It therefore seems probable that there are only two co-axial molecules and that these are unequally spaced along the fibre axis.

Thus, while we do not attempt to offer a complete interpretation of the fibre-diagram of structure B, we may state the following conclusions. The structure is probably helical. The phosphate groups lie on the outside of the structural unit, on a helix of diameter about 20 A. The structural unit probably consists of two co-axial molecules which are not equally spaced along the fibre axis, their mutual displacement being such as to account for the variation of observed intensities of the innermost maxima on the layer lines ; if one molecule is displaced from the other by about three-eighths of the fibre-axis period, this would account for the absence of the fourth layer line maxima and the weakness of the sixth. Thus our general ideas are not inconsistent with the model proposed by Watson and Crick in the preceding communication.

The conclusion that the phosphate groups lie on the outside of the structural unit has been reached previously by quite other reasoning[1]. Two principal lines of argument were invoked. The first derives from the work of Gulland and his collaborators[7], who showed that even in aqueous solution the —CO and —NH_2 groups of the bases are inaccessible and cannot be titrated, whereas the phosphate groups are fully accessible. The second is based on our own observations[1] on the way in which the structural units in structures A and B are progressively separated by an excess of water, the process being a continuous one which leads to the formation first of a gel and ultimately to a solution. The hygroscopic part of the molecule may be presumed to lie in the phosphate groups ($(C_2H_5O)_2PO_2Na$ and $(C_3H_7O)_2PO_2Na$ are highly hygroscopic[8]), and the simplest explanation of the above process is that these groups lie on the outside of the structural units. Moreover, the ready availability of the phosphate groups for interaction with proteins can most easily be explained in this way.

We are grateful to Prof. J. T. Randall for his interest and to Drs. F. H. C. Crick, A. R. Stokes and M. H. F. Wilkins for discussion. One of us (R. E. F.) acknowledges the award of a Turner and Newall Fellowship.

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Quiet debut for the double helix

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Past discoveries usually become aggrandized in retrospect, especially at jubilee celebrations, and the double helix is no exception. The historical record reveals a muted response by the scientific community to the proposal of this structure in 1953. Indeed, it was only when the outlines appeared of a mechanism for DNA's involvement in protein synthesis that the biochemical community began to take a serious interest in the structure.

"... we may expect genetic chemistry to become in time an integrating core for cellular biochemistry." Robert Sinsheimer, in a lecture delivered at the California Institute of Technology, 1956 (published in ref. 1, p. 1128).

To recall the year 1953 is to visit — and for some of us to revisit — another world, when *Nature* did not use the abbreviation DNA for deoxyribonucleic acid. In June that year, Elizabeth II, Queen of the United Kingdom, was crowned amidst much pomp and ceremony. In March, British scientists prepared to construct an atomic power station by the Calder River. Two months later, Mount Everest was conquered. At the University of London my biochemistry teacher enthused about Frederick Sanger's success in the first sequencing of the units of a protein, insulin. But deoxyribonucleic acid (DNA) was not even mentioned. Yet in 1953 *Nature* published seven papers on the structure and function of DNA[2–8], but only one national British newspaper — the *News Chronicle* — referred to the double helix[9] (see facsimile below).

Reception to the double helix

Fifty years on it is hard to believe the double helix had such a lukewarm reception. But turn to *Nature* and to *Science* in the 1950s and what do we find? Figure 1 records the number of papers in *Nature* reporting on any aspects of DNA, and of these the number that mention the Watson–Crick model or cite any of the 1953 papers on DNA structure. Through the decade *Nature*'s volumes increased in size, and in 1960 the number of volumes published per year was doubled. This increase was accompanied by an increase in the number of papers on some aspect of DNA, but references to the double helix did not increase. The pattern of citation in *Science* is similar.

At the time the structure of DNA was discovered, there was already a considerable ongoing programme of research on DNA (see time line in Box 1). These studies include the physical properties of DNA, methods of extraction, and whether the content and composition of DNA is the same for all the cells of the same organism. Also discussed were the damaging effects of ultraviolet light and ionizing radiation on DNA, and differing views over the involvement of nucleic acids in protein synthesis.

Researchers working on DNA at that time were principally biochemists and physical chemists, and their institutional locations and funding were chiefly medically related. Their interests and means of support related to two main concerns of the time — the action of 'mutagens' (agents that cause mutations in DNA), a subject important to the international debate on the effects of ionizing radiation and radioactive materials (see accompanying article by Friedberg, page 122), and the nature of protein synthesis, of great interest to biochemists in the light of its importance in growth and nutrition, in addition to cancer research.

In the light of the muted reception of the structure, let us take a different angle and ask what justification was there in the 1950s for giving the DNA double helix more than passing attention? At the time, most scientists reading *Nature* viewed DNA as a 'conjugated protein', owing to its association with protein; it was important as such, but not in its own right. This was despite the remarkable work of Oswald Avery, Colin MacLeod and Maclyn McCarty in 1944 (ref. 10; and see accompanying article by McCarty, page 92), followed by Al Hershey and Martha Chase's demonstration in 1952 (ref. 11) that most of the material entering a bacterium from an infecting bacterial virus is nucleic acid not protein. These studies made DNA look very much like the hereditary material.

Why you are you

Nearer secret of life

By RITCHIE CALDER
The Science Editor

AN exciting discovery about what makes YOU the sort of person you are will be discussed today by one of Britain's foremost scientists.

It was Sir Laurence Bragg, director of the Cavendish Laboratory, Cambridge, who used the word "exciting" yesterday.

He will be talking to [illegible] Hospital medical school about the discovery two groups of young scientists have made with X-rays.

One group is at King's College, London; the other at the Cavendish.

They have found the structure of the chemical which transmits—from one generation to another—inherited characteristics like the colour of the eyes, the shape of the nose and even intelligence.

Vast field opens

They think it is a chemically pure substance which can be isolated from the living cell and crystallised. They call it "DNA" (short for deoxyribose nucleic acid) and they have produced a model of its structure.

Sir Lawrence could "tell me: 'It provides the first rational explanation of how a chemical can reproduce itself.'"

One could go further and say that it means to the study of the living processes what Rutherford's early descriptions of the structure of the nucleus of the atom meant to physics.

It will open up a vast new field of research into the secret of life.

On four groups of elements, according to their arrangement, depend the characteristics passed from generation to generation.

No one suggests these groupings can yet be arranged artificially. Discovering how these chemical "cards" are shuffled and paired will keep the scientists busy for the next 50 years.

Ritchie Calder's report on the discovery of the structure of DNA on page 1 of the *News Chronicle*, 15 May 1953.

No one suggests these groupings can yet be arranged artificially. Discovering how these chemical "cards" are shuffled and paired will keep the scientists busy for the next 50 years.

Connecting structure to function

More information was needed to convince the scientific community. What was there about the chemistry of DNA to justify its role in inheritance? An answer came with the structure put forward by Watson and Crick. Chief among its "novel features" of "considerable biological interest"[2], Watson and Crick described the pairing of the bases, where adenine forms hydrogen bonds with thymine, and guanine with cytosine. This pairing, they wrote, "immediately suggests a possible copying mechanism for the genetic material."[2] Expanding on this in a subsequent paper appearing in *Nature* a month later, they wrote of DNA: "Until now, however, no evidence has been

BETTMANN/CORBIS

Six of the Nobel winners of 1962 display their diplomas after formal ceremonies in Stockholm's Concert Hall. From left to right: Maurice Wilkins (Medicine), Max Perutz (Chemistry), Francis Crick (Medicine), John Steinbeck (Literature), James Watson (Medicine) and John Kendrew (Chemistry).

presented to show how it might carry out the essential operation required of a genetic material, that of exact self-duplication."[5]

With these words Watson and Crick claimed their priority on a mechanism for DNA replication, but admitted there were problems with their scheme: how do the chains unwind and separate "without everything getting tangled"[5]? What is the exact mechanism by which gene duplication occurs? How does the genetic material "exert a highly specific influence on the cell"[12] when the sequence of bases assumed to encode the specificity is on the inside of the helical molecule?

The 'unwinding problem' dominated much of the early discussions that followed the discovery of the DNA structure. In 1953, Watson and Crick admitted it was "formidable"[12], but support for their structure came in 1958, when Matthew Meselson and Franklin Stahl proved the semi-conservative nature of DNA replication[13]: each of the two new daughter DNA molecules formed during DNA replication consists of one strand from the original parent molecule and a new strand synthesized from the parent strand, which served as a template. This confirmed Watson and Crick's theoretical prediction from the structure that replication would proceed in a semi-conservative manner. Later that same year, Arthur Kornberg announced the partial purification of an enzyme that catalyses DNA synthesis later called DNA polymerase[14]. This first linked enzymology to the double helix, for not long thereafter Kornberg provided biochemical evidence that DNA polymerase synthesizes new strands from opposite directions of the two chains of the molecule[15].

In 1957, Crick defined biological 'information' as the sequence of the bases in the nucleic acids and of the amino acids in proteins, and proposed the now famous 'central dogma' according to which information so defined flows between the nucleic acids and proteins only in one direction — from the former to the latter[16]. Just four years later, Marshall Nirenberg and Heinrich Matthaei successfully synthesized a polypeptide constituted of only one kind of amino acid (phenylalanine) using an RNA composed only of one kind of base (uracil). They concluded that "one or more [of these RNA bases] appear to be the code for phenylalanine."[17] Meanwhile, Crick, Sydney Brenner and Leslie Barnett had been using genetic analysis to investigate mutagenesis. This led them to the important concept of a form of mutation in which there is a 'frame shift' in the sequence of the bases in DNA, from which they went on to infer that the genetic message is composed of single or multiple triplets of bases, and that the message is read starting at a fixed point and proceeds always in the same

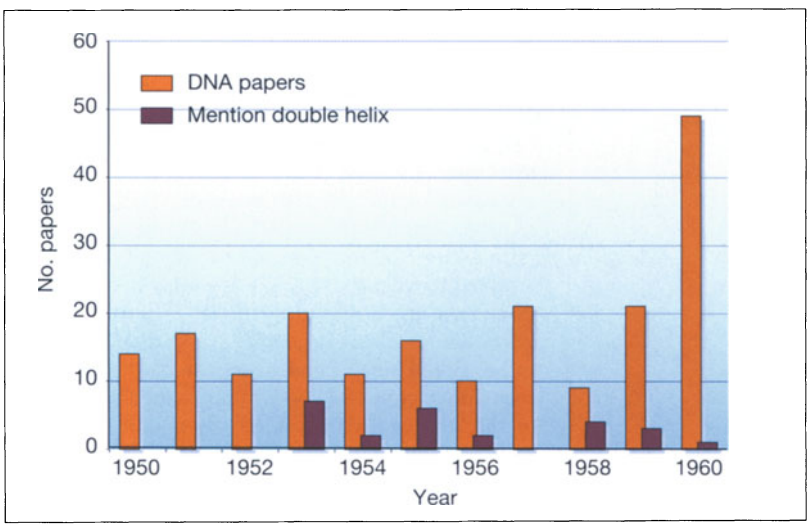

Figure 1 Papers published in *Nature* referring to DNA and the extent of their reference to the double helix 1950–1960.

Box 1
Time line of the discovery of the structure of DNA

1869 Fritz Miescher discovers that the nuclei of pus cells contain an acidic substance to which he gave the name 'nuclein'. Later he finds that nuclein is composed of a protein and a compound to which the name nucleic acid, and subsequently DNA, will be given.

1919 Phoebus Aaron Levene proposes the 'tetranucleotide' structure of DNA, whereby the four bases of DNA were arranged one after another in a set of four.

1928 Frederick Griffith finds that a substance in heat-killed bacteria can cause heritable changes in the live bacteria alongside them. He calls the phenomenon 'transformation'.

1938 Rudolf Signer, Torbjorn Caspersson and Einer Hammarsten find molecular weights for DNA between 500,000 and 1,000,000 daltons. Levene's tetranucleotide must be a polytetranucleotide.

1944 Oswald Avery, Colin MacLeod and Maclyn McCarty establish the chemical identity of Griffith's transforming principle as DNA, and they suggest that it may function as the genetic material.

1949 Erwin Chargaff reports that DNA base composition varies from one species to another, yet the ratio between the quantities of the two purine bases, adenine and guanine, and that between the quantities of the two pyrimidine bases, thymine and cytosine, remains about the same, namely one to one.

1949 Roger and Colette Vendrely, together with André Boivin find half as much DNA in the nuclei of sex cells as they find in the body cells, thus paralleling the reduction in the number of chromosomes, making DNA look like the genetic material.

1951 Rosalind Franklin distinguishes two forms of DNA, the paracrystalline B form and the crystalline A form.

1952 Al Hershey and Martha Chase find that DNA but scarcely any protein from an infecting bacterial virus enters the bacterial cell and can be recovered from the progeny virus particles.

1952 Rosalind Franklin and Raymond Gosling produce a magnificent X-ray diffraction pattern of the B form of DNA.

1953 James Watson and Francis Crick, Rosalind Franklin and Raymond Gosling, Maurice Wilkins, W. E. Seeds, Alec Stokes and Herbert Wilson, and Bertil Jacobson all publish on the structure of DNA[2–8].

1954 George Gamow suggests a DNA code for the synthesis of proteins.

1955 Seymour Benzer analyses the fine structure of the genetic material of a bacterial virus at a level close to the distances that separate the individual bases along the DNA chain.

1957 Francis Crick proposes 'the sequence hypothesis' and 'the central dogma'.

1958 Matthew Meselson and Franklin Stahl demonstrate the semi-conservative replication of DNA.

1959 Arthur Kornberg and colleagues isolate the enzyme DNA polymerase.

1961 Marshall Nirenberg and Johann Heinrich Matthaei show that a sequence of nucleotide can encode a particular amino acid, laying the foundations for deciphering the genetic code.

1962 The Nobel prize in medicine is awarded to James Watson, Francis Crick and Maurice Wilkins.

direction[18]. Thus was the stage set for the subsequent unravelling of the entire genetic code.

From a muted reception in 1953 to accelerating momentum towards the end of the decade, one is tempted to infer that the DNA double helix was not taken seriously until a mechanism for its involvement in protein synthesis began to take shape. There was, to be sure, a small band of scientists who from the start either built their careers upon the implications of the structure (such as Meselson and Alexander Rich) or redirected their research to follow it up (including Seymour Benzer and Sydney Brenner). However, many scientists, notably Erwin Chargaff and Alexander Dounce, did not refer to the structure in their scientific papers in the mid-fifties, even though it was clearly relevant and presumably known to them. Such omissions suggest that some biochemists had their own agendas, and the double helix was not at first seen as an aid to their work.

Biochemists debate protein synthesis

Biochemists' reservations about the double helix stemmed in part from the fact that evidential support for it in 1953 was far from strong. Watson and Crick themselves admitted that it "could in no sense be considered proved", although it was "most promising"[19]. In part the biochemists' coolness owed much to the debates among them over the mechanism of protein synthesis. The paper by Peter Campbell and Thomas Work, published in *Nature* on 6 June 1953, portrayed this debate vividly. They identified two contrasting theories under discussion on how proteins are made: first, the peptide theory (also known as the multi-enzyme theory), where proteins are made by "stepwise coupling of many small peptide units"; and second, the template theory, involving "synthesis on templates, each template being specific for a single protein structure and probably identifiable as a gene."[20]

The peptide model was, for a very long time, supported by many prominent biochemists, including Joseph Fruton. The conviction behind it was the power of enzymes to both synthesize and break down their substrates, with a high degree of specificity attributed to both actions. Synthesis was proposed to involve the formation of a succession of peptides, ultimately yielding the protein molecule, and enzymes synthesize only those peptide bonds that they also hydrolyse. But the problem with this theory was that, except for a very few special cases, the alleged peptides constituting the intermediaries in protein synthesis could neither be detected in the cell nor incorporated into the protein being synthesized. Amino acids, however, could be incorporated, indicating they were the building blocks of proteins.

The second model of protein synthesis, which assumed synthesis on a template, had been advocated by Dounce in 1952. He pictured polypeptide chains being laid down on RNA molecules, and the RNA sequence determining the sequence of amino acids incorporated (on a one-to-one basis). Thus, DNA in the nucleus would control the order of bases in the RNA[21].

After weighing up the merits and difficulties of Dounce's scheme, Campbell and Work voiced their distaste for the genetic control of protein synthesis, remarking in 1953 that: "...the gene is essentially an abstract idea and it may be a mistake to try to clothe this idea in a coat of nucleic acid or protein... if we must have a gene it should have a negative rather than a positive function so far as protein synthesis is concerned."[20] Only three years later, however, Robert Sinsheimer concluded a lecture at the California Institute of Technology with the following words: "The gene, once a formal abstraction, has begun to condense, to assume form and structure and defined activity."[1]

But those three years were a scene of pronounced change. By January 1957, when Fruton revised the second edition of his widely used textbook *General Biochemistry*, his remarks on the peptide theory were

cautious and were followed by a discussion of the role of RNA on which, he noted, there have been "stimulating speculations about the role of nucleic acids as 'templates' in protein synthesis."[22] Earlier in the book he devoted a paragraph to the double helix, describing it as an 'ingenious speculation'. The only diagram was of the base pair adenine–thymine, rather than the helical model of the structure.

Kornberg had shown in 1957 that DNA replication follows the rules of base pairing, whereby DNA polymerase adds a base to the newly synthesized strand that is complementary to the opposing base in the template strand (A is always opposite T, and C always opposite G). But his interest in the subject had not been stimulated by Watson and Crick's discovery. Rather, in 1953 he was preoccupied with how coenzymes (non-protein compounds needed for enzyme activity) are synthesized from nucleotides. He was led to wonder how DNA and RNA might be made from thousands of nucleotides. "The significance of the double helix," he recalled, "did not intrude" into his work until 1956, after he had shown that a "moderately purified fraction" of what he was later to call DNA polymerase "appeared to increase the size of a DNA chain."[23,24]

Conclusion

The two once enigmatic processes — DNA replication and protein synthesis — intersected ongoing research programmes in the physical, organic and biological chemistry of the early 1950s. After the discovery of the double helix, those grappling with the problem of replication found its molecular foundation in the structure of DNA, although it took more than two decades to deduce the intricate mechanism of its operation in the cell (see accompanying article by Alberts, page 117). Those working on protein synthesis found the source of its specificity lay in the base sequence of DNA.

But why celebrate this one discovery? Why not celebrate the golden jubilee of Max Perutz's solution to the 'phase problem' for proteins in 1953, without which the subsequent discovery of the structure of myoglobin and haemoglobin would not have been possible? What about the year 2005 for celebrating the golden jubilee of Sanger's determination of the complete amino-acid sequence of a protein? Undoubtedly, the double helix has remarkable iconic value that has contributed significantly to its public visibility, something that has not been achieved by any of the protein structures (see accompanying article by Kemp, page 102). There is, too, a degree of notoriety attaching to the manner of its discovery and the characters involved that has given spice to the story, as widely publicized by James Watson's account of the discovery in *The Double Helix*, published in 1968 (ref. 25), and Brenda Maddox's recent illuminating biography of Rosalind Franklin[26]. But there is a centrality about DNA that relates to the centrality of heredity in general biology.

The silver and golden jubilees of the Queen's accession to the throne have come and gone, nuclear power stations are no longer being built in the United Kingdom, and mountaineer after mountaineer has ascended Mount Everest without a fanfare of press reports. But DNA is very much in the news — whether it be as a tool for studying evolution, a forensic test for rape, a source of genetic information or a path to designer drugs. And what better emblem or mascot is there for molecular biology than the double helix, and its spartan yet elegant representation in the original paper[2] from the pen of Odile Crick, Francis's wife, fifty years ago? □

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Discovering genes are made of DNA

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Maclyn McCarty is the sole surviving member of the team that made the remarkable discovery that DNA is the material of inheritance. This preceded by a decade the discovery of the structure of DNA itself. Here he shares his personal perspective of those times and the impact of the double helix.

Editor's note — For a long time, biologists thought that 'genes', the units of inheritance, were made up of protein. In 1944, in what was arguably the defining moment for nucleic acid research, Oswald Avery, Maclyn McCarty and Colin MacLeod, at Rockefeller Institute (now University) Hospital, New York, proved that DNA was the material of inheritance, the so-called stuff of life. They showed that the heritable property of virulence from one infectious strain of pneumococcus (the bacterial agent of pneumonia) could be transferred to a noninfectious bacterium with pure DNA[1]. They further supported their conclusions by showing that this 'transforming' activity could be destroyed by the DNA-digesting enzyme DNAase[2,3].

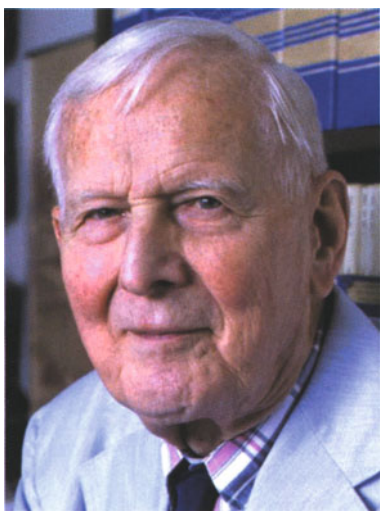
This work first linked genetic information with DNA and provided the historical platform of modern genetics. Their discovery was greeted initially with scepticism, however, in part because many scientists believed that DNA was too simple a molecule to be the genetic material. And the fact that McCarty, Avery and MacLeod were not awarded the Nobel prize is an oversight that, to this day, still puzzles.

"The pivotal discovery of 20th-century biology." Joshua Lederberg, Rockefeller University, 1994, referring to the discovery by McCarty, Avery and MacLeod.

At the time of our discovery and publication in 1944 (ref. 1) of the research showing that DNA is heritable, my personal view, which I shared with MacLeod, was that there was little doubt that genes are made of DNA, and that this would ultimately be accepted. I was not sure of the best approach to use in pursuing research on the subject, but suspected that clarification of the structure of DNA was necessary.

But this was not an area of research in which I had received any training. Additionally, I had planned to make my career in disease-oriented research, and knowledge of the gene did not seem likely to become applicable in this area for some years. Thus, when invited to lead my own laboratory in the Rockefeller Hospital, investigating streptococcal infection and the pathogenesis of rheumatic fever, I decided to leave Avery's laboratory for this new position in July 1946.

Rollin Hotchkiss joined Avery at this point, and together with Harriett Taylor (a recent PhD graduate in genetics who had joined the laboratory in 1945), carried out studies increasing the purity of the transforming DNA mixture by further reducing any contaminating traces of protein. Together with other investigators, they also showed that properties of the pneumococcus other than just specific polysaccharide components of its cell wall could be transferred by the DNA preparations, indicating that the purified DNA also contained other genes of the bacterium.



Maclyn McCarty at The Rockefeller University.

THE ROCKEFELLER UNIVERSITY

Our findings continued to receive little acceptance for a variety of reasons, the most significant being that the work on the composition of DNA, dating back to its first identification 75 years earlier, had concluded that DNA was too limited in diversity to carry genetic information. Even those biologists who had considered the possibility had dropped the idea, and the prevailing dogma was that if genes are composed of a known substance, it must be protein.

There were a few biologists who took a different view, the most notable being Erwin Chargaff, who changed his area of research to DNA after reading our 1944 paper[1]. His work revealed the great diversity in DNA isolated from various sources, and that despite this diversity the amount of adenine always equalled that of thymine, and the amount of guanine that of cytosine. The latter finding was an important factor in the next significant advance in the field — the Watson–Crick determination of the double helical structure of DNA.

After the change in my research activity, I continued to give talks on our work on pneumococcal transformation and found the acceptance of the probable genetic role of DNA still to be minimal. However, I was convinced that it was only a matter of time before our results would become established.

Even though I was no longer involved in research on the subject, I continued to follow the developments as they appeared in the literature. Thus, when the papers of Watson and Crick describing the double helical structure of DNA were published in *Nature* in 1953, I certainly grasped the significance of their findings and was pleased to see such illuminating results come from a structural approach. I was not so pleased, however, that they failed to cite our work as one reason for pursuing the structure of DNA.

The concept of the double helix also hastened the silencing of those who had clung to the idea of genes as proteins. As a progressively larger body of investigators joined the study of the genetic role of DNA, there was an expanding amount of new information, starting with the resolution of the genetic code. By the end of the twentieth century, subsequent work on the mechanisms by which DNA is replicated with each cell division, is reshuffled with each generation, and is repaired when mistakes arise — the importance of which can in each case be traced back to the finding that DNA is the hereditary material — has transformed research in all areas of biology, technology and medicine. □

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The double helix and the 'wronged heroine'

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In 1962, James Watson, Francis Crick and Maurice Wilkins received the Nobel prize for the discovery of the structure of DNA. Notably absent from the podium was Rosalind Franklin, whose X-ray photographs of DNA contributed directly to the discovery of the double helix. Franklin's premature death, combined with misogynist treatment by the male scientific establishment, cast her as a feminist icon. This myth overshadowed her intellectual strength and independence both as a scientist and as an individual.

"Science and everyday life cannot and should not be separated. Science, for me, gives a partial explanation of life. In so far as it goes, it is based on fact, experience and experiment." Rosalind Franklin, in a letter to her father, summer 1940.

In late February 1953, Rosalind Franklin, a 33-year-old physical chemist working in the biophysics unit of King's College in London, wrote in her notebooks that the structure of DNA had two chains. She had already worked out that the molecule had its phosphate groups on the outside and that DNA existed in two forms.

Two weeks later James Watson and Francis Crick, at the Cavendish Laboratory at Cambridge, built their now celebrated model of DNA as a double helix. They did it not only through brilliant intuition and a meeting of compatible minds, but also on the basis of Franklin's unpublished experimental evidence, which had reached them through irregular routes. She did not know that they had seen either her X-ray photograph (Fig. 1), showing unmistakable evidence of a helical structure, or her precise measurements of the unit cell (the smallest repeating unit), and the crystalline symmetry, of the DNA fibres.

As Watson was to write candidly, "Rosy, of course, did not directly give us her data. For that matter, no one at King's realized they were in our hands." When this admission appeared in Watson's best-selling, much-acclaimed book of the discovery, *The Double Helix*, published in 1968 (ref. 1), he was a Harvard professor and Nobel laureate (he had shared the prize for medicine and physiology in 1962, with Crick and Maurice Wilkins of King's College.) By then Franklin had died — in 1958, at the age of 37, from ovarian cancer.

Other comments dismissive of "Rosy" in Watson's book caught the attention of the emerging women's movement in the late 1960s. "Clearly Rosy had to go or be put in her place [...] Unfortunately Maurice could not see any decent way to give Rosy the boot". And, "Certainly a bad way to go out into the foulness of a [...] November night was to be told by a woman to refrain from venturing an opinion about a subject for which you were not trained."

A feminist icon

Such flamboyantly chauvinist phrases were sufficient to launch the legend of Franklin, the wronged heroine. So too was Watson's insistence on judging Franklin by her appearance rather than by her performance as a scientist. (She was, when she came to King's from the French government laboratory where she had worked from 1947 to the end of 1950, a recognized expert on the structure of coals, carbons and disordered crystals, with many publications to her credit.)

The Franklin myth has continued to grow, abetted by the fact of her tragically early death. Franklin has become a feminist icon — the Sylvia Plath of molecular biology — seen as a genius whose gifts were sacrificed to the greater glory of the male. Her failure to win the Nobel prize has been given as a prime example

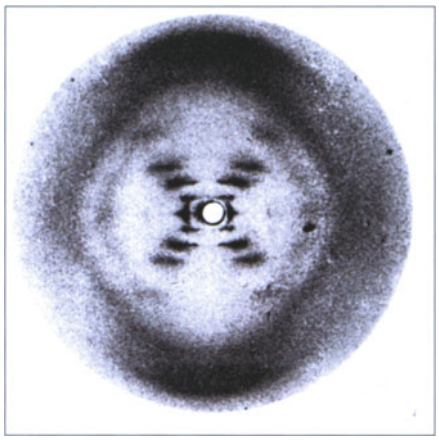

Figure 1 "Her photographs are among the most beautiful X-ray photographs of any substance every taken." — J. D. Bernal, 1958. Franklin's X-ray diagram of the B form of sodium thymonucleate (DNA) fibres, published in *Nature* on 25 April 1953, shows "in striking manner the features characteristic of helical structures"[5].

of the entrenched misogyny of the science establishment, rather than the consequence of the Nobel statute against posthumous awards.

Watson's caricature of the bad-tempered "Rosy" drew a counter-blast from her good friend, the American writer Anne Sayre, in *Rosalind Franklin and DNA*, published in 1975 (ref. 2). Sayre's book provided a much-needed corrective portrait, but was marred by a feminist bias. For example, it grossly underestimated the number of women scientists at King's in the early 1950s. Sayre maintained there was only one other than Franklin, whereas there were at least eight on the senior staff. She insisted, moreover, that women's exclusion from the King's senior common room deprived Franklin of the intellectual companionship of her colleagues. In fact, most of the scientific staff preferred to eat in the joint dining room, men and women together, and the women, in general, felt well treated at King's.

Reassessing the facts

As a biographer writing nearly three decades later and given access to Franklin's personal correspondence, I found a more attractive, capable woman than Watson had suggested, and a King's College more congenial and welcoming to women scientists than Sayre had allowed. I also found that Franklin felt singularly unhappy at King's, not so much because of her gender, but because of her class and religion: a wealthy Anglo-Jew felt out of place in a Church of England setting dominated by swirling cassocks and students studying for the priesthood. "At King's," she wrote to Sayre (albeit inaccurately), "there are neither Jews nor foreigners".

She was, in fact, so unhappy at King's that, in early 1953, getting out as fast as possible was far more important to her than finishing her work on DNA. How far she had advanced was reported in two articles in *Nature*[3,4] by Sir Aaron Klug, Franklin's closest collaborator at Birkbeck College, London, where she moved to from King's. He concluded that she had come very close to discovering the structure of DNA herself.

An irony of the story is that her own manuscript (coauthored by her student, R. G. Gosling and dated 17 March 1953) summarizing her results was already prepared by the time news reached King's that Watson and Crick had cracked the DNA secret. Thus she inserted a hand-written amendment to her manuscript — which was published in *Nature* on 25 April 1953 (ref. 5), along with the now-celebrated Watson and Crick paper and another by Wilkins, Herbert Wilson and Alec Stokes of King's — to say "Thus our general ideas are not inconsistent with the model proposed by Watson and Crick in the preceding communication". And so they should have been, for the Watson-Crick findings were based on her data.

There is no evidence that she knew that in late January 1953 Wilkins had innocently shown her Photograph 51, with its stark cross of black reflections (Fig. 1), to Watson, who was visiting King's. Nor did she know that in February 1953 Max Perutz, then at the Cavendish Laboratory, had let Watson and Crick see his copy of the Medical Research Council's report summarizing the work of all principal researchers, including Franklin's.

At the same time there is no evidence that Franklin felt bitter about their achievement or had any sense of having been outrun in a race that nobody but Watson and Crick knew was a race. Indeed, she could accept the Watson–Crick model as a hypothesis only. She wrote in *Acta Crystallographica* in September 1953 that "discrepancies prevent us from accepting it in detail"[6].

Belated credit

Watson and Crick seem never to have told Franklin directly what they subsequently have said from public platforms long after her death — that they could not have discovered the double helix of DNA in the early months of 1953 without her work. This is all the more surprising in view of the close friendship that developed among the three of them — Watson, Crick and Franklin — during the remaining years of her life. During this time, she was far happier at non-sectarian Birkbeck than she ever was at King's, and led a spirited team of researchers studying tobacco mosaic virus (TMV).

From 1954 until months before her death in April 1958, she, Watson and Crick corresponded, exchanged comments on each other's work on TMV, and had much friendly contact. At Wood's Hole, Massachusetts, in the summer of 1954 Watson offered Franklin a lift across the United States as he was driving to her destination, the California Institute of Technology. In the spring of 1956 she toured in Spain with Crick and his wife Odile and subsequently stayed with them in Cambridge when recuperating from her treatments for ovarian cancer. Characteristically, she was reticent about the nature of her illness. Crick told a friend who asked that he thought it was "something female".

In the years after leaving King's, Franklin published 17 papers, mainly on the structure of TMV (including four in *Nature*). She died proud of her world reputation in the research of coals, carbons and viruses. Given her determination to avoid fanciful speculation, she would never have imagined that she would be remembered as the unsung heroine of DNA. Nor could she have envisaged that King's College London, where she spent the unhappiest two years of her professional career, would dedicate a building — the Franklin–Wilkins building — in honour of her and the colleague with whom she had been barely on speaking terms. □

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The mosaic that is our genome

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The discovery of the basis of genetic variation has opened inroads to understanding our history as a species. It has revealed the remarkable genetic similarity we share with other individuals as well as with our closest primate relatives. To understand what make us unique, both as individuals and as a species, we need to consider the genome as a mosaic of discrete segments, each with its own unique history and relatedness to different contemporary and ancestral individuals.

The discovery of the structure of DNA[1], and the realization that the chemical basis of mutations is changes in the nucleotide sequence of the DNA, meant that the history of a piece of DNA could be traced by studying variation in its nucleotide sequence found in different individuals and in different species. But it was not until rapid and inexpensive methods became available for probing DNA sequence variation in many individuals that the efficient study of molecular evolution in general — and of human evolution in particular — became feasible. Thus, the development in the 1980s of techniques for efficiently scoring polymorphisms with restriction enzymes and amplifying DNA[2,3] enabled the study of molecular evolution to become a truly booming enterprise.

What follows is a personal and, by necessity, selective attempt to consider what the accelerating pace of exploration of human genetic variation over the past two decades has taught us about ourselves as a species, as well as some suggestions for what may be fruitful areas for future studies.

Primate relations

The first insight of fundamental importance for our understanding of our origins came from comparisons of DNA sequences between humans and the great apes. These analyses showed that the African apes, especially the chimpanzees and the bonobos, but also the gorillas, are more closely related to humans than are the orangutans in Asia[4]. Thus, from a genetic standpoint, humans are essentially African apes (Fig. 1). Although there had been hints of this from molecular comparisons of proteins[5,6], it was a marked shift from the earlier common belief that humans represented their own branch separate from the great apes.

Our sense of uniqueness as a species was further rocked by the revelation that human DNA sequences differ by, on average, only 1.2 per cent from those of the chimpanzees[7], as a consequence of humans and apes sharing a recent common ancestry. It should be noted that the dating of molecular divergences has uncertainties of unknown magnitude attached, not least because of calibration based on palaeontological data. Nevertheless, it seems clear that the human evolutionary lineage diverged from that of chimpanzees about 4–6 million years ago, from that of gorillas about 6–8 million years ago, and from that of the orangutans about 12–16 million years ago[7]. Before the advent of molecular data, the human–chimpanzee divergence was widely believed to be about 30 million years old.

In fact, we have recently come to realize that the relationship between humans and the African apes is so close as to be entangled. Although the majority of regions in our genome are most closely related to chimpanzees and bonobos, a non-trivial fraction is more closely related to gorillas[7]. In yet other regions, the apes are more closely related to each other than to us (Fig. 2). This is because the speciation events that separated these lineages occurred so closely in time that genetic variation in the first ancestral species, from which the gorilla lineage diverged, survived until the second speciation event between the human and chimpanzee lineages[8]. Thus, there is not one history with which we can describe the relationship of our genome to the genomes of the African apes, but instead different histories for different segments of our genome. In this respect, our genome is a mosaic, where each segment has its own relationship to that of the African apes.

Modern humans

The mosaic nature of our genome is even more striking when we consider differences in DNA sequence between currently living humans. Our genome sequences are about 99.9 per cent identical to each other. The variation found along a chromosome is structured in 'blocks' where the nucleotide substitutions are associated in so-called haplotypes (Figs 2b and 3). These 'haplotype blocks' are likely to result from the fact that recombination, that is, the re-shuffling of chromosome segments that occurs during formation of sex cells (meiosis), tends to occur in certain areas of the chromosomes more often than in others[9–11]. In addition, the chance occurrence of recombination events at certain spots and not at others in the genealogy of human chromosomes will influence the structure of these blocks. Thus, any single human chromosome is a mosaic of different haplotype blocks, where each block has its own pattern of variation. Although the delineation of such blocks depends on the methods used to define them, they are typically 5,000–200,000 base pairs in length, and as few as four to five common haplotypes account for most of the variation in each block (Fig. 3).

Of 928 such haplotype blocks recently studied in humans from Africa, Asia and Europe[12], 51 per cent were found on all three

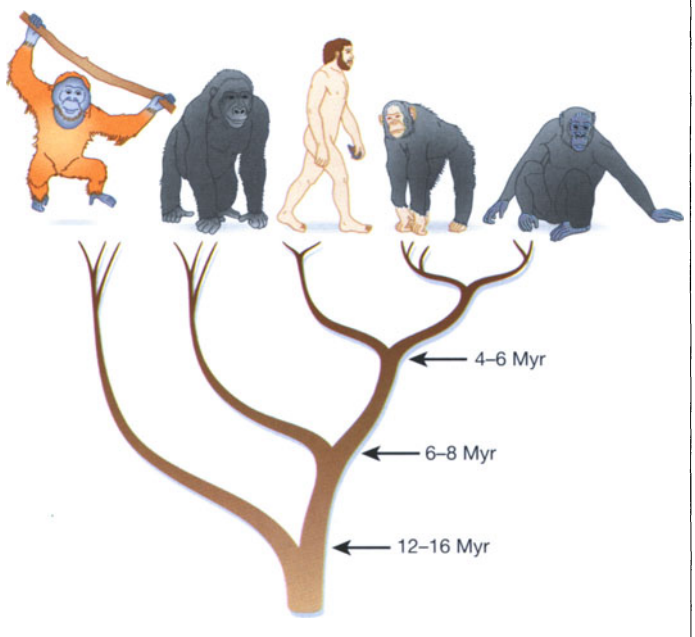

Figure 1 Tree showing the divergence of human and ape species. Approximate dates of divergences are given for, from left to right, orangutan, gorilla, human, bonobo and chimpanzee.

continents, 72 per cent in two continents and only 28 per cent on one continent. Of those haplotypes that were on one continent only, 90 per cent were found in Africa, and African DNA sequences differ on average more among themselves than they differ from Asian or European DNA sequences[13]. Therefore, within the human gene pool, most variation is found in Africa and what is seen outside Africa is a subset of the variation found within Africa.

Two parts of the human genome can be regarded as haplotype blocks where the history is particularly straightforward to reconstruct, as no recombination occurs at all. The first of these is the genome of the mitochondrion (the cellular organelle that produces energy and has its own genetic material), which is passed on to the next generation from the mother's side; the second is the Y chromosome, which is passed on from the father's side. Variation in DNA sequences from both the mitochondrial genome[14–16] and the Y chromosome[17], as well as many sections of the nuclear genome[13,18–20], have their geographical origin in Africa. Because other evidence suggest that humans expanded some 50,000 to 200,000 years ago[21] from a population of about 10,000 individuals, this suggests that we expanded from a rather small African population. Thus, from a genomic perspective, we are all Africans, either living in Africa or in quite recent exile outside Africa.

Ancient humans

What happened to the other hominids that existed in the Old World from about 2 million years ago until about 30,000 years ago? For instance, the Neanderthals are abundant in the fossil record and persisted in western Europe until less than 30,000 years ago. Analysis of Neanderthal mitochondrial DNA has shown that, at least with respect to the mitochondrial genome, there is no evidence that Neanderthals contributed to the gene pool of current humans[22–25]. It is possible, however, that some as yet undetected interbreeding took place between modern humans and archaic hominids, such as *Homo erectus* in Asia or Neanderthals in Europe[22,26,27].

But any interbreeding would not have significantly changed our genome, as we know that the variation found in many haplotype blocks in the nuclear genome of contemporary humans is older than the divergence between Neanderthals and humans. Thus, the divergence of modern humans and Neanderthals was so recent that Neanderthal nuclear DNA sequences were probably more closely related to some current human DNA sequences than to other Neanderthals. In other words, the overlapping genetic variation that is likely to have existed between different ancient hominid forms makes it difficult to resolve the extent to which any interbreeding occurred.

Nevertheless, the limited variation among humans outside Africa, as well palaeontological evidence[28], suggest that any contribution cannot have been particularly extensive. Thus, it seems most likely that modern humans replaced archaic humans without extensive interbreeding and that the past 30,000 years of human history are unique in that we lack the company of the closely related yet distinct hominids with which we used to share the planet.

Human variation and 'race'

Comparisons of the within-species variation among humans and among the great apes have shown that humans have less genetic variation than the great apes[29,30]. Furthermore, early data that only about 10 per cent of the genetic variation in humans exist between so-called 'races'[31] is borne out by DNA sequences which show that races are not characterized by fixed genetic differences. Rather, for any given haplotype block in the genome, a person from, for example, Europe is often more closely related to a person from Africa or from Asia than to another person from Europe that shares his or her complexion (for example, see ref. 32; Fig. 2).

Claims about fixed genetic differences between races (see ref. 33 for example) have proved to be due to insufficient sampling[34]. Furthermore, because the main pattern of genetic variation across the globe is one of gene-frequency gradients[35], the contention that significant differences between races can be seen in frequencies of various genetic markers[36] is very likely due to sampling of populations separated by vast geographical distances. In this context it is worth noting that the colonization history of the United States has resulted in a sampling of the human population made up largely of people from western Europe, western Africa and southeast Asia. Thus, the fact that 'racial groups' in the United States differ in gene frequencies cannot be taken as evidence that such differences represent any true subdivision of the human gene pool on a worldwide scale.

Rather than thinking about 'populations', 'ethnicities' or 'races', a more constructive way to think about human genetic variation is to consider the genome of any particular individual as a mosaic of haplotype blocks. A rough calculation (Fig. 3) reveals that each individual carries in the order of 30 per cent of the entire haplotype variation of the human gene pool. Although not all of our

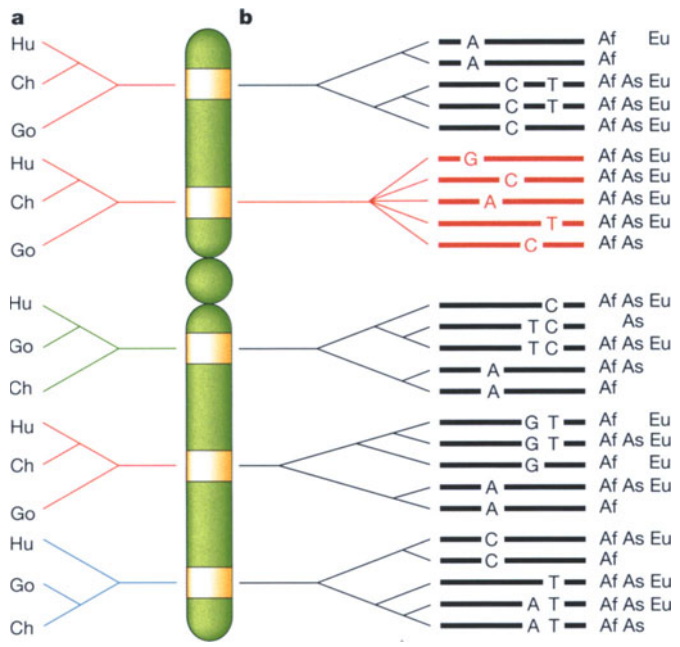

Figure 2 Within- and between-species variation along a single chromosome. **a**, The interspecies relationships of five chromosome regions to corresponding DNA sequences in a chimpanzee and a gorilla. Most regions show humans to be most closely related to chimpanzees (red) whereas a few regions show other relationships (green and blue). **b**, The among-human relationships of the same regions are illustrated schematically for five individual chromosomes. Most DNA variants are found in people from all three continents, namely Africa (Af), Asia (As) and Europe (Eu). But a few variants are found on only one continent, most of which are in Africa. Note that each human chromosome is a mosaic of different relationships. For example, a chromosome carried by a person of European descent may be most closely related to a chromosome from Asia in one of its regions, to a chromosome from Africa in another region, and to a chromosome from Europe in a third region. For one region (red), the extent of sequence variation within humans is low relative to what is observed between species. The relationship of this sequence among humans is illustrated as star-shaped owing to a high frequency of nucleotide variations that are unique to single chromosomes. Such regions may contain genes that contribute to traits that set humans apart from the apes.

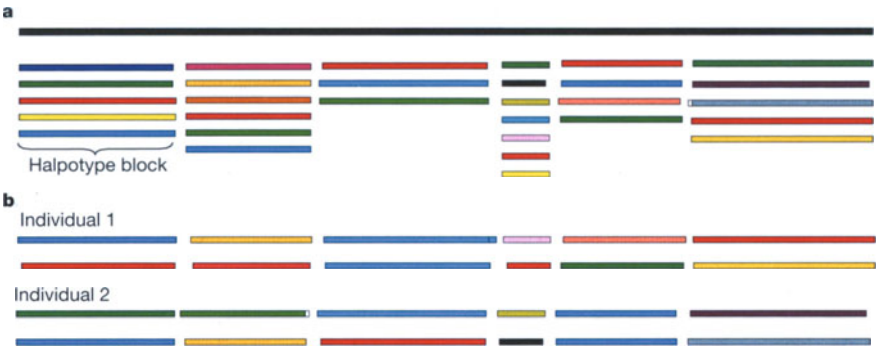

Figure 3 The mosaic structure of human genetic variation. **a**, Each human chromosome is made up of regions, called 'haplotype blocks', which are stretches of DNA sequence where three to seven variants (at frequencies above 5 per cent in the human population) account for most of the variation found among humans. Each such haplotype found in a block is illustrated here as a bar of different colour. The catalogue of haplotypes for every block makes up the 'haplotype map' of the human genome. **b**, The chromosomes of two hypothetical individuals are shown. Each individual carries two copies of each block (as humans carry two sets of chromosomes). As the chance that the two haplotypes carried at a block are identical is about 20 per cent, each of us carries an average of about 1.8 different haplotypes per block. Since there is on average 5.5 haplotypes for every block, each individual carries about 30 per cent of the total haplotype diversity of the entire human species. Haplotype blocks tend to be shorter in Africa than elsewhere; as a result, African variation will probably have to be used to define the species-wide block lengths, which may be an average of around 10,000 base pairs. Note that not all of the human genome may have a clearly definable haplotype-block structure.

genome may show a typical haplotype-block structure and more research is needed to fully understand the haplotype landscape of our genome, this perspective clearly indicates that each of us contain a vast proportion of the genetic variation found in our species. In the future, we therefore need to focus on individuals rather than populations when exploring genetic variation in our species.

Tracking human traits

What are the frontiers ahead of us in human evolutionary studies? One of them, to my mind, is to identify gene variants that have been selected and fixed in all humans during the past few hundred thousand years. These will include genes involved in phenotypic traits that set humans apart from the apes and at least some archaic human forms (for example, genes involved in complex cognitive abilities, language and longevity). However, an important obstacle in this respect is that there is little detailed knowledge of many of the relevant traits in the great apes. For example, only recently has the extent to which apes possess the capability for language[37] and culture[38] begun to be comprehensively described. As a consequence, we have come to realize that almost all features that set humans apart from apes may turn out to be differences in grade rather than absolute differences.

Many such differences are likely to be quantitative traits rather than single-gene traits. To have a chance to unravel the genetic basis of such traits, we will need to rigorously define the differences between apes and humans — for instance, how we learn, how we communicate and how we age. In the next few years, geneticists will therefore need to consider insights from primatology and psychology, and more studies will be required that directly compare humans to apes.

There are, however, ways in which we can contribute towards the future unravelling of functionally important genetic differences between humans and apes. For example, we can identify regions of the human genome where the patterns of variation suggest the recent occurrence of a mutation that was positively selected and swept through the entire human population. The sequencing of the chimpanzee genome, as well as the haplotype-map project, will greatly help in this. Further prerequisites include the capability to determine the DNA sequence of many human genomes and the development of tools and methods to analyse the resulting data; in particular, a more realistic model of human demographic history is required.

Collectively these studies will allow us to identify regions in the human genome that have recently been acted upon by selection and thus are likely to contain genes contributing to human-specific traits (Fig. 2). Other interesting candidate genes for human-specific traits are genes duplicated or deleted in humans[39], genes that have changed their expression in humans[40], and genes responsible for disorders affecting traits unique to humans, such as language[41] and a large brain size[42].

A problem inherent in studying genes that are involved in traits unique to humans, such as language, is that functional experiments cannot be performed, as no animal model exists, and transgenic humans or chimpanzees cannot be constructed. A further difficulty is that many genes that enable humans to perform tasks of interest may exert their effects during early development where our ability to study their expression both in apes and humans is extremely limited.

A challenge for the future is therefore to design ways around these difficulties. This will involve *in vitro* as well as *in silico* approaches that study how genes interact with each other to influence developmental and physiological systems. As these goals are achieved, we will be able to determine the order and approximate times of genetic changes during the emergence of modern humans that led to the traits that set us apart among animals. □

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Nature, nurture and human disease

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What has been learnt about individual human biology and common diseases 50 years on from the discovery of the structure of DNA? Unfortunately the double helix has not, so far, revealed as much as one would have hoped. The primary reason is an inability to determine how nurture fits into the DNA paradigm. We argue here that the environment exerts its influence at the DNA level and so will need to be understood before the underlying causal factors of common human diseases can be fully recognized.

"We used to think our fate was in our stars. Now we know, in large measure, our fate is in our genes." J. D. Watson, quoted in *Time* magazine, 20 March 1989 (ref. 1).

The double helix, in its simplicity and beauty, is the ultimate modern icon of contemporary biology and society. Its discovery provided the bridge between the classical breeding definition and the modern functional definition of genetics, and permanently united genetics with biochemistry, cell biology and physiology. The DNA structure provided an immediate explanation for mutation and variation, change, species diversity, evolution and inheritance. It did not, however, automatically provide a mechanism for understanding how the environment interacts at the genetic level.

One gene, one disease

Recognition that genes have a role in human disease dates back to the rediscovery of the rules that govern the inheritance of genes by Gregor Mendel — the so-called Mendelian laws of inheritance. So far, human geneticists have been most successful at understanding single-gene disorders, as their biological basis, and thus presumed action, could be predicted from inheritance patterns. Mendelian diseases are typically caused by mutation of a single gene that results in an identifiable disease state, the inheritance of which can readily be traced through generations.

The landmark sequencing of the human genome provided some important lessons about the role of genes in human disease. Notably, mutations in specific genes lead to specific biological changes, and rarely do mutations in multiple genes lead to an identical set of characteristics that obey 'Mendelian inheritance'. Additionally, sequence diversity of mutations is large and, consequently, individual mutations are almost always rare, showing relatively uniform global distributions.

But a few exceptions do exist. Some recessive mutations (mutations that influence a person only if both copies of the gene are altered) are surprisingly common in specific populations. This defiance of general mutation patterns arises either from chance increases in frequency in isolated populations, such as in the Old Order Amish[2], or from the protective effect of a deleterious mutation in a single copy, such as the genetic mutation that on the one hand causes sickle-cell anaemia, but on the other hand offers protection against malaria[3]. These examples show that human history, geography and ecology of a particular people are relevant to understanding their present-day molecular disease burden[4].

For over 90 years, the association between DNA mutations and a vast variety of single-gene disorders has repeatedly emphasized the notion that human disease results from faults in the DNA double helix (see, for example, the Online Mendelian Inheritance in Man database at www.ncbi.nlm.nih.gov/omim/, which provides a catalogue of human genes and genetic disorders). Is it then too extrapolative to suggest that all diseases and traits, each of which has some familial and imputed inherited component, will be caused by a corrupted piece of double helix?

Is our fate encoded in our DNA?

Is Watson's genetic aphorism of human disease really true? The excitement of genetics, and the perceived medical importance of the human genome sequence, is pegged to the promise of an understanding of common chronic disease and not rare Mendelian diseases. In theory, one might hope that approaches used successfully to identify single-gene diseases could simply be applied to the common causes of world-wide morbidity and mortality, such as cancer, heart disease, psychiatric illness and the like. This would enable a boon for diagnosis, understanding and the eventual treatment of these common maladies[5].

The reality is that progress towards identifying common disease mutations has been slow, and only recently have there been some successes[6]. It is now appreciated that although genes are one contributor to the origin of common diseases, the mutations they contain must have properties that are different from the more familiar, deterministic features of single-gene mutations. Indeed, the underlying genes are likely to be numerous, with no single gene having a major role, and mutations within these genes being common and imparting small genetic effects (none of which are either necessary or sufficient[7]).

Moreover, there is a suspicion that these mutations both interact with one another and with the environment and lifestyle, although the molecular specificity of inter-

actions is unproven[8]. To complicate matters, common disorders frequently show large population differences that have led to health disparities and, as is becoming more evident, the incidence of these disorders can show significant changes over time[9].

Interplay of DNA and environment

The inability of geneticists to easily identify common disease genes has been seen as a vindication of the importance of nurture. This is too simplistic; the influence of nature and nurture cannot be neatly divided, as it is clear that nurture is important to biology through its actions on DNA and its products. The environment must affect the regulation of critical genes by some mechanism and so, seen another way, mutations are not the only agent for altering gene function.

The scientific literature of cancer research reveals that despite having heterogeneous origins — both inherited and acquired — a specific tumour develops only from altering the expression (activity) of specific sets of genes[10]. That is, a variety of exposures and mutations collaborate to change the activity of specific genes and, consequently, interrupt precise aspects of cell metabolism. The regulation of circadian rhythm is another example of how external environmental cues influence DNA functions[11].

Thus, the double helix inevitably interacts with the environment, directly and indirectly, to predispose or protect us from disease. If perturbations of multiple genes contribute to a disorder, then the activities of these genes can be affected by any combination of mutation and environmental exposure altering their function. It is our opinion that genes have a stronger, maybe even a pervasive, role in all diseases and traits, with the understanding that it is the collective action of genes and nurture that underpins ultimate disease outcome.

Rather than dismissing the role of environment, our view embraces it directly, and, by that, expands the meaning of the term 'genetic'. It also emphasizes the work that remains to be done to understand gene regulation and, in particular, how genes and their products are modulated by external cues and how homeostasis is disrupted in human disease. Human beings are each the product of a unique genome and a unique set of experiences. Both need to be understood to intervene effectively in disease causation.

Implications for medicine

What does this mean in practice? The assessment of the quantitative role of genes in human traits is derived largely from studies on identical and fraternal twins (Fig. 1). By this measure, all common disorders have a 'genetic' basis, but the contribution varies from slight in some cancers and multiple sclerosis, to moderate in diabetes, heart diseases, migraine and asthma, to high in disorders such as psoriasis[12]. Critically, the discordance between identical twins — where twins show different diseases despite being genetically identical — illustrates the influence of exogenous factors, but does not prove the lack of influence of genes: of course, environmental factors over a lifetime affect an individual's chance of developing disease.

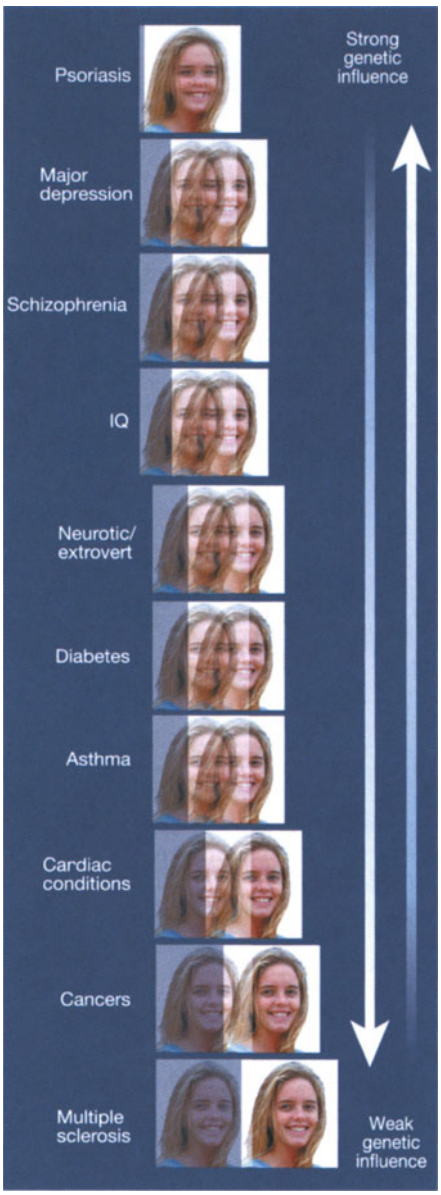

Figure 1 Studies of identical twins have revealed that some conditions, such as psoriasis, have a strong genetic component and are less influenced by environmental and lifestyle factors — identical twins are more likely to share these diseases. But other conditions, such as multiple sclerosis, are only weakly influenced by genetic makeup and therefore twins may show differences depending on their exposure to various environmental factors.

Let us assume, for the sake of argument, that all of the relevant genetic and environmental factors are identified that lead to a disease. Appreciating the relationship of genetic variation and environment suggests that a number of presently fashionable ideas about genetics are simplistic; two in particular are the 'bar code' view of genetic diagnosis and the 'right medicine for the right patients'.

Common genetic variations are essentially binary — either an adenine or guanine base, or a cytosine or thymine base — at a given position in the sequence. Unfortunately, this leads to a tendency to define genetic individuality as a binary pattern, a so-called 'bar code' for each individual. Some genetic variants convey susceptibility to a disease, but they typically convey risk rather than certainty of being afflicted with a condition.

Knowledge based on the sequence could have significant public health implications, and even be predictive at the population level. But a human DNA bar code would provide uncomfortable, perhaps even intolerable, knowledge of likely outcomes, with no certainty, only probabilities. Most individuals, we suspect, are ill equipped to deal with the knowledge that they have a 50 per cent chance of succumbing to an illness; equally, society has had great difficulty in knowing how to respond to such information, hence the concerns regarding genetic discrimination[13]. The reality is that the genetic bar code is weakly predictive and individuals may find this threatening, life enhancing or just irrelevant; in any event, much work is needed to enable the predictive revolution in medicine.

Human genetic individuality has forced the recognition that medicine has to refocus on the individual. This has been the rallying cry, particularly within the pharmaceutical business, of pharmacogenomics (the application of genome-scale understanding to the development of medicines), and there is no doubt that understanding of the variation within drug-metabolizing enzymes has exploded in the past 20 years[14]. The underpinning idea is enormously attractive — if genetic analysis of key DNA variations can be used to understand how individuals might respond to drugs, then it could be possible to eliminate the difficult, sometimes lethal, hit-and-miss approaches to medication that are a necessary feature of present medical practice.

Unfortunately, the influence of lifestyle is just as much a feature of drug response as it is of any other genetically influenced condition. The classic case of the influence of drinking grapefruit juice on the levels of many drugs[15] illustrated that there can be no such thing as 'the patient', because the patient is living in a complex world that changes by the minute. Once again, predictions for the population do not have the same predictive power for individuals.

Future challenges

The challenges that lifestyle presents to genetic studies are considerable. We believe

that the next 50 years will bring a genuine revolution of far greater individual significance than that delivered by genetics over the past 50 years. This is because lifestyle can conceivably be analysed, and in so doing, it should be possible to develop a genuinely personalized medicine.

Researchers can now think seriously about how to identify lifestyle influences: such studies will have to be on an unprecedented scale and one of the first of these, proposed to comprise 500,000 individuals in the United Kingdom, has already started[16]. These kinds of studies are a bold venture into relatively uncharted territory and face substantial technical, biological and science-culture challenges.

Scientifically, it is necessary to understand a deceptively simple equation: genes + environment = outcome. The difficulty here is the uncertainty surrounding both terms in the equation; ideally, one set of genetic factors will interact with one set of environmental influences to produce identical outcomes, but it is unknown whether this is always going to be the case. A far more difficult relationship would exist if multiple genetic factors interacted with multiple environments to achieve the same outcome. The example of glutathione *S*-transferase mutations, smoking and incidence of lung cancer[17] shows it is possible to detect some interactions, but it is unclear how, or even if, statistical methods might be developed for addressing the more complex possibilities.

Perhaps the greatest unknown in undertaking these projects is human psychology; the consequences of smoking have been known for many decades, but people still smoke. Advice does not imply acceptance. How to turn knowledge into practical outcomes must be an increasing focus of attention for both researchers and funding agencies.

Psychology is also in play in the initial decision to undertake this research; for researchers, funding agencies and politicians there is great risk implicit in undertaking a hugely expensive project with complex outcome. People would like to live in a simpler world, with simpler decisions, but the vision of such a project is enormous: once complete, as much will be known about the origins of human disorders as can be discovered by using such epidemiological and genetic studies. Perhaps more important, the beginnings of a new medicine will emerge, one focused uniquely and completely upon the individual, upon the combination of genetic uniqueness and personal choices that are the very essence of individual lives.

If we are collectively bold in our present decisions and accept the risk of action, a world can be created where medicine is a guide, not a place of last resort. If the past 50 years has seen the revolution of DNA, then the revolution cannot be completed without an appreciation of both genetic and environmental individuality; only then will individuals understand the meaning of their inheritance. □

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The double helix in clinical practice

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The discovery of the double helix half a century ago has so far been slow to affect medical practice, but significant transformations are likely over the next 50 years. Changes to the way medicine is practised and new doctors are trained will be required before potential benefits are realized.

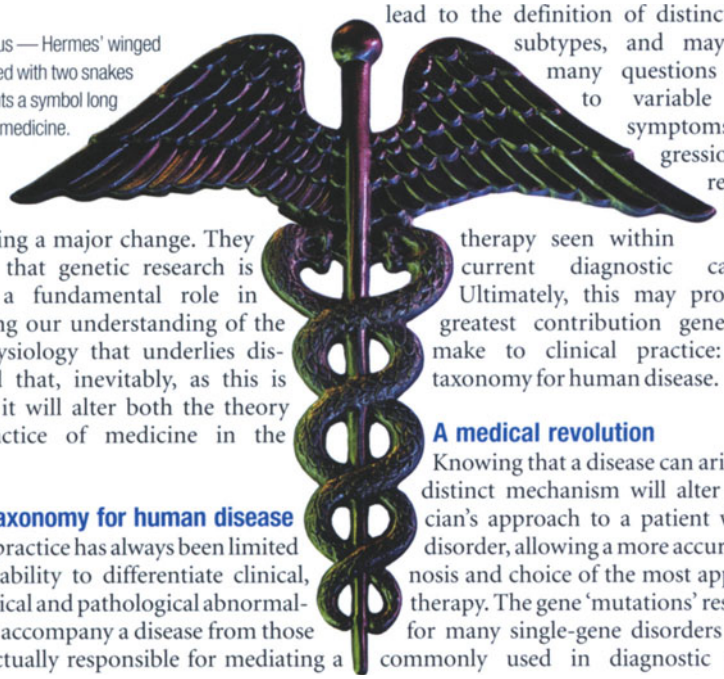
"It is much more important to know what kind of patient has a disease than to know what kind of disease a patient has." Caleb Parry, 18th century physician, Bath.

The structure of DNA established the basic framework that would develop into the field of molecular genetics. The information gleaned from this scientific endeavour continues to have a profound influence on our understanding of biological systems[1]. As most human diseases have a significant heritable component, it was soon recognized that the characterization of the genetic determinants of disease would provide remarkable opportunities for clinical medicine, potentially altering the way disease was understood, diagnosed and treated.

But despite the obvious potential applications to medicine, the development of significant genetic advances relevant to clinical practice could take generations. This is in marked contrast to many other medically related discoveries that occurred around the same time and which were translated rapidly into clinical practice. For instance, the development of penicillin by Ernst Chain and Howard Florey in 1941 was saving thousands of lives within months of their discovery of how to efficiently produce the antibiotic[2]. Discoveries relating to disease aetiology, such as the recognition in 1950 of a relationship between smoking and lung cancer, have had a profound effect on mortality[3]. This was despite the convictions of at least one distinguished statistical geneticist who argued against the causality of this observation, implying that a common genetic factor caused both lung cancer and a predilection to smoking cigarettes[4]!

Although other important discoveries have had demonstrably more impact on health care at the time of their fiftieth anniversaries than has the double helix, its slower transition from discovery to clinical implementation will be balanced by its potentially profound impact across all medical disciplines. Progress has been slow, but mounting evidence suggests that, while public health and antibiotics produced important healthcare outcomes in the past 50 years, the next 50 are likely to belong to genetics and molecular medicine.

The potential impact of genetics on clinical practice has been questioned by some observers[5] who believe that the positive predictive value of genetic testing for most common disease genes will be insufficient to provide the beneficial effects seen with single-gene disorders, which affect only a tiny proportion of the population. Many advocates of genetics argue, on the other hand, that our understanding of disease is



The caduceus — Hermes' winged staff entwined with two snakes — represents a symbol long adopted by medicine.

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undergoing a major change. They contend that genetic research is playing a fundamental role in improving our understanding of the pathophysiology that underlies disease and that, inevitably, as this is applied, it will alter both the theory and practice of medicine in the future[6].

A new taxonomy for human disease

Clinical practice has always been limited by its inability to differentiate clinical, biochemical and pathological abnormalities that accompany a disease from those events actually responsible for mediating a disease process. Clinicians may have moved on from calling 'fever' a disease[7], but they still rely on phenotypic criteria to define most diseases, and yet these may obscure the underlying mechanisms and often mask significant heterogeneity. As Thomas Lewis pointed out in 1944, diagnosis of most human disease provides only "insecure and temporary conceptions"[8]. Of the main common diseases, only the infectious diseases have a truly mechanism-based nomenclature.

An understanding of the genetic basis of maladies is providing a new taxonomy of disease, free from the risk that the diagnostic criteria related to events are secondary to the disease process, rather than to its cause. Genetic information has allowed us to identify mechanistically distinct forms of diabetes, defining an autoimmune form of the disease associated with human leukocyte antigens (a highly diverse complex of immune-system genes), and recently has implicated dysfunction of factors that affect both expression and modification of gene products in mediating the adult form of the disorder[9]. Similarly, we are now aware of a range of molecules and pathways previously not recognized in the pathogenesis of asthma[10–12].

A clearer understanding of the mechanisms and pathways that mediate disease will lead to the definition of distinct disease subtypes, and may resolve many questions relating to variable disease symptoms, progression and response to therapy seen within current diagnostic categories. Ultimately, this may provide the greatest contribution genetics will make to clinical practice: a new taxonomy for human disease.

Table 1 **Molecular genetics in clinical practice**

- Mechanistically based diagnostic criteria
- Predisposition testing and screening
- Rapid molecular diagnostic testing of pathogens
- Pharmacogenetics
- Identification of new drug targets
- Tools for molecular medicine (for example, recombinant DNA methodology)
- Recombinant expression of therapeutic proteins
- Gene therapy

A medical revolution

Knowing that a disease can arise from a distinct mechanism will alter a physician's approach to a patient with that disorder, allowing a more accurate prognosis and choice of the most appropriate therapy. The gene 'mutations' responsible for many single-gene disorders are now commonly used in diagnostic practice, whereas those associated with common complex diseases are just being characterized. Although their predictive value will be less than with single-gene disorders, their contribution as risk factors will be similar to other risk factors such as blood pressure, cholesterol levels and environmental exposures. Because much of clinical practice involves evaluating and acting on risk probabilities, the addition of genetic risk factors to this process will be an important extension of existing practice. The overall effect of genetic risk factors is likely to be significant. For example, recent estimates in breast cancer suggest that the attributable genetic risks are likely to exceed the predictive value of a range of existing non-genetic risk factors[13].

Other potential applications of genetics in health care may be realized in a shorter timeframe. Individual variation in response to drugs and in drug toxicity is a significant problem, both in clinical practice and in the development of new therapeutic agents. Clear examples now exist of genetic variants that alter metabolism, drug response or risk of toxicity[14,15]. Such information provides an opportunity to direct therapy at individuals most likely to benefit from an intervention, thereby reducing cost and toxicity, and improving methods for drug development.

The discovery of the structure of DNA not only led to an ability to characterize genetic determinants in disease, but also provided the tools necessary for the revolution in molecular medicine that has occurred in the past 25 years. The description of the double helix was the first important step in the development of techniques to cut, ligate and amplify DNA. The application of these molecular biology and DNA-cloning techniques has already had a profound impact on our understanding of the basic cellular and molecular processes that underlie disease.

Molecular biology has improved our ability to study proteins and pathways involved in disease and has provided the technology necessary to generate new sets of targets for small-molecule drug design. It has also enabled the creation and production of a new range of biological therapeutics — recombinant proteins such as interferon, erythropoietin and insulin, as well as therapeutic antibodies, which are one of the fastest growing classes of new treatments. Further extensions of this methodology will see the inevitable introduction of DNA-based therapies that will produce proteins of interest in the appropriate cellular setting. DNA-based vaccines represent the first wave of such novel gene-therapy approaches to disease and many more are expected to follow.

We are undergoing a revolution in clinical practice that depends upon a better understanding of disease mechanisms and pathways at a molecular level. Much has already been achieved: an enhanced understanding of disease-related pathways, new therapies, novel approaches to diagnostics and new tools for identifying those at risk. But more remains to be done before the full impact of genetics on medicine is realized. Complex disease, with multiple susceptibility determinants (both environmental and genetic), will take time to dissect. This information must then be moved into the clinic and evaluated for its benefits.

As the practice of medicine moves to one more scientifically founded in disease mechanisms, many aspects of clinical practice will need to be transformed. Individual genetic variation is likely to explain a significant part of the heterogeneity seen clinically in the natural history of disease and in response to therapy. Tools to tailor medicine to an individual's needs rather than directing it at a population will inevitably become available. Similarly, as predictions of risk improve, early or preventative therapy of high-risk populations will become a reality, with screening programmes targeted to those at particularly high risk.

Transforming clinical practice

For fundamental changes to take place in clinical practice, sweeping transformation will be needed to healthcare provision, economic management and training. It is currently difficult to predict the cost–benefit ratio for such changes — certainly the present impact of molecular medicine has not made medicine less expensive. Few medical schools adequately train their students to think mechanistically about disease; indeed, the trend towards pattern-recognition medicine, away from basic science training, means that we are still far from educating the next generation of clinicians to apply the knowledge and tools bequeathed to us by the double helix. The evolution in health care that will incorporate these new principles of early diagnosis and individualized therapy will be a daunting

challenge in an era of uncertainty for healthcare systems worldwide.

The influence of genetic and molecular medicine on the health of patients is already sufficiently ubiquitous that it will have an impact on most common diseases. Its influence will grow over the next few decades (Table 1). It will not, however, answer all of the questions about human health, nor will it provide all the answers for optimizing clinical practice. The reductionism that accompanies molecular genetics will identify the pieces in the jigsaw, but assembling these to understand how complex systems malfunction will require a substantially more integrated approach than is available at present.

The crucial role played by environmental determinants of disease will perhaps become more tractable when combined with an understanding of genetic susceptibility. Sceptics, rightly, will wish to see more data before they acknowledge that molecular medicine will be truly transformed over the next 50 years, despite the fact that its influence on diagnostics and new therapeutics is already clearly apparent. A transition is underway, the direction of travel is clear, but managing the change in clinical practice may prove at least as challenging as resolving the original structure of the helix. □

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BRIDGEMAN/GETTY IMAGES

The *Mona Lisa* of modern science

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No molecule in the history of science has reached the iconic status of the double helix of DNA. Its image has been imprinted on all aspects of society, from science, art, music, cinema, architecture and advertising. This review of the *Mona Lisa* of science examines the evolution of its form at the hands of both science and art.

"A monkey is a machine that preserves genes up trees, a fish is a machine that preserves genes in water; there is even a small worm that preserves genes in German beer mats. DNA works in mysterious ways." Richard Dawkins in *The Selfish Gene* (Oxford University Press, 1976).

History has thrown up a few super-images, which have so insinuated themselves into our visual consciousness that they have utterly transcended their original context. This is epitomized by the *Mona Lisa*, painted by Leonardo da Vinci around 1503. The double helix of DNA is unchallenged as the image epitomizing the biological sciences. Both images speak to audiences far beyond their respective specialist worlds, and both carry a vast baggage of associations.

In the worlds of popular image diffusion, particularly on the Internet, the double helix is beginning to rival the *Mona Lisa* as a playground for eccentrics and obsessives (Fig. 1). There is an apparent difference, of course. Leonardo's panel painting is the product of human artifice, whereas DNA is a naturally occurring, large organic molecule. But Leonardo claimed that his art represented a systematic remaking of nature on the basis of a rational understanding of causes and effects. His painting is the result of a complex, nonlinear interaction between concept, subject, plan of action, acquired knowledge, skill, medium and the evolving image itself. In *The Art of Genes*[1], Enrico Coen argues that "biological development and human creativity are highly interactive processes in which events unfold rather than being necessarily pre-planned or anticipated. In other words, in both cases there is no easy separation between plan (or programme) and execution."

Looking at the investigation and representations of the double helix, we can say that they are cultural activities no less than any painting. Behind the discovery lies the vast infrastructure of a scientific culture that led to the development of the knowledge, theories, institutions, techniques and equipment that made the quest both possible and desirable. The very natures of scientific models and representations, using whatever technique, are integral to the vehicles of science communication. Their visual look is compounded from a complex set of factors, ranging from technical to aesthetic. But, in case anyone should be getting the wrong impression, I acknowledge that the cultural vehicles are designed to deliver non-arbitrary information that is open to rational scrutiny as a way of working towards real knowledge of the physical constitution of the world.

Looked at from a popular perspective (and even from the standpoint of reputation within science), James Watson and Francis Crick are identified with DNA no less than Leonardo is identified with the *Mona Lisa*. The researchers were in a very real sense the 'authors' or 'artists' of the acts of visualization that generated their models of the molecule. But their brilliant achievement was not necessarily of a higher order than that of the other pioneers of molecular modelling, such as the Braggs, John Kendrew, Max Perutz, Maurice Wilkins and Linus Pauling. Rather, they were uniquely fortunate that their molecule was both visually compelling, as a supreme example of nature's 'sculpture', and

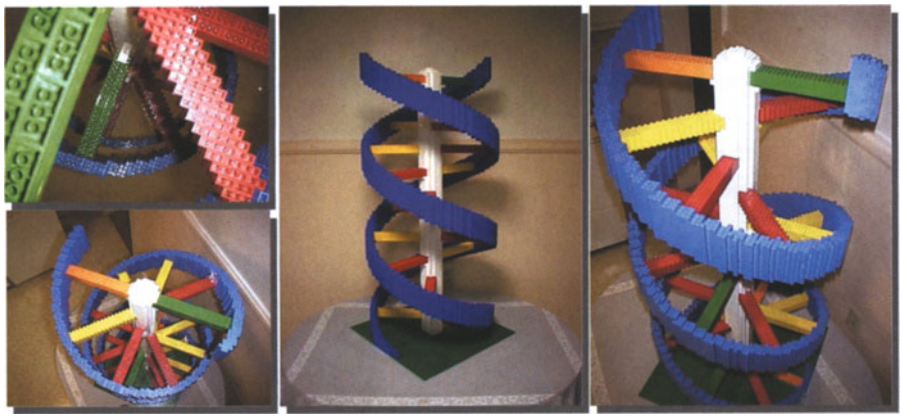


Figure 1 LEGO model of the DNA double helix (in reverse!) by Eric Harshbarger (2001), who also used his mastery of the coloured units of LEGO to compose a 'pixelated' LEGO version of the *Mona Lisa*. (Images courtesy of E. Harshbarger.)

Figure 2 Structure of DNA, drawn by Francis Crick's wife Odile Crick, which was published as the sole figure in Watson and Crick's seminal paper in *Nature*, 25 April 1953 (ref. 2).

lay at the heart of the twentieth-century version of the quest to unravel the ultimate secret of life.

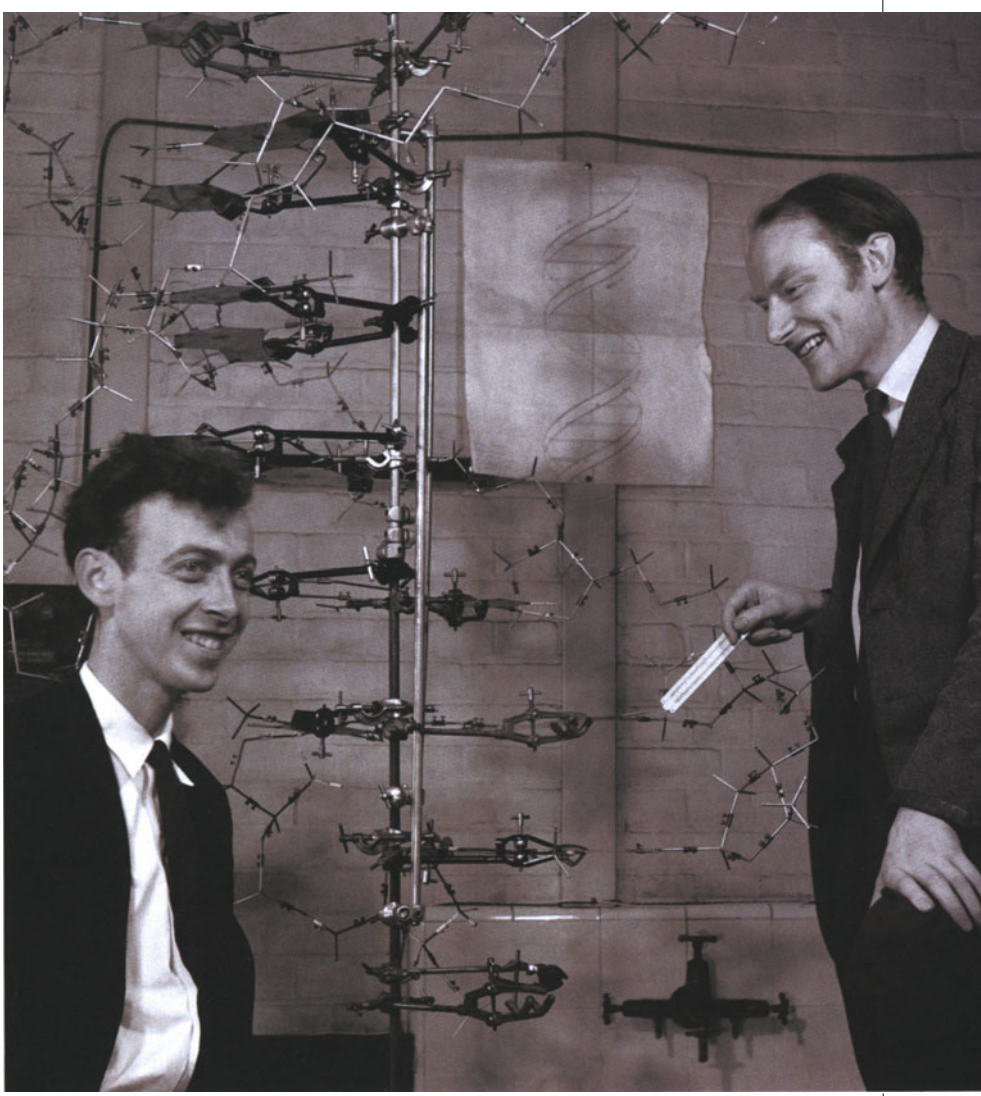
The 50-year journey of the DNA molecule from the reticent line diagram in Watson and Crick's seminal article[2] (Fig. 2) to its position in today's world of global imagery is extraordinary. It is therefore timely to look at some of the representational issues involved in science communication, and then at a few selected instances of the various guises in which the molecule has replicated itself within varied visual habitats.

A model of communication

Looking back on the laconic article in *Nature* that announced the structure of DNA, which we tend to assume in retrospect provided the definitive solution, it is remarkable how little was actually given away. This is true of the article's sole diagram, drawn by Odile Crick, Francis's wife, which represented the sugar chains as directional ribbons, while the bases were rudimentary rods represented flat on (Fig. 2). Along the vertical axis runs the central pole, depicted as a thick line that is broken where the bases lie in front. This axis is a visually useful point of reference, but its early ubiquity seems to depend on the structural necessities of physical models. The developed model, composed from standard brass components with tailor-made metal bases, provided a more detailed and explicit entity for debate and large-scale publicity, although the famous photographs by Anthony Barrington Brown (Fig. 3), taken for an article in *Time* magazine, were actually staged a few months later.

The model of the double helix — like those of other molecules, such as the model of haemoglobin by Perutz — played an important role in scientific understanding, being both based upon and in turn affecting the acts of scientific conceptualization. Overtaken by more refined models made at King's College London, including the widely illustrated space-filling model with Van der Waals surfaces by Wilkins (Fig. 4), the ramshackle masterpiece of Watson and Crick passed the way of so many obsolete bits of scientific paraphernalia. When, 23 years after its making, some of the specially cut plates (Fig. 5) resurfaced in Bristol, they were incorporated into a pious reconstruction by Farooq Hussain of King's College. Like an ancient Greek vase reassembled from chards, the semi-original model is now a treasured cultural icon, displayed in the Science Museum in London.

Communicating the complex structure and, in due course, the awesomely intricate behaviour of the modular molecule, has provided an unparalleled challenge for



A. BARRINGTON BROWN/SPL

Figure 3 Anthony Barrington Brown's photograph of Watson and Crick with their model of DNA at the Cavendish Laboratory in Cambridge, 21 May 1953.

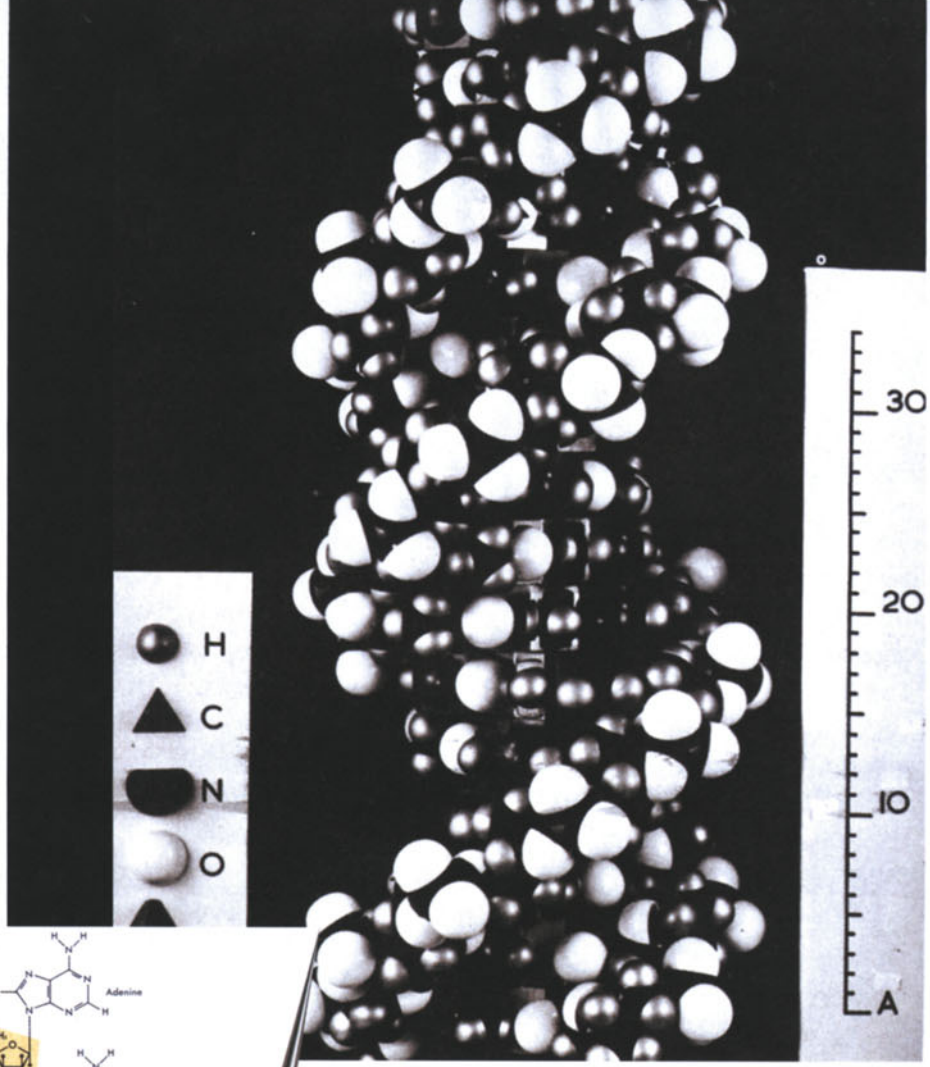


Figure 4 Main image shows Maurice Wilkins' space-filling model of DNA. Inset: a series of lucid and inventive graphics by Keith Roberts appeared in Watson's *Molecular Biology of the Gene*[3]. Here a flat chemical diagram is enhanced through two-colour printing to provide schematic emphasis upon the sugar phosphate chains or 'ribbons'. (Images reproduced from ref. 3, with permission.)

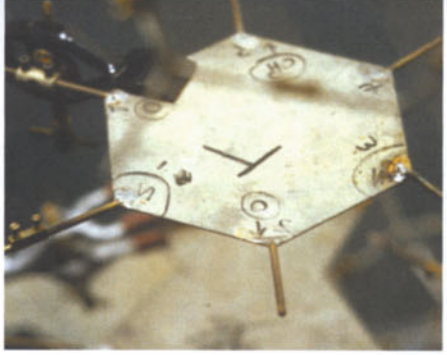


Figure 5 One of the specially cut plates used by Watson and Crick in their model of the structure of DNA.

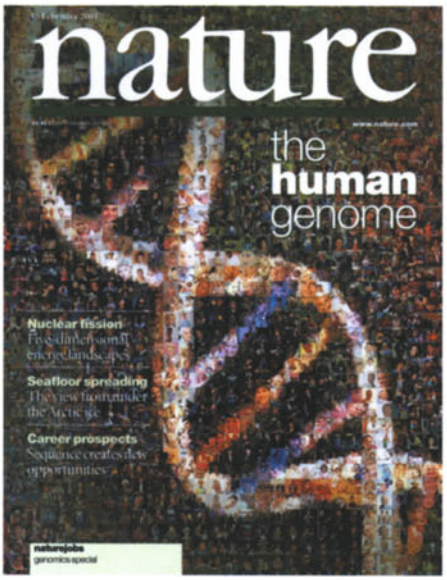


Figure 6 Cover of *Nature* human genome issue, published on 15 February 2001. The image, by Eric Lander, was created by Runaway Technology Inc. using PhotoMosaic by Robert Silvers from original artwork by Darryl Leja (courtesy of the Whitehead Institute for Biomedical Research). Gregor Mendel, James Watson and Francis Crick are amongst the crowd.

biological illustrators and model-makers. This is vividly shown by Keith Roberts's illustrations in successive editions of Watson's *Molecular Biology of the Gene*, beginning in 1965, which chart the complex interplay between developing science, graphic ingenuity and technologies of reproduction[3] (see Fig. 4, inset).

As the complex functioning of DNA became increasingly elucidated, so methods and conventions of illustration that privileged behaviour over structure played an ever more conspicuous role. As with sets of illustrations in any science, the visual conventions not only reflect what scientists want to show, but also provide an important framework for thinking and visualization in the process of research itself. Subsequently, the resources of computer design, stereoscopy and, in particular, animation have provided a vivid sense of spatial and temporal processes, only partly possible in conventional text and illustration. Three-dimensional contrivances have had a crucial role from the outset, unsurprisingly given a structure that taxes our powers of spatial visualization. Even in the age of computer graphics, there is still a pedagogic and popular market for kits using a variety of space-filling units.

A number of notable models of DNA and other large molecules have become revered items, typically displayed in protective cases in the foyers of laboratories, where they form part of the visual furniture that speaks of the enterprise of biological science in general and that of the institution in particular. For the sub-species of biologist known as 'molecular', the seductive geometry of DNA helps to underline the fundamental 'hardness' of their science, compared to natural historians and ecologists from whom they have become institutionally distinguished. It is in this spirit, less of didactic instruction than of emblematic signalling, that the double helix has become the icon for the communication of a generalized message. Few have any trouble in recognizing the ghostly twist that emerges from the mosaic of faces on the cover of the *Nature* issue devoted to the human genome on 15 February 2001 (Fig. 6). Similarly, any hint of the double twist in any logo of a laboratory or biotech company is immediately identifiable.

Aesthetics and meaning

Given the role of aesthetic intuitions in the processes that led to its discovery, and its recognition as 'right', it is understandable that the double helix has itself assumed the guise of a work of art, not least in three-dimensional form. For artists, the attraction of a form that is both beautiful and full of all kinds of scientific and social significance is considerable.

Some grand structures have been commissioned by academic institutions, whereby the artist has basically been given a brief to make a sculpture representing DNA, much like a sculptor might be commissioned to produce an anatomically accurate image of a naked figure. For example, in 1998 Roger Berry produced a huge sculpture hanging down the central well of a multi-story staircase at the University of California at Davis (Fig. 7). Another rendering of the helical structure, *Spirals Time — Time Spirals*, resides on a hillock in the grounds of the Cold Spring Harbor Laboratory (Fig. 8). Designed by the artist, architect and theorist of post-

PERRY A. JOHNSON/UC DAVIS

MIRIAM CHUA, COLD SPRING HARBOR LAB.

Figure 7 (left) *Portrait of a DNA Sequence* by Roger Berry (1998) at the Life Sciences Addition building, University of California, Davis. **Figure 8** *Spirals Time — Time Spirals* by Charles Jencks (2000) at Cold Spring Harbor Laboratory.

modernism, Charles Jencks, it stands at the heart of a programme of commissioning and collecting artwork that expresses the vision of Watson — who became director of Cold Spring Harbor Laboratory in 1968 and president in 1994 — of an environment in which the visual stimulation of the surroundings is integral to the conduct of high-level mental activity.

In pursuit of structural aesthetics, the British sculptor, Mark Curtis, proposed a reformed molecular structure for DNA. As an artist concerned with geometrical logic and symmetries, Curtis was worried about the 'ugly' engineering of the Watson–Crick version. Rather than using the sugar phosphate backbones to control the helices, he proposed that stacked base pairs, coupled in an opposite orientation from the accepted bonding, comprises a helix of pentagonal plates around a central void of decagonal cross-section. The geometrical and structural probity of Curtis's models, which eschew a central pole, made it on to a British millennium stamp (Fig. 9), if not into the world of scientific orthodoxy. In a real sense, the molecular biologists' rejection of Curtis's effort to re-design DNA on the basis of *a priori* principles represents an extreme example of the tension within science itself between the polar instincts of the modellers and the empiricists.

Alongside such sculptural exploitations of the inherent beauty of the double helix has run a strand of artistic iconography that has been more overtly concerned with meaning. The tone for the more fantastical exploitations was set by the flamboyant surrealist, Salvador Dali, as ever concerned with the metaphysical potential implicit in scientific imagery. During the late 1950s and 1960s, the DNA molecule features as a symbolic vision, lurking in a surreal hinterland between galactic mystery and spiritual significance (as a kind of Jacob's Ladder). His *Butterfly Landscape, The Great Masturbator in Surrealist Landscape with DNA* (1957–8; Fig. 10) locates a prettified evocation of a space-filling model in one of Dali's typically barren landscapes inhabited by sub-Freudian enigmas, designed to conjure up a dreamworld of obscure sexual fantasy[4]. Subsequent artists, particularly those who have engaged with the social implication of molecular biology and genetic engineering, have located images of DNA in contexts of meaning that are less obscure and more polemic.

This savagely selective glance at DNA art — omitting such contemporary luminaries of genetic art as Suzanne Anker[5] (Fig. 11; www.geneculture.org), David Kremers

ROYAL MAIL

Figure 9 Paintings of DNA models on a 'Millennium Collection' stamp, designed by Mark Curtis (1999–2000), from the UK Royal Mail's Scientists' Tale collection.

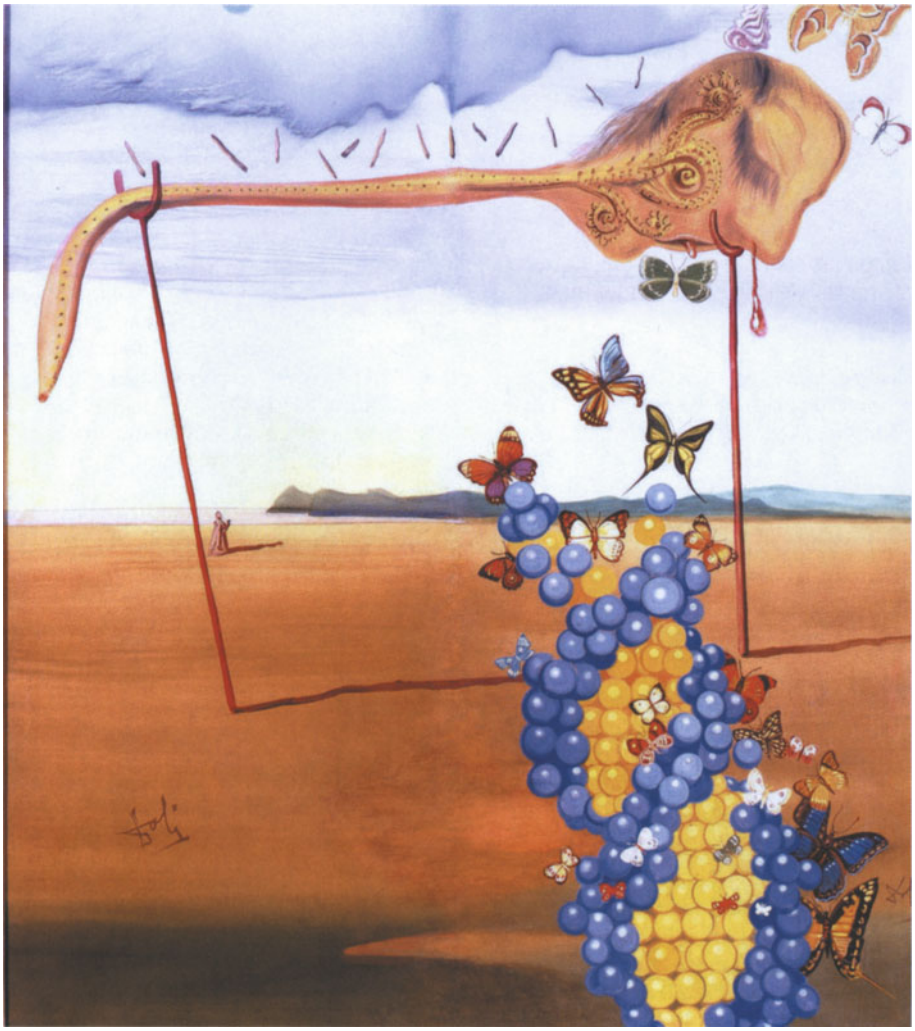


Figure 10 *Butterfly Landscape, The Great Masturbator in Surrealist Landscape with DNA* by Salvador Dali, 1957–8. Private collection.

Figure 11 *Zoosemiotics: Primates, Frog, Gazelle, Fish* (detail) by Suzanne Anker (1993).

(http://davidkremers.caltech.edu/), Ellen Levy (http://www.geneart.org/genome-levy.htm), Sonya Rapoport (http://users.lmi.net/sonyarap/transgenicbagel/) and Gary Schneider (http://www.icp.org/exhibitions/schneider/) — can barely claim to be representative even of the main range of possibilities. In particular, exploitation of the replicating potential of DNA to generate self-organizing images — exemplified by Marc Quinn's genetic portrait of Sir John Sulston from Sulston's own DNA, fragmented and replicated in bacterial colonies on plates of agar jelly[6] — shows that some artists' engagement with DNA is maturing beyond iconographical opportunism.

Box 1

2003 exhibitions celebrating art in the age of the double helix

2003 is to throw up a series of exhibitions, including:

- Representations of the Double Helix, at the Whipple Museum of the History of Science, Department of History and Philosophy of Science, University of Cambridge, UK. The exhibition is curated by Soraya de Chadarevian and Harmke Kamminga, with the assistance of Corrina Bower, and will run from January to December 2003.
- Genetic Expressions: Art After DNA, at the Heckscher Museum of Art, Huntington, New York. Curated by Elizabeth Meryman and Lynn Gamwell, the exhibition will run from 28 June to 7 September 2003.
- From Code to Commodity: Genetics and Visual Art, at the New York Academy of Sciences. The exhibition runs from 13 February to 11 April 2003 and is curated by Dorothy Nelkin and Suzanne Anker. Cold Spring Harbor Laboratory Press are publishing a book by Nelkin and Anker entitled *The Molecular Gaze: Art in the Genetic Age*.
- PhotoGENEsis: Opus 2 — Artists' Response To the Genetic Information Age, at the Santa Barbara Museum of Art, 9 November 2002–9 February 2003.

But as with the *Mona Lisa*, opportunism will always be the name of a prominent public game. Typical of this tendency is the introduction by the perfume company Bijan in 1993 of a fragrance named DNA. Ironically, we learn from the maker's blurb that "DNA is recommended for casual use". Such is the destiny of one of the greatest popular icons. □

doi:10.1038/nature01403

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Acknowledgements

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Original reference: *Nature* **421,** 416–420 (2003).

Portrait of a molecule

Philip Ball

Nature, *The Macmillan Building, 4 Crinan Street, London N1 9XW, UK (e-mail: p.ball@nature.com)*

The double helix is idealized for its aesthetic elegant structure, but the reality of DNA's physical existence is quite different. Most DNA in the cell is compressed into a tangled package that somehow still exposes itself to meticulous gene-regulatory control. Philip Ball holds a mirror up to what we truly know about the mysteries of DNA's life inside a cell.

"Each level of organization represents a threshold where objects, methods and conditions of observation suddenly change . . . Biology has then to articulate these levels two by two, to cross each threshold and unveil its peculiarities of integration and logic." François Jacob, in *The Logic of Life* (Penguin, London, 1989).

Rather like those of Albert Einstein, DNA's popular images are hardly representative. While it is fashionable in these post-genome days to show it as an endless string of A's, C's, G's and T's, this year's anniversary will surely be replete with two kinds of picture. One shows the famous double helix, delightfully suggesting the twin snakes of Wisdom and Knowledge intertwining around the caduceus, the staff of the medic's god Hermes. The other reveals the X-shaped symbol of inheritance, the chromosome.

But it is rare that DNA looks this good. For only a couple of hours during the early stages of the cell cycle, as the cell prepares to divide, the genome is compacted into its distinctive chromosomal fragments (Fig. 1). The rest of the time you will search the eukaryotic cell in vain for those molecular tetrapods. What you find instead in the cell nucleus is, apparently, a tangled mess.

And don't think that this will, on closer inspection, turn out to be woven from that elegant, pristine double helix. Rather, the threads are chromatin — a filamentary assembly of DNA and proteins — in which only very short stretches of the naked helix are fleetingly revealed. Although chromosomes are often equated with DNA, there is actually about twice as much protein as DNA in chromatin. And about 10 per cent of the mass of a chromosome is made up of RNA chains, newly formed (or in the act of forming) on the DNA template in the process called transcription.

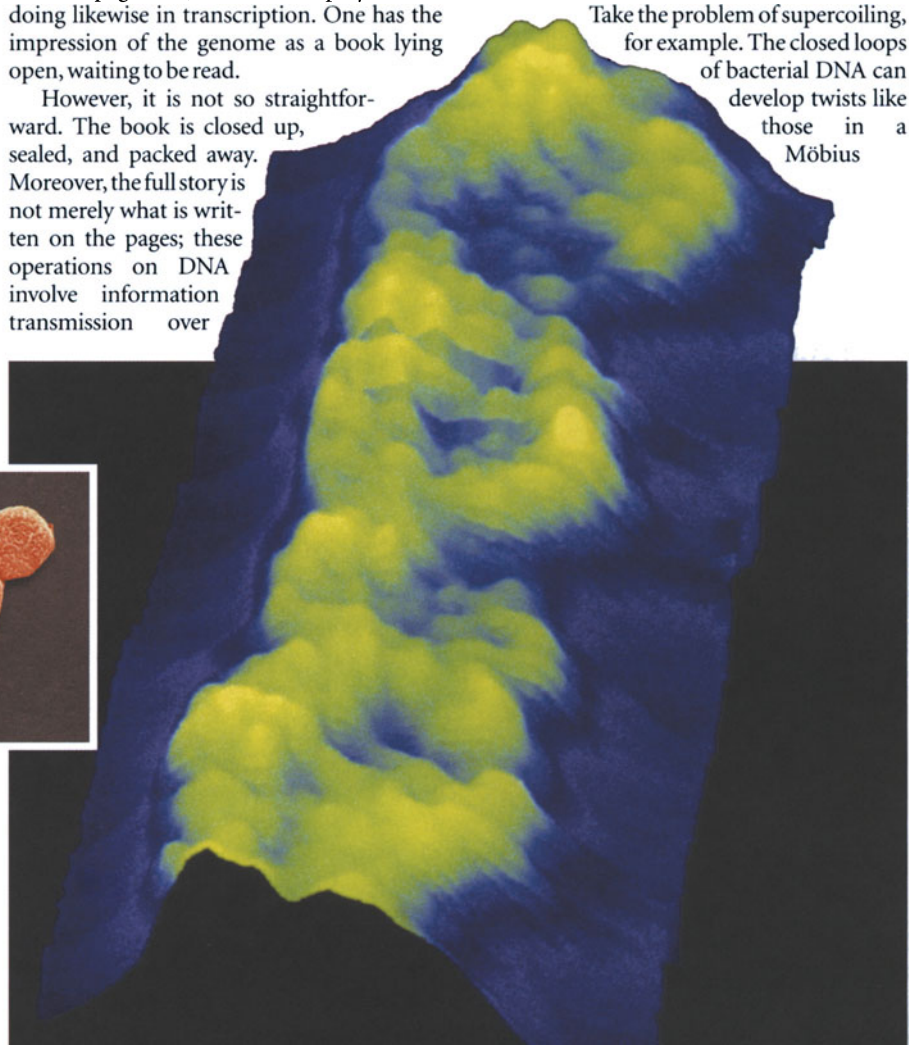

Figure 1 Inset: coloured scanning electron micrograph of a pair of human chromosomes. Main image: scanning tunnelling micrograph showing approximately three turns of a DNA double helix. The image is created by scanning a fine point just above the surface of a DNA molecule and electronically recording the height of the point as it moves across the specimen.

INSET: A. SYRED/SPL. MAIN IMAGE: DRISCOLL, YOUNGQUIST & BALDESCHWIELER, CALTECH/SPL

Zooming in on DNA

If we want to know how DNA really functions, it is not enough to zoom in to the molecular level with its beautifully simple staircase of base pairs. Textbooks tend, understandably, to show replication as the steady progress of the DNA-synthesizing enzyme DNA polymerase along a linear single strand laid out like a railway line (see accompanying article by Alberts, page 117), and RNA polymerase doing likewise in transcription. One has the impression of the genome as a book lying open, waiting to be read.

However, it is not so straightforward. The book is closed up, sealed, and packed away. Moreover, the full story is not merely what is written on the pages; these operations on DNA involve information transmission over many length scales. Perhaps those who do not routinely have to delve into the intricacies of genome function have acquired such a simplistic picture of it all because, until relatively recently, these length scales were considered largely out of bounds for molecular science. We know about molecules; we know about cells and organelles; but the stuff in between is messy and mysterious.

We speak of molecular biology and cell biology, but no one really talks of mesobiology. Yet that is the level of magnification at which much of the action takes place: the scale of perhaps a few to several hundred nanometres. How DNA is arranged on these scales seems to be central to the processes of replication and transcription that we have come to think of in terms of neat base pairings, yet it is precisely here that our understanding remains the most patchy.

Partly that's because the mesoscale represents, quite literally, a difficult middle ground. It encompasses too many atoms for one to apply straightforward molecular mechanics, with its bond bending and breaking; yet the graininess still matters, the continuum has not yet become a good approximation. As Bustamante and colleagues show elsewhere in this collection (see page 109), looking at DNA on a scale where it flexes and twists like a soft rod reveals how the mechanical and the molecular interact.

Take the problem of supercoiling, for example. The closed loops of bacterial DNA can develop twists like those in a Möbius

strip, which either 'overwind' or 'underwind' the helix. Generally there is some degree of underwinding (negative supercoiling) such that there is one negative supercoil for every 200 base pairs (bp) or so. This has an energy cost of around –9 kcal mol^{-1}, which manifests itself in physiological effects. In bacteria, too much supercoiling can inhibit growth, which is why enzymes called topoisomerases exist to release it. On the other hand, negative supercoiling tends to unwind the double helix, which is needed to initiate strand separation for DNA replication.

Although the chromosomal DNA of eukaryotes has free ends, it too is prone to supercoiling, as it seems typically to be attached in large loops to a filamentous structure called the nuclear matrix that coats the inside of the nuclear membrane. The attachment may in fact be necessary for both replication and transcription to take place.

Packaging problem

Stretched into a linear double helix, the three billion or so base pairs of human DNA would measure 1.8 m. This strand, snipped into 46 chromosomes, has to be packed into a nucleus just 6 μm or so across. As a result, the DNA chains are far from the idealized picture of molecules floating in an infinite solvent. They have a density of around 100 mg per ml, comparable to that of a highly viscous polymer gel.

The packing ratio for the chains is therefore enormous. In the smallest human chromosome, a length of DNA 14 mm long is compressed into a chromosome about 2 μm long: a packing ratio of 7,000. The first stage in solving this packaging problem is to wind the DNA around protein disks to form a bead-like nucleosome (see accompanying article by Felsenfeld and Groudine, page 134). Each disk is an octamer of four types of histone protein; a fifth histone, called H1, seals the DNA to the disk at the point where the winding starts and ends. Each nucleosome, 6 nm high by 11 nm in diameter, binds around 200 bp of DNA in two coils, and there is very little 'free' DNA between adjacent nucleosomes: sometimes as little as 8 bp.

The string of nucleosomes forms a fibre about 10 nm thick, which is then packaged into a filament three times as wide. This 30-nm fibre is the basic element of chromatin — yet we still don't know its structure. It is widely held to be composed of nucleosomes arranged in a solenoid, but hard evidence for this is scanty. How many celebrations of the double helix will admit that, 50 years on, we don't really know what DNA at large in the cell looks like?

The 30-nm fibre is further folded and condensed to give a packing ratio of around 1,000 in chromosomes during interphase (the time between cell divisions), and around ten times that in the X-shaped chromosomes of mitosis (cell division). How this happens is even more of a mystery. For mitotic chromosomes it was thought until only recently that there might be a contiguous protein scaffold holding the whole affair together; but now it seems that the structural integrity must come from chromatin crosslinking[1]. All the histones seem to have higher-order structural functions. Multi-subunit protein complexes in yeast called SWI/SNF and RSC (both of which seem to have human homologues) are chromatin-remodelling machines, which distort histone–DNA contacts or transfer histones between DNA molecules, exposing the DNA to attack by DNA-cleaving enzymes called nucleases. How they work remains hazy[2]. According to one recent study[3], DNA engaged by such complexes 'behaves as if it were free and bound at the same time'. Or in other words, as if 'free' and 'bound' were notions too simplistic to have much meaning here. What is clear is that these chromatin-shaping machines are important in transcription: cells lacking RSC are no longer viable.

There are in fact two types of chromatin in the nucleus of an interphase eukaryotic cell. Euchromatin is the most abundant: it is relatively dispersed, with the tangled-net appearance of a polymer gel. Heterochromatin is much denser (virtually solid-like), comparable to the density of mitotic chromosomes, and is confined to a few small patches. The invitation is to regard euchromatin as 'active' DNA, unpacked enough to let the transcription apparatus get to work on it, while heterochromatin is compressed, like a big data file, until needed. But like just about any other generalization about DNA's structure and behaviour, this one quickly breaks down. Clearly only a fraction of a cell's euchromatin is made up of transcribable DNA in the first place (so why not pack the rest away?); and even chromosomes containing a large amount of heterochromatin can be transcriptionally active. Some researchers think that 'euchromatin' and 'heterochromatin' are just blanket terms for many things we don't understand: further hierarchies of DNA organization yet to be revealed.

Structured chaos

Certainly, there seems to be more to the nucleus than a disorderly mass of DNA. It is a constantly changing structure, but not randomly: there is method in there somewhere. Specific chromosomes occupy discrete nuclear positions during interphase, and these positions can change in a deterministic way in response to changes in the cell's physiological state.

And the euchromatin itself has an internal logic, albeit one only partly decoded. It has been proposed that DNA has sequences called scaffold/matrix-attached regions (S/MARs), recurring typically every 10–100 kbp, that bind to the nuclear matrix to divide up the chromosome into loops[2]. Yet the existence of not only S/MARs, but also the nuclear scaffold itself, has been questioned. There is no sign of the scaffold during mitosis, and the material it is thought to be composed of may be nothing more than a mess of denatured proteins.

Be that as it may, the organization of the loops seems to be important for compaction of DNA and for the regulation of gene expression, and each loop may act as an independent unit of gene activity. In other words, there is at least one level of superstructural organization in the chromosomes that makes its influence felt at the scale of molecular information transfer. Topoisomerase II is one of several proteins that bind specifically to the putative S/MARs, suggesting that these points are important for controlling supercoiling in the strands.

With all this high-level structure, transcription of DNA is not so much a matter of slotting the parts in place as tugging on the rope. DNA is highly curved around the nucleosomes, the inward-facing groove compressed and the outer one widened. RNA polymerase, at 13 by 14 nm, is about the same size as the nucleosome, yet it binds to a region of DNA around 50 bp long: about a quarter of the entire histone-bound length. So clearly some DNA must leave the surface of the histone core for transcription to proceed. But this core need not be displaced completely. The histone disk actually has a considerable amount of mobility, sometimes described as a corkscrew motion through the DNA coil. The reality is undoubtedly more complex, involving a kind of diffusion of localized defects in the DNA–histone contact.

If all of this destroys the pretty illusion created by the iconic model of Watson and Crick, it surely also opens up a much richer panorama. The fundamental mechanism of information transfer in nucleic acids — complementary base pairing — is so elegant that it risks blinding us to the awesome sophistication of the total process. These molecules do not simply wander up to one another and start talking. They must first be designated for that task, and must then file applications at various higher levels before permission is granted, forming a complex regulatory network (see accompanying article by Hood and Galas, page 130). For those who would like to control these processes, and those who seek to mimic them in artificial systems, the message is that the biological mesoscale, far from being a regime where order and simplicity descend into unpredictable chaos, has its own structures, logic, rules and regulatory mechanisms. This is the next frontier at which we will unfold the continuing story of how DNA works. □

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Ten years of tension: single-molecule DNA mechanics

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The basic features of DNA were elucidated during the half-century following the discovery of the double helix. But it is only during the past decade that researchers have been able to manipulate single molecules of DNA to make direct measurements of its mechanical properties. These studies have illuminated the nature of interactions between DNA and proteins, the constraints within which the cellular machinery operates, and the forces created by DNA-dependent motors.

The physical properties of the DNA double helix are unlike those of any other natural or synthetic polymer. The molecule's characteristic base stacking and braided architecture lend it unusual stiffness: it takes about 50 times more energy to bend a double-stranded DNA (dsDNA) molecule into a circle than to perform the same operation on single-stranded DNA (ssDNA). Moreover, the phosphates in DNA's backbone make it one of the most highly charged polymers known.

The protein machinery involved in copying, transcribing and packaging DNA has adapted to exploit these unique physical properties (see article by Alberts, pages 117). For example, RNA polymerases (which synthesize RNA from a DNA template) and helicases (which unwind the double helix to provide single-stranded templates for polymerases) have evolved as motors capable of moving along torsionally constrained DNA molecules. DNA-binding proteins can use the polymer's electrostatic potential to cling to DNA while they diffuse along the molecule in search of their target sequences. Topoisomerases break and rejoin the DNA to relieve torsional strain that accumulates ahead of the replication fork.

During the past ten years, direct manipulation of single molecules of DNA has expanded our understanding of the mechanical interactions between DNA and proteins, following a pattern in which basic investigations of DNA elasticity have laid the groundwork for real-time, single-molecule assays of enzyme mechanism.

DNA as a worm-like chain

Although mechanical properties vary according to local sequence and helical structure, the relevant physics of DNA in many biological contexts is usefully described using a coarse-grained treatment such as the worm-like chain (WLC) model[1], which characterizes a polymer using a single parameter, the flexural persistence length (A). The WLC model imagines a polymer as a line that bends smoothly under the influence of random thermal fluctuations. The value of A defines the distance over which the direction of this line persists: correlation between the orientations of two polymer segments falls off exponentially (with decay length A) according to the contour length that separates them. For dsDNA in physiological buffer, $A = \sim 50$ nm.

There is a simple relationship between A and the bending rigidity κ of the polymer represented as an elastic rod[2]: $k_B TA = \kappa$, where k_B is Boltzmann's constant and T is the temperature. According to this relation, the energy required to bend a segment of DNA of length L through an angle θ and a radius of curvature R/L is:

$$E(\theta) = \frac{k_B TAL}{2R^2} = \frac{k_B TA}{2L}\theta^2$$

This model, therefore, predicts that it is energetically more favourable to bend the molecule smoothly, spreading the strain over large distances, than to bend it sharply at discrete locations. This mechanical property is central to interactions with regulatory proteins that bend DNA severely upon binding. The biological relevance of these bends is demonstrated by the enhancement of DNA recombination and gene transcription observed when specific protein-binding sites for activators are replaced by intrinsically bent DNA sequences[3] or by binding sequences for unrelated DNA-bending proteins in the presence of these proteins[4].

To bend DNA, proteins must convert part of their binding energy into mechanical work, as illustrated by an experiment in which a binding sequence was pre-bent towards the major groove by placing it in a DNA minicircle. The affinity of a transcription factor (TBP) for this binding site was found to be 300-fold higher (equivalent to a free-energy change of 3.4 kcal mol^{-1}) when the sequence was pre-bent in the same direction as TBP-induced bending, relative to pre-bending in the opposite direction[5]. This increase can largely be accounted for by the difference in bending energy between the two initial DNA conformations, which by the equation above is predicted to be 3.2 kcal mol^{-1}.

The high linear charge density of the double helix provides one mechanism for converting binding energy into work. DNA's structure is pre-stressed by electrostatic self-repulsion, as a result of the negatively charged phosphate backbone of the double helix. Therefore, asymmetric neutralization of the DNA helix (for example, by a DNA-binding protein that presents a positively charged face) can lead to compression and bending of DNA towards the neutralized face. This

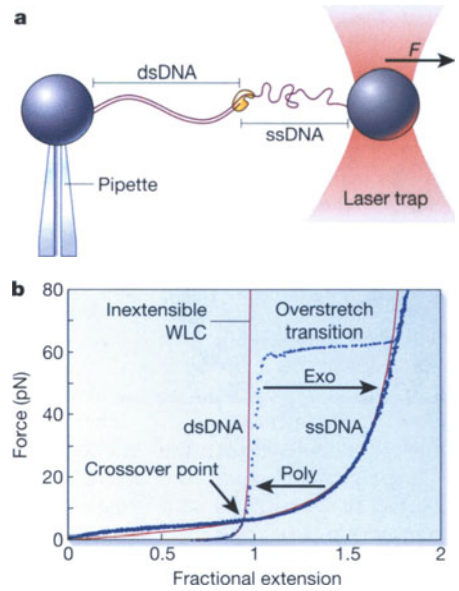

Figure 1 Single-molecule assays of replication[12,13]. **a**, A DNA molecule is stretched between beads held in a micropipette and a force-measuring optical trap[12]. The measured extension is the sum of contributions from the single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) segments. **b**, Force versus extension for dsDNA and ssDNA molecules, obtained in the instrument in panel **a**. Arrows show changes in extension observed at constant tension during polymerization (Poly) or force-induced exonuclease activity (Exo).

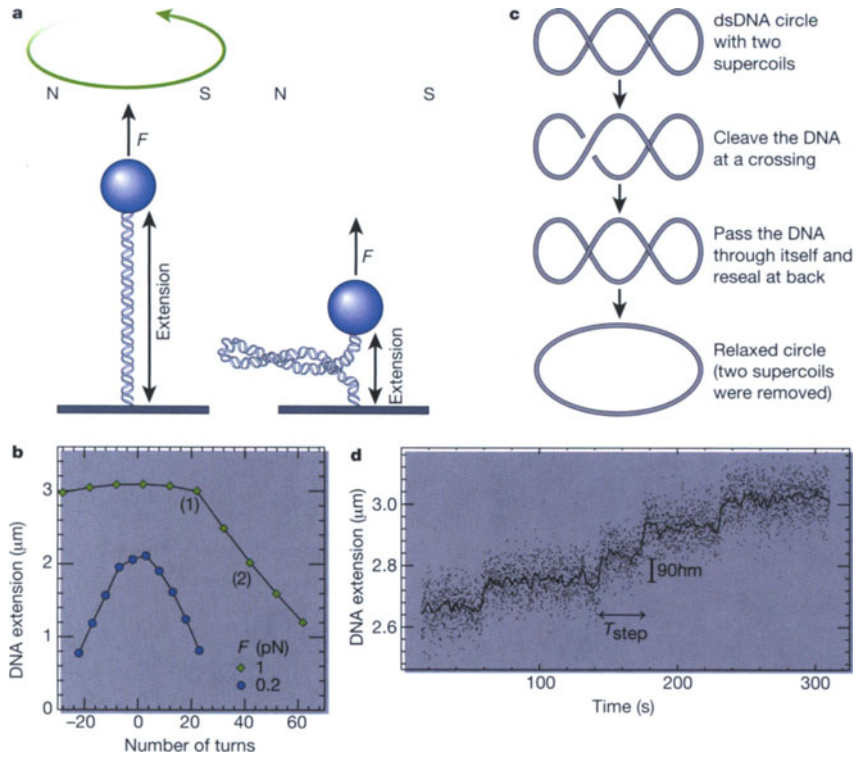

Figure 2 The elastic behaviour of supercoiled DNA molecules[49] forms the basis of single-molecule topoisomerase assays[15–17]. **a**, Molecules are stretched and twisted in magnetic tweezers. Under sufficient torsional strain, a twisted DNA molecule buckles to form plectonemes, shortening the measured extension. **b**, Extension as a function of turns introduced into the molecule remains nearly constant until the buckling transition is reached (1), after which the molecule contracts linearly (2). Underwinding at 1 pN does not cause reduced extension because the strained molecule forms alternative underwound structures (for example, melted DNA) in preference to buckling. **c**, The accepted model of type II topoisomerase action: the enzyme binds to supercoiled DNA, cleaves both strands, and passes the double helix through itself, leading to the removal of two turns. (Redrawn from ref. 50, with permission.) **d**, When topoisomerase II is added to a plectonemed molecule in the magnetic tweezers, 90-nm steps in extension are seen, corresponding to the removal of two turns[15], as predicted by the model.

effect has been elegantly demonstrated by incorporating neutral phosphate analogues or tethered cations onto one face of a DNA molecule[6].

DNA elasticity

The bending elasticity of DNA has consequences beyond short-range interactions with proteins: the WLC model explicitly connects local bending mechanics with the statistics of global conformations. Thus, a polymer with smaller bending rigidity tends to adopt a more compact random-coil structure.

This preference is reflected in the phenomenon of entropic elasticity, which is responsible for the elastic properties of common polymeric materials such as rubber[7]. A flexible polymer coils randomly in solution, resulting in an average end-to-end distance much shorter than its contour length. Pulling the molecule into a more extended chain is entropically unfavourable, as there are fewer possible conformations at longer extensions, with only a single possible conformation (a perfectly straight line) for maximum extension. The resulting entropic force increases as a random coil is pulled from the ends; tensions on the order of k_BT/A (~0.1 pN for dsDNA or 5 pN for ssDNA) are required to extend the molecule significantly.

Direct measurements of force and extension on single molecules of DNA provide the most rigorous test to date of theories of entropic elasticity. When magnets and fluid flow[8] and later optical traps[9,10] were used to stretch DNA molecules attached to micron-scale beads (Fig. 1), the entropic force–extension behaviour of dsDNA was found to agree closely with the WLC model[11]. Tensions of ~6 pN, within the range of forces exerted by characterized molecular motors, stretch dsDNA to ~95% of its contour length.

The intrinsic flexibility of ssDNA causes it to maintain very compact conformations, so that its extension per base pair is shorter than that of dsDNA for forces smaller than ~6 pN. At higher forces, however, the situation is reversed. A single strand is not constrained to follow a helical path, so it becomes nearly twice as long as dsDNA as it is pulled straight (Fig. 1).

From elasticity to enzymology

A quantitative appreciation of the different elastic properties of ssDNA and dsDNA has allowed researchers to observe replication of single DNA molecules[12,13]. In these studies, a molecule of ssDNA was stretched between two surfaces, and a DNA polymerase was allowed to replicate the stretched molecule at a given constant tension. As ssDNA was converted into dsDNA by the polymerase, replication could be followed in real time by monitoring the extension (below 6 pN) or contraction (above 6 pN) of the molecule (Fig. 1).

These studies showed that the rate-limiting step of DNA replication, which involves closing a structural 'fingers' domain of the enzyme, is sensitive to DNA tension and is capable of generating forces as high as 35 pN. Small forces can accelerate the enzyme's activity, probably by helping it to stretch the proximal collapsed template strand into the correct geometry for polymerization. A surprising result was the induction of a strong exonuclease activity (removal of nucleotides) at tensions above 40 pN (ref. 12). This effect provides a novel assay to investigate the proofreading mechanism of DNA polymerases.

Studies of the force–extension behaviour of single supercoiled DNA molecules further illustrate the progression from elasticity measurements to enzymology (Fig. 2). DNA tethers were stretched between a surface and a magnetic bead that could be rotated using magnets[14]. Because the molecule was attached at each end through multiple linkages on both strands, rotation of the bead led to the build up of torsional strain in the molecule. Under tension, such a molecule behaves roughly like a twisted rubber tube: as turns are added, the extension remains nearly constant until a critical amount of torque accumulates and the tube buckles, trading twist for writhe to form plectonemes (units of supercoiled DNA that project out of the molecular axis), thus reducing its apparent extension with each subsequent turn. As tension is increased, so does the energetic penalty for buckling; therefore, more turns must be added to reach the buckling transition.

The activity of topoisomerase II, an enzyme that relaxes supercoils in eukaryotic cells, has been analysed on single plectonemed DNA tethers[15]. Under conditions of limiting ATP, discrete steps in extension were observed that were attributable to single enzymatic turnovers. The size of these steps corresponded to the removal of two turns, confirming the

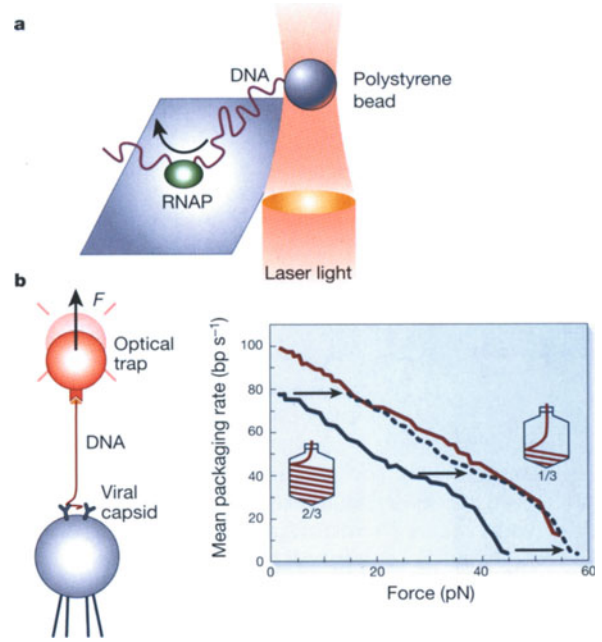

Figure 3 Force generation by DNA-dependent motors. **a**, Transcription. A surface-bound RNA polymerase (RNAP) transcribes against a force exerted by an optical trap[24]. Its velocity remains unchanged against forces up to ~20 pN (implying that translocation is not rate-limiting), and then falls steeply with increasing force. **b**, Bacteriophage DNA packaging. As DNA is pulled in by the ϕ29 portal motor to fill the viral capsid, the extension of the external DNA becomes shorter. The force–velocity relation for packaging when the capsid is two-thirds full (solid blue) must be shifted by ~15 pN (dashed blue) to match the curve for one-third-full capsids (red), implying the presence of an additional internal force that builds up during DNA packaging, due to highly compressed DNA[32].

accepted model of topoisomerase II action in which the double helix is passed through itself, changing the linking number by two (Fig. 2). Later experiments applied the same methodology to bacterial topoisomerases I and IV (refs 16 and 17, respectively).

Single-molecule assays of topoisomerase IV (ref. 17) helped reveal that the enzyme has a chiral substrate specificity: it relaxes overwound DNA substantially more efficiently than underwound DNA. This allows the enzyme to relax the positive supercoils formed during replication while avoiding counterproductive relaxation of the negative supercoils present in non-replicating DNA.

These studies also showed that topoisomerase IV relaxes DNA (of either handedness) an order of magnitude faster than had been estimated from bulk studies, helping to resolve a dilemma in which the enzyme's apparent low turnover rate *in vitro* had seemed to be at odds with its demonstrated ability to counteract rapid supercoil formation at the replication fork *in vivo*. This result reflects a general caveat for bulk enzyme kinetics: the presence of inactive enzymes in solution can lead to gross underestimation of the turnover rate per enzyme. Single-molecule assays, including those based on DNA manipulation, can sidestep this issue by selecting only the active fraction for analysis.

DNA unzipped

DNA helicases must generate force to unzip the parental strands during replication (see article by Alberts, page 117). Mechanical unzipping forces for dsDNA were first measured by attaching one strand to a surface (through a dsDNA linker) and pulling on the other strand using a glass needle[18] or optical tweezers[19] as a force transducer. DNA from a bacterial virus, called lambda phage, was unzipped (and re-zipped) at forces between 10 and 15 pN, depending on the local sequence. The pattern of force fluctuations during unzipping of a particular sequence was remarkably reproducible, and could be rationalized from a simple model incorporating the known difference in stability between adenine–thymine and guanine–cytosine base pairs.

Future studies might use this experimental geometry to investigate helicase function directly. Such an experiment could provide insight into the rates, processivity, force generation and sequence dependence of helicases, complementing the results of previous single-molecule helicase assays which observed translocation without measuring or applying forces[20,21].

In a new application of mechanical unzipping[22], the extra force needed to separate the DNA strands past DNA-binding proteins has been used to map the positions of target sequences, and (by noting the fraction of occupied sites as a function of protein concentration) to measure dissociation constants.

Forces in DNA transcription and packaging

The ability to apply forces on DNA has altered the way we think about DNA-dependent enzymes, by revealing these enzymes to be powerful motors (Fig. 3). Optical tweezers have been used to follow transcription by *Escherichia coli* RNA polymerase against external loads[23–25]. This enzyme can generate forces exceeding those of cytoskeletal motors that drive transport processes within the cell[26]. Its velocity remains unchanged against forces of up to ~20 pN (refs 24,25), showing that the translocation step (which must by definition be force sensitive) is not rate limiting.

An external load can, however, affect the tendency of an enzyme[25] to pause or arrest during transcription. The application of force in an 'aiding' direction[27] reduces pausing and arrest probabilities, presumably by preventing the polymerase from sliding backwards along the template during entry into these inactive states[28]. The same 'backsliding' phenomenon may be responsible for the steep drop in transcriptional velocity seen as opposing force is increased above ~20 pN (ref. 24).

In eukaryotes, forces generated by RNA polymerase or by chromatin-remodelling enzymes might help to displace nucleosomes that would otherwise impede transcription. In support of this idea, several groups have pulled on single chromatin fibres and found that nucleosomes can be removed from DNA by applying a tension of ~20 pN (refs 29–31). At lower forces (~6 pN), reversible modifications of the chromatin structure (such as partial DNA release[31] or disruption of internucleosomal interactions[29]) are observed. These tension-inducible structural rearrangements might be exploited by RNA polymerase or other cellular factors to modulate access of the transcriptional apparatus to chromosomal DNA.

The machine that packs DNA into the viral capsid of the bacteriophage ϕ29 (a virus that infects bacteria) generates higher forces than have been seen so far for any other translocating (displacing) molecular motor[32] (Fig. 3). A comparison of the force–velocity relation of the motor when the capsid is mostly empty with that when it is nearly full of DNA revealed the presence of a large internal force (up to ~50 pN) pushing back on the motor. This pressure, which must be overcome in order to package the viral genome, presumably arises from the combined effects of configurational entropy loss, elastic bending energy, electrostatic self-repulsion, and changes in hydration of the DNA upon packaging. The potential energy thus stored by the motor in the form of a pre-compressed 'spring' should provide some of the driving force for DNA ejection into the bacterial cytoplasm when the virus infects.

Extreme forms of DNA

The helical structure of DNA is highly adaptable and can assume various forms[33]. Although the helix of dsDNA is typically right-handed and extended in aqueous solution (B form), it can become shorter and wider (A form) in dehydrating solution. Molecules with specific base sequences (alternating purines and pyrimidines) easily assume the left-handed Z form, which is longer than B form and has reverse twist. Recently, single-molecule manipulation experiments

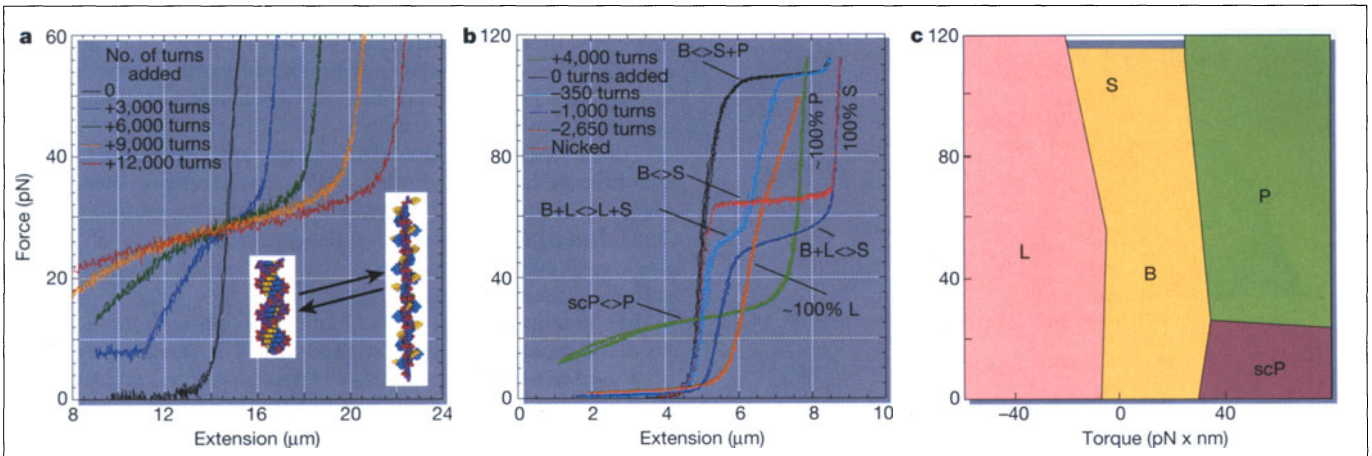

Figure 4 Mechanically induced structural transitions in twist-constrained DNA. **a**, Force–extension curves for a 44.4-kilobase DNA molecule overwound by successively larger numbers of turns (Z.B., M. D. Stone, S.B.S., N. R. Cozzarelli and C.B., unpublished data). As was seen in ref. 39, the high-force curves can by interpreted as the sum of contributions from a fraction of the molecule that remains in B form and a progressively larger fraction that is converted to 'P form', whose structure (proposed from molecular mechanics modelling[39]) is shown in the inset (courtesy of R. Lavery). The curves cross at an 'isosbestic point' marking the force at which B-DNA and P-DNA have equal extensions. **b**, Multiple plateaux occur in force–extension curves for torsionally constrained DNA[35]. (Data shown are for a 14.8-kilobase molecule twisted and stretched in 100 mM Na^+; Z.B., M. D. Stone, S.B.S., N. R. Cozzarelli and C.B., unpublished data.) **c**, These multiple plateaux can be explained by a 'phase diagram' for DNA under torque and tension (adapted from ref. 41). Coloured regions represent conditions under which pure phases occur; lines indicate conditions for phase coexistence within a molecule. S, overstretched; P, Pauling, sc, supercoiled (shortened by forming plectonemes). L is used here in place of 'Z'[35,41] to denote a phase with an average left-handed twist. Other studies have concluded that this form contains exposed bases, consistent with melted DNA[49]; a mixture of non-canonical forms may in fact be present. A nicked DNA molecule (red curve in **b**) remains at zero torque and therefore crosses the B–S coexistence line at 65 pN.

have revealed the existence of additional helical forms of DNA stabilized by external forces and torques (Fig. 4).

When tension in a nicked DNA molecule is increased to 65 pN, it displays a reversible, cooperative transition to an extended form that is ~70 per cent longer than normal B-DNA[9,34] and with substantially reduced twist[35] (Figs 1b, 4b). But, what is the form of this overstretched dsDNA? Do the strands associate in some specific base-paired structure, dubbed 'S form'[9,34], or does overstretched DNA simply comprise two independent strands of ssDNA[36]? Evidence exists for both models, so the question remains open; a further challenge in single-molecule mechanics is the development of methods to probe the high-resolution structure of manipulated molecules[37].

Twisting of stretched DNA can lead to other structural transitions[35,38,39]. For example, after a critical amount of overwinding has been introduced into a molecule (Fig. 4a), it gets progressively longer with additional twisting, implying cooperative conversion to an overextended form with greatly increased helicity (~2.6 base pairs per turn, compared with 10.5 base pairs per turn for B-DNA). The evidence[39] suggests an inside-out double helix reminiscent of the structure proposed by Linus Pauling in 1953 (ref. 40) and therefore dubbed Pauling DNA (P-DNA).

Complex force–extension curves with multiple force plateaux are seen when single DNA molecules are twisted in either direction and pulled to high forces (ref. 35 and Fig. 4b). A simple model to account for these features assumes that DNA has five interconvertible structural forms[41]. This model predicts a force–torque 'phase diagram'(Fig. 4c), thus framing mechanically induced structural transitions in terms of coexistence lines, critical stresses, and triple-points. Such a model might be tested by direct measurements of torque on stretched and twisted DNA — so far, this quantity has been inferred only indirectly from force–extension experiments. It remains to be determined whether molecular motors can generate sufficient concomitant torque and tension to generate 'extreme forms' of DNA in a biological context.

From mechanics to nanotechnology

Single-molecule manipulation of DNA has illuminated the mechanical basis of interactions between DNA and the molecular machinery involved in transcription, replication and recombination. Over the next decade, these studies are likely to expand to include detailed analyses of the mechanical interactions of many factors involved in these fundamental cellular processes. Because of the potentially large class of motors that track the DNA helix (as demonstrated elegantly for RNA polymerase[42]), necessary technical improvements will include direct measurement of torque in experiments that decouple twisting from bending.

Outside of traditional DNA biology, the ease of synthesis and well-characterized elasticity of DNA make it an ideal material for stiff molecular 'handles' to manipulate other molecules. So far, such handles have been used to mechanically unfold molecules of RNA[43], but covalent attachment of DNA segments to protein molecules has also been demonstrated[44], opening the door for the next generation of forced protein (un)folding studies[45] and perhaps mechanical assays of domain motion in enzymes.

Engineers have recently exploited the properties of DNA to construct self-assembled nanomachines, such as artificial DNA-based devices driven by strand displacement[46,47] or chemically induced structural rearrangements (ref. 48; and see article by Seeman, page 113). DNA micromanipulation techniques will help assess the utility of this new class of molecular machines for which force and torque generation have yet to be measured. The past decade has provided a new perspective of the mechanical nature of the double helix. The next decade promises deeper insight into its interactions with the cellular machinery and its potential for constructing sophisticated nanomachines. □

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DNA in a material world

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The specific bonding of DNA base pairs provides the chemical foundation for genetics. This powerful molecular recognition system can be used in nanotechnology to direct the assembly of highly structured materials with specific nanoscale features, as well as in DNA computation to process complex information. The exploitation of DNA for material purposes presents a new chapter in the history of the molecule.

"The nucleic-acid 'system' that operates in terrestrial life is optimized (through evolution) chemistry incarnate. Why not use it ... to allow human beings to sculpt something new, perhaps beautiful, perhaps useful, certainly unnatural." Roald Hoffmann, writing in *American Scientist*, 1994 (ref. 1).

The DNA molecule has appealing features for use in nanotechnology: its minuscule size, with a diameter of about 2 nanometres, its short structural repeat (helical pitch) of about 3.4–3.6 nm, and its 'stiffness', with a persistence length (a measure of stiffness) of around 50 nm. There are two basic types of nanotechnological construction: 'top-down' systems are where microscopic manipulations of small numbers of atoms or molecules fashion elegant patterns (for example, see ref. 2), while in 'bottom-up' constructions, many molecules self-assemble in parallel steps, as a function of their molecular recognition properties. As a chemically based assembly system, DNA will be a key player in bottom-up nanotechnology.

The origins of this approach date to the early 1970s, when *in vitro* genetic manipulation was first performed by tacking together molecules with 'sticky ends'. A sticky end is a short single-stranded overhang protruding from the end of a double-stranded helical DNA molecule. Like flaps of Velcro, two molecules with complementary sticky ends — that is, their sticky ends have complementary arrangements of the nucleotide bases adenine, cytosine, guanine and thymine — will cohere to form a molecular complex.

Sticky-ended cohesion is arguably the best example of programmable molecular recognition: there is significant diversity to possible sticky ends (4^N for N-base sticky ends), and the product formed at the site of this cohesion is the classic DNA double helix. Likewise, the convenience of solid support-based DNA synthesis[3] makes it is easy to program diverse sequences of sticky ends. Thus, sticky ends offer both predictable control of intermolecular associations and predictable geometry at the point of cohesion. Perhaps one could get similar affinity properties from antibodies and antigens, but, in contrast to DNA sticky ends, the relative three-dimensional orientation of the antibody and the antigen would need to be determined for every new pair. The nucleic acids seem to be unique in this regard, providing a tractable, diverse and programmable system with remarkable control over intermolecular interactions, coupled with known structures for their complexes.

Branched DNA

There is, however, a catch; the axes of DNA double helices are unbranched lines. Joining DNA molecules by sticky ends can yield longer lines, perhaps with specific components in a particular linear

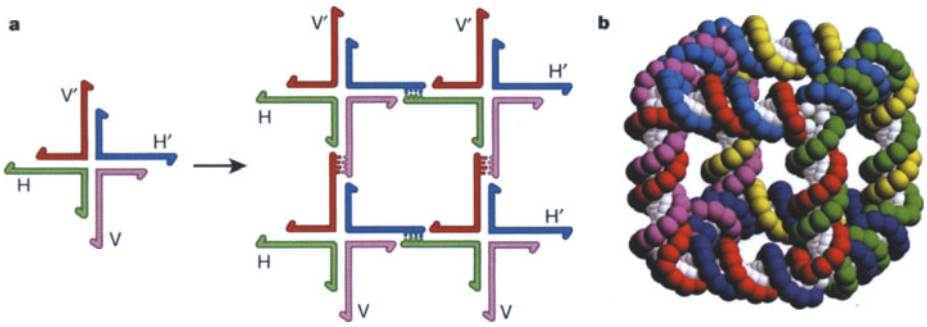


Figure 1 Assembly of branched DNA molecules. **a**, Self-assembly of branched DNA molecules into a two-dimensional crystal. A DNA branched junction forms from four DNA strands; those strands coloured green and blue have complementary sticky-end overhangs labelled H and H′, respectively, whereas those coloured pink and red have complementary overhangs V and V′, respectively. A number of DNA branched junctions cohere based on the orientation of their complementary sticky ends, forming a square-like unit with unpaired sticky ends on the outside, so more units could be added to produce a two-dimensional crystal. **b**, Ligated DNA molecules form interconnected rings to create a cube-like structure. The structure consists of six cyclic interlocked single strands, each linked twice to its four neighbours, because each edge contains two turns of the DNA double helix. For example, the front red strand is linked to the green strand on the right, the light blue strand on the top, the magenta strand on the left, and the dark blue strand on the bottom. It is linked only indirectly to the yellow strand at the rear.

or cyclic order in one dimension. Indeed, the chromosomes packed inside cells exist as just such one-dimensional arrays. But to produce interesting materials from DNA, synthesis is required in multiple dimensions and, for this purpose, branched DNA is required.

Branched DNA occurs naturally in living systems, as ephemeral intermediates formed when chromosomes exchange information during meiosis, the type of cell division that generates the sex cells (eggs and sperm). Prior to cell division, homologous chromosomes pair, and the aligned strands of DNA break and literally cross over one another, forming structures called Holliday junctions. This exchange of adjacent sequences by homologous chromosomes — a process called recombination — during the formation of sex cells passes genetic diversity onto the next generation.

The Holliday junction contains four DNA strands (each member of a pair of aligned homologous chromosomes is composed of two DNA strands) bound together to form four double-helical arms flanking a branch point (Fig. 1a). The branch point can relocate throughout the molecule, by virtue of the homologous sequences. In contrast, synthetic DNA complexes can be designed to have fixed branch points containing between three and at least eight arms[4,5]. Thus, the prescription for using DNA as the basis for complex materials with nanoscale features is simple: take synthetic branched DNA molecules with programmed sticky ends, and get them to self-assemble into the desired structure, which may be a closed object or a crystalline array (Fig. 1a).

Other modes of nucleic acid interaction aside from sticky ends are available. For example, Tecto-RNA molecules[6], held together by loop–loop interactions, or paranemic crossover (PX) DNA, where cohesion derives from pairing of alternate half turns in inter-wrapped double helices[7]. These new binding modes represent programmable cohesive interactions between cyclic single-stranded molecules that do not require cleavage to expose bases to pair molecules together. Nevertheless, cohesion using sticky ends remains the most prominent intermolecular interaction in structural DNA nanotechnology.

DNA constructions

It is over a decade since the construction of the first artificial DNA structure, a stick-cube, whose edges are double helices[8] (Fig. 1b). More complex polyhedra and topological constructs[9], such as knots and Borromean rings (consisting of three intricately interlinked circles), followed. But the apparent floppiness of individual branched junctions led to a hiatus before the next logical step: self-assembly into two-dimensional arrays.

This step required a stiffer motif, as it was difficult to build a periodic well-structured array with marshmallow-like components,

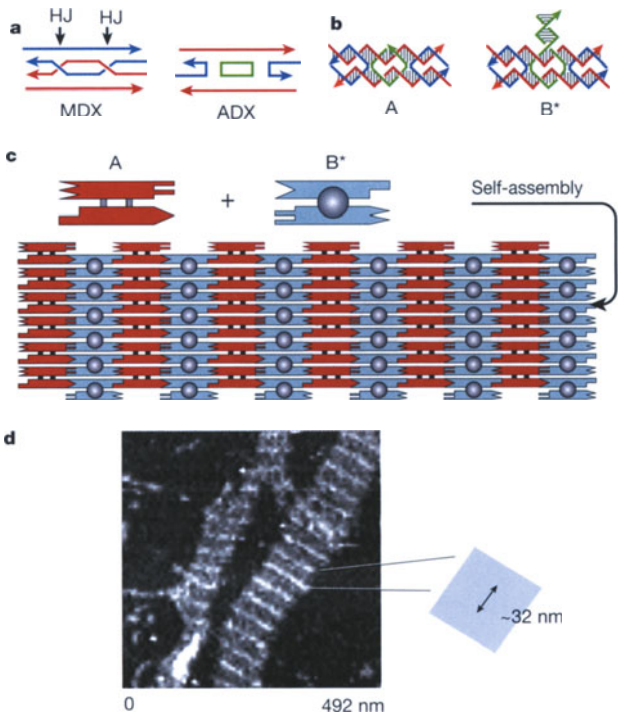


Figure 2 Two-dimensional DNA arrays. **a**, Schematic drawings of DNA double crossover (DX) units. In the meiotic DX recombination intermediate, labelled MDX, a pair of homologous chromosomes, each consisting of two DNA strands, align and cross over in order to swap equivalent portions of genetic information; 'HJ' indicates the Holliday junctions. The structure of an analogue unit (ADX), used as a tiling unit in the construction of DNA two-dimensional arrays, comprises two red strands, two blue crossover strands and a central green crossover strand. **b**, The strand structure and base pairing of the analogue ADX molecule, labelled A, and a variant, labelled B*. B* contains an extra DNA domain extending from the central green strand that, in practice, protrudes roughly perpendicular to the plane of the rest of the DX molecule. **c**, Schematic representations of A and B* where the perpendicular domain of B* is represented as a blue circle. The complementary ends of the ADX molecules are represented as geometrical shapes to illustrate how they fit together when they self-assemble. The dimensions of the resulting tiles are about 4 × 16 nm and are joined together so that the B* protrusions lie about 32 nm apart. **d**, The B* protrusions are visible as 'stripes' in tiled DNA arrays under an atomic force microscope.

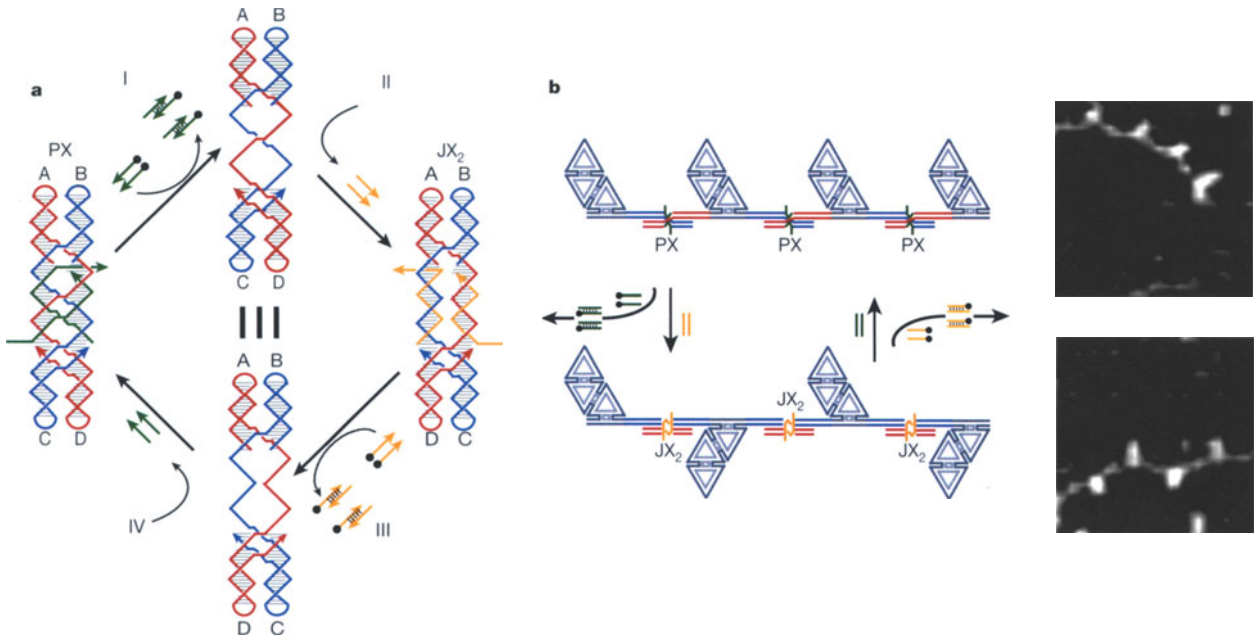


Figure 3 A rotary DNA nanomachine. **a**, The device works by producing two different conformations, depending on which of two pairs of strands (called 'set' strands) binds to the device framework. The device framework consists of two DNA strands (red and blue) whose top and bottom double helices are each connected by single strands. Thus, they form two rigid arms with a flexible hinge in between and the loose ends of the two strands dangling freely. The two states of the device, PX (left) and JX_2 (right), differ by a half turn in the relative orientations of their bottom helices (C and D on the left, D and C on the right). The difference between the two states is analogous to two adjacent fingers extended, parallel to each other (right), or crossed (left). The states are set by the presence of green or yellow set strands, which bind to the frame in different ways to produce different conformations. The set strands have extensions that enable their removal when complementary strands are added (steps I and III). When one type of set strand is removed, the device is free to bind the other set strands and switch to a different state (steps II and IV). **b**, The PX–JX_2 device can be used to connect 20-nm DNA trapezoid constructs. In the PX state, they are in a parallel conformation, but in the JX_2 state, they are in a zig-zag conformation, which can be visualized on the right by atomic force microscopy.

even with a well-defined blueprint (sticky-ended specificity) for their assembly. The stiffer motif was provided by the DNA double-crossover (DX) molecule[10], analogous, once again, to the double Holliday-junction intermediate formed during meiosis (MDX, Fig. 2a). This stiff molecule contains two double helices connected to each other twice through crossover points. It is possible to program DX molecules to produce a variety of patterned two-dimensional arrays just by controlling their sticky ends[11–13] (Fig. 2b).

DNA constructions

In addition to objects and arrays, a number of DNA-based nanomechanical devices have been made. The first device consisted of two DX molecules connected by a shaft with a special sequence that could be converted from normal right-handed DNA (known as B-DNA) to an unusual left-handed conformation, known as Z-DNA[14]. The two DX molecules lie on one side of the shaft before conversion and on opposite sides after conversion, which leads to a rotation. The problem with this device is that it is activated by a small molecule, $Co(NH_3)_6^{3+}$, and with all devices sharing the same stimulus, an ordered collection of DX molecules would not produce a diversity of responses.

This problem was solved by Bernard Yurke and colleagues, who developed a protocol for a sequence-control device that has a tweezers-like motion[15]. The principle behind the device is that a so-called 'set' strand containing a non-pairing extension hybridizes to a DNA-paired structural framework and sets a conformation; another strand that is complementary to the 'set' strand is then added, which binds to both the pairing and non-pairing portions, and removes it from the structure, leaving only the framework.

A robust rotary device was developed based on this principle[16] (Fig. 3), in which different set strands can enter and set the conformation to different structural end-states. In this way, the conformation of the DNA device can readily be flipped back and forth simply by adding different set strands followed by their complements. A variety of different devices can be controlled by a diverse group of set strands.

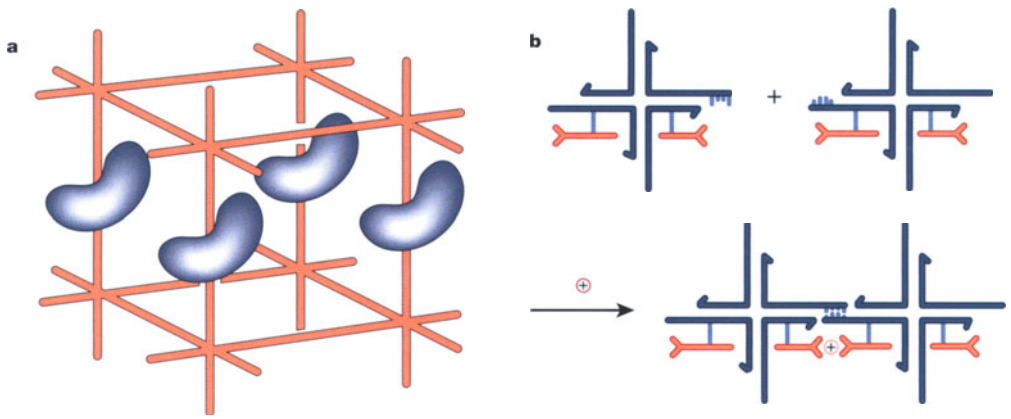


Figure 4 Applications of DNA scaffolds. **a**, Scaffolding of biological macromolecules. A DNA box (red) is shown with protruding sticky ends that are used to organize boxes into crystals. Macromolecules are organized parallel to each other within the box, rendering them amenable to structure determination by X-ray crystallography. **b**, DNA scaffolds to direct the assembly of nanoscale electrical circuits. Branched DNA junctions (blue) direct the assembly of attached nanoelectronic components (red), which are stabilized by the addition of a positively charged ion.

DNA nanomachines

What is the purpose of constructing DNA arrays and nanodevices? One prominent goal is to use DNA as scaffolding to organize other molecules. For example, it may be possible to use self-assembled DNA lattices (crystals) as platforms to position biological macromolecules so as to study their structure by X-ray crystallography[4] (Fig. 4a). Towards this goal, programming of DNA has been used to bring protein molecules in proximity with each other to fuse multiple enzymatic activities[17]. However, the potential of this approach awaits the successful self-assembly of three-dimensional crystals.

Another goal is to use DNA crystals to assemble nanoelectronic components in two- or three-dimensional arrays[18] (Fig. 4b). DNA has been shown to organize metallic nanoparticles as a precursor to nanoelectronic assembly[19–22], but so far it has not been possible to produce multidimensional arrays containing nanoelectronic components with the high-structural order of the naked DNA arrays described earlier.

There has been some controversy over whether DNA can be used as an electrical conductor (for example, ref. 23), although the resolution of this debate is unlikely have any impact on the use of DNA as a scaffold. Recently, the effects of DNA conformational changes on conduction in the presence of an analyte were shown to have potential as a biosensor[24].

Replicating DNA components

A natural question to ask of any assembly system based on DNA is whether the components can be replicated. To produce branched DNA molecules whose branch points do not move, they must have different sequences in opposite branches but, as a consequence, these structures are not readily reproduced by DNA polymerase; the polymerase would produce complements to all strands present, leading only to double helical molecules. One option is to use topological tricks to convert structures like the DNA cube into a long single strand by adding extra stretches of DNA bases. The single strand could then be replicated by DNA polymerase and the final replicated product induced to fold into the original shape, with any extraneous segments cleaved using restriction enzymes. Although this would produce a molecule with sticky ends ready to participate in self-assembly, it would be a cumbersome process[25].

Günter von Kiedrowski and colleagues have recently developed a way of replicating short, simple DNA branches in a mixed organic–DNA species. Their branched molecule consists of three DNA single strands bonded to an organic triangle-shaped linker. To replicate the branched molecule, the single-stranded complement of each of these strands is bound to the molecule, so that one end of each complement molecule is close to the same end of the other complement molecule. In the final step, the juxtaposed complements are connected together by bonding their neighbouring ends to another molecule of the organic linker[26]. Extension of this system to the next level, such as objects like the cube, will need to solve topological problems involved in the separation of the two components, or it will be limited to unligated systems.

Future prospects

Many separate capabilities of DNA nanotechnology have been prototyped — it is now time to extend and integrate them into useful systems. Combining sequence-dependent devices with nanoscale arrays will provide a system with a vast number of distinct, programmable structural states, the *sine qua non* of nanorobotics. A key step in realizing these goals is to achieve highly ordered three-dimensional arrays, both periodic and, ultimately, algorithmic.

Interfacing with top-down nanotechnology will extend markedly the capabilities of the field. It also will be necessary to integrate biological macromolecules or other macromolecular complexes into DNA arrays in order to make practical systems with nanoscale components. Likewise, the inclusion of electronic components in highly ordered arrays will enable the organization of nanoelectronic circuits. Chemical function could be added to DNA arrays by adding nucleic acid species evolved *in vitro* to have specific binding properties ('aptamers') or enzymatic activities ('ribozymes' or 'DNAzymes'). A further area that has yet to have an impact on DNA nanotechnology is

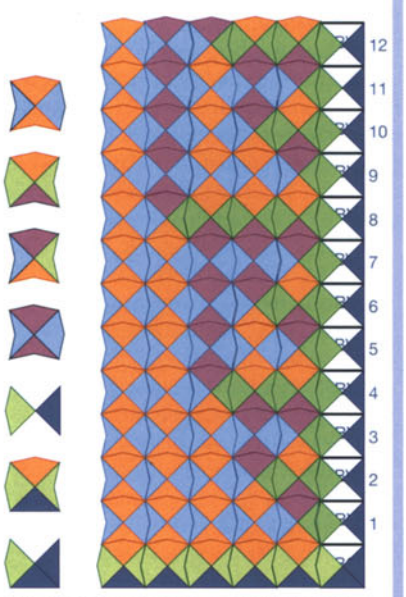
Box 1

DNA computers

An assembly of DNA strands can process data in a similar way as an electronic computer, and has the potential to solve far more complex problems and store a greater amount of information, for substantially less energy costs than do electronic microprocessors. DNA-based computation dates from Leonard Adleman's landmark report in 1994 (ref. 27), where he used DNA to solve the 'Hamiltonian path' problem, a variant of the 'travelling salesman' problem. The idea is to establish whether there is a path between two cities, given an incomplete set of available roads. Adleman used strands of DNA to represent cities and roads, and encoded the sequences so that a strand representing a road would connect (according to the rules of base pairing) to any two strands representing a city. By mixing together the strands, joining the cities connected by roads, and weeding out any 'wrong answers', he showed that the strands could self-assemble to solve the problem.

It is impossible to separate DNA nanotechnology from DNA-based computation: many researchers work in both fields and the two communities have a symbiotic relationship. The first link between DNA computation and DNA nanotechnology was established by Erik Winfree, who suggested that short branched DNA molecules could be 'programmed' to undergo algorithmic self-assembly and thus serve as the basis of computation[28].

Periodic building blocks of matter, such as the DNA molecules shown in Fig. 1a, represent the simplest algorithm for assembly. All components are parallel, so what is on one side of a component is also on the other side, and in every direction. Given this parallelism, if the right side complements the left, the top complements the bottom and the front complements the back, a crystal should result. Even more complex algorithms are possible if one uses components of the same shape, but with different sticky ends. For example, Winfree has shown that, in principle, DNA tiles can be used to 'count' (see figure below) by creating borders with programmable sizes for one-, two- and possibly three-dimensional assemblies[29]. If this scheme can be realized, self-assembly of precisely sized nanoscale arrays will be possible. A computation using self-assembly has been prototyped in one dimension, thereby lending some credence to the viability of algorithmic assembly[30].



Box 1 Figure Counting with self-assembled DNA tiles. DNA tiles are represented by squares with coloured edges that are protruded or indented. Seven component tiles are shown on the left: three border tiles on the bottom and four tiles with the values **0** or **1**. The array illustrates binary counting from 1 (bottom row) to 12 (top row). Assembly is assumed to proceed by forming the reverse L-shaped border first, followed by binding the tiles that fit into the sites containing two (but not one) edges. Thus, the border determines the **1** tile in its bend, then that **1** tile and the horizontal-border tile on its left determine the **0** tile that fits, while the **1** tile and the vertical-border tile above it determine the (different) **0** tile that fits. (Adapted from ref. 29.)

combinatorial synthesis, which may well lead to greater diversity of integrated components. DNA-based computation and algorithmic assembly is another active area of research, and one that is impossible to separate from DNA nanotechnology (see Box 1).

The field of DNA nanotechnology has attracted an influx of researchers over the past few years. All of those involved in this area have benefited from the biotechnology enterprise that produces DNA-modifying enzymes and unusual components for synthetic DNA molecules. It is likely that applications in structural DNA nanotechnology ultimately will use variants on the theme of DNA (for example, peptide nucleic acids, containing an unconventional synthetic peptide backbone and nucleic acid bases for side chains), whose properties may be better suited to particular types of applications.

For the past half-century, DNA has been almost exclusively the province of biologists and biologically oriented physical scientists, who have studied its biological impact and molecular properties. During the next 50 years, it is likely they will be joined by materials scientists, nanotechnologists and computer engineers, who will exploit DNA's chemical properties in a non-biological context. □

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DNA replication and recombination

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Knowledge of the structure of DNA enabled scientists to undertake the difficult task of deciphering the detailed molecular mechanisms of two dynamic processes that are central to life: the copying of the genetic information by DNA replication, and its reassortment and repair by DNA recombination. Despite dramatic advances towards this goal over the past five decades, many challenges remain for the next generation of molecular biologists.

"Though facts are inherently less satisfying than the intellectual conclusions drawn from them, their importance should never be questioned." James D. Watson, 2002.

DNA carries all of the genetic information for life. One enormously long DNA molecule forms each of the chromosomes of an organism, 23 of them in a human. The fundamental living unit is the single cell. A cell gives rise to many more cells through serial repetitions of a process known as cell division. Before each division, new copies must be made of each of the many molecules that form the cell, including the duplication of all DNA molecules. DNA replication is the name given to this duplication process, which enables an organism's genetic information — its genes — to be passed to the two daughter cells created when a cell divides. Only slightly less central to life is a process that requires dynamic DNA acrobatics, called homologous DNA recombination, which reshuffles the genes on chromosomes. In reactions closely linked to DNA replication, the recombination machinery also repairs damage that inevitably occurs to the long, fragile DNA molecules inside cells (see accompanying article by Friedberg, page 122).

The model for the DNA double helix[1] proposed by James Watson and Francis Crick is based on two paired DNA strands that are complementary in their nucleotide sequence. The model had striking implications for the processes of DNA replication and DNA recombination. Before 1953, there had been no meaningful way of even speculating about the molecular mechanisms of these two central genetic processes. But the proposal that each nucleotide in one strand of DNA was tightly base-paired with its complementary nucleotide on the opposite strand — either adenine (A) with thymine (T), or guanine (G) with cytosine (C) — meant that any part of the nucleotide sequence could act as a direct template for the corresponding portion of the other strand. As a result, any part of the sequence can be used either to create or to recognize its partner nucleotide sequence — the two functions that are central for DNA replication and DNA recombination, respectively.

In this review, I discuss how the discovery of the structure of DNA half a century ago opened new avenues for understanding the processes of DNA replication and recombination. I shall also emphasize how, as our understanding of complex biological molecules and their interactions increased over the years, there have been profound changes in the way that biologists view the chemistry of life.

Structural features of DNA

The research that immediately followed the discovery of the double helix focused primarily on understanding the structural properties

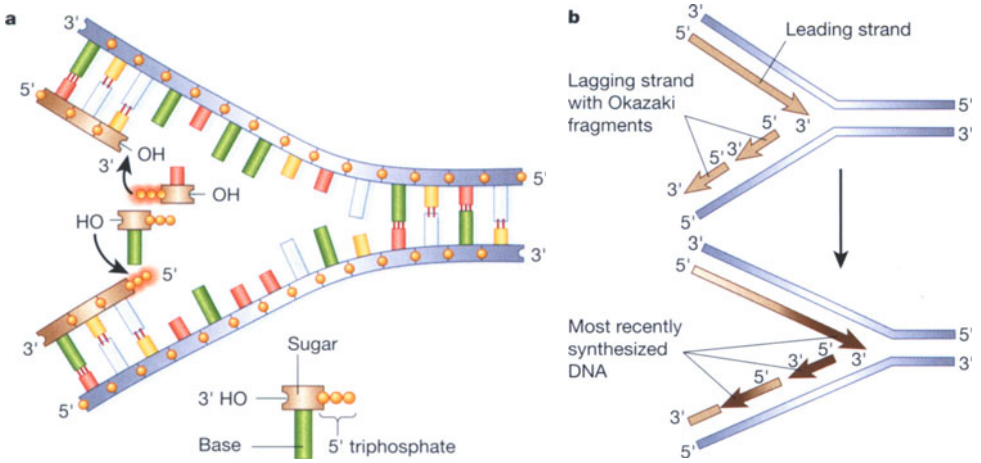


Figure 1 The DNA replication fork. **a**, Nucleoside triphosphates serve as a substrate for DNA polymerase, according to the mechanism shown on the top strand. Each nucleoside triphosphate is made up of three phosphates (represented here by yellow spheres), a deoxyribose sugar (beige rectangle) and one of four bases (differently coloured cylinders). The three phosphates are joined to each other by high-energy bonds, and the cleavage of these bonds during the polymerization reaction releases the free energy needed to drive the incorporation of each nucleotide into the growing DNA chain. The reaction shown on the bottom strand, which would cause DNA chain growth in the 3′ to 5′ chemical direction, does not occur in nature. **b**, DNA polymerases catalyse chain growth only in the 5′ to 3′ chemical direction, but both new daughter strands grow at the fork, so a dilemma of the 1960s was how the bottom strand in this diagram was synthesized. The asymmetric nature of the replication fork was recognized by the early 1970s: the 'leading strand' grows continuously, whereas the 'lagging strand' is synthesized by a DNA polymerase through the backstitching mechanism illustrated. Thus, both strands are produced by DNA synthesis in the 5′ to 3′ direction. (Redrawn from ref. 27, with permission.)

of the molecule. DNA specifies RNA through the process of gene transcription, and the RNA molecules in turn specify all of the proteins of a cell. This is the 'central dogma' of genetic information transfer[2]. Any read-out of genetic information — whether it be during DNA replication or gene transcription — requires access to the sequence of the bases buried in the interior of the double helix. DNA strand separation is therefore critical to DNA function. Thus, the Watson–Crick model drove scientists to a search for conditions that would disrupt the hydrogen bonds joining the complementary base pairs, so as to separate the two strands of the DNA double helix.

Physical chemists found that heating a solution of DNA to temperatures near boiling (100 °C), or subjecting it to extremes of pH, would cause the strands to separate — a change termed 'DNA denaturation'. The so-called 'melting temperature' (or T_m) of a stretch of DNA sequence depends on its nucleotide composition: those DNAs with a larger proportion of G–C base pairs exhibit a higher T_m because of the three hydrogen bonds that Watson and Crick had predicted to hold a G–C base pair together, compared with only two for the A–T base pair. At physiological salt concentrations, the T_m of mammalian DNA is nearly 90 °C, owing to the particular mix of its base pairs (47% G–C and 53% A–T)[3].

Initially it seemed inconceivable that, once separated from its complementary partner, a DNA strand could reform a double helix again. In a complex mixture of DNA molecules, such a feat would require finding the one sequence match amongst millions during random collisions with other sequences, and then rapidly rewinding with a new partner strand. The dramatic discovery of this unexpected phenomenon[4], called 'DNA renaturation', shed light on how sequences could be rearranged by DNA recombination. And it also provided a critical means by which DNA could be manipulated in the laboratory. The annealing of complementary nucleotide sequences, a process called hybridization, forms the basis of several DNA technologies that helped launch the biotechnology industry and modern genomics. These include gene cloning, genomic sequencing, and DNA copying by the polymerase chain reaction (see article by Hood and Galas on page 130).

The arrangement of DNA molecules in chromosomes presented another mystery for scientists: a long, thin molecule would be highly sensitive to shear-induced breakage, and it was hard to imagine that a mammalian chromosome might contain only a single DNA molecule. This would require that a typical chromosome be formed from a continuous DNA helix more than 100 million nucleotide pairs long — a massive molecule weighing more than 100 billion daltons, with an end-to-end distance of more than 3 cm. How could such a giant molecule be protected from accidental fragmentation in a cell only microns in diameter, while keeping it organized for efficient gene readout and other genetic functions?

There was no precedent for such giant molecules outside the world of biology. But in the early 1960s, autoradiographic studies revealed that the chromosome of the bacterium *Escherichia coli* was in fact a single DNA molecule, more than 3 million nucleotide pairs in length[5]. And when — more than a decade later — innovative physical techniques demonstrated that a single huge DNA molecule formed the basis for each mammalian chromosome[6], the result was welcomed by scientists with little surprise.

DNA replication forks

How is the enormously long double-stranded DNA molecule that forms a chromosome accurately copied to produce a second identical chromosome each time a cell divides? The template model for DNA replication, proposed by Watson and Crick in 1953 (ref. 7), gained universal acceptance after two discoveries in the late 1950s. One was an elegant experiment using density-labelled bacterial DNAs that confirmed the predicted template–anti-template scheme[8]. The other was the discovery of an enzyme called DNA polymerase, which uses one strand of DNA as a template to synthesize a new complementary strand[9]. Four deoxyribonucleoside triphosphate nucleotides — dATP, dTTP, dGTP and dCTP — are the precursors to a new daughter DNA strand, each nucleotide selected by pairing with its complementary nucleotide (T, A, C or G, respectively) on the parental template strand. The DNA polymerase was shown to use these triphosphates to add nucleotides one at a time to the 3′ end of the newly synthesized DNA molecule, thereby catalysing DNA chain growth in the 5′ to 3′ chemical direction.

Although the synthesis of short stretches of DNA sequence on a single-stranded template could be demonstrated in a test tube, how an enormous, twisted double-stranded DNA molecule is replicated was a puzzle. Inside the cell, DNA replication was observed to occur at a Y-shaped structure, called a 'replication fork', which moves steadily along a parental DNA helix, spinning out two daughter DNA helices behind it (the two arms of the 'Y')[5]. As predicted by Watson and Crick, the two strands of the double helix run in opposite chemical directions. Therefore, as a replication fork moves, DNA polymerase can move continuously along only one arm of the Y — the arm on

Box 1
Core proteins at the DNA replication fork

Proteins at the Y-shaped DNA replication fork are illustrated schematically in panel **a** of the figure below, but in reality, the fork is folded in three dimensions, producing a structure resembling that of the diagram in the inset **b** (cartoons redrawn from ref. 27, with permission).

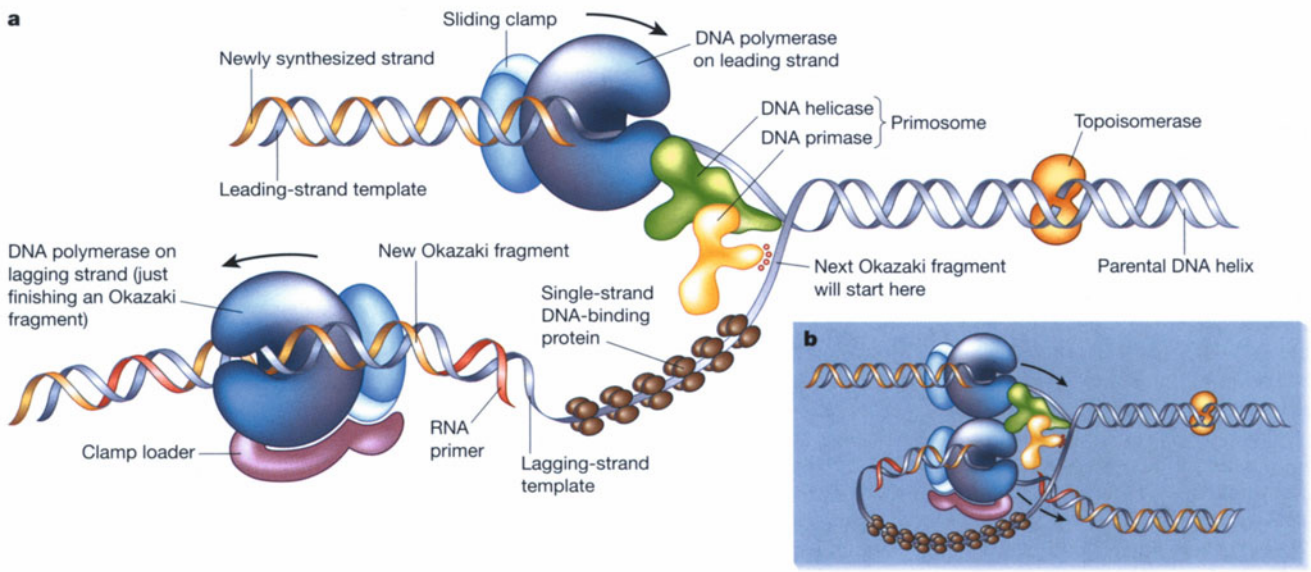
Focusing on the schematic illustration in **a**, two DNA polymerase molecules are active at the fork at any one time. One moves continuously to produce the new daughter DNA molecule on the leading strand, whereas the other produces a long series of short 'Okazaki DNA fragments' on the lagging strand. Both polymerases are anchored to their template by polymerase accessory proteins, in the form of a sliding clamp and a clamp loader.

A DNA helicase, powered by ATP hydrolysis, propels itself rapidly along one of the template DNA strands (here the lagging strand), forcing open the DNA helix ahead of the replication fork. The helicase exposes the bases of the DNA helix for the leading-strand polymerase to copy. DNA topoisomerase enzymes facilitate DNA helix unwinding.

In addition to the template, DNA polymerases need a pre-existing DNA or RNA chain end (a primer) onto which to add each nucleotide. For this reason, the lagging strand polymerase requires the action of a DNA primase enzyme before it can start each Okazaki fragment. The primase produces a very short RNA molecule (an RNA primer) at the 5′ end of each Okazaki fragment onto which the DNA polymerase adds nucleotides. Finally, the single-stranded regions of DNA at the fork are covered by multiple copies of a single-strand DNA-binding protein, which hold the DNA template strands open with their bases exposed.

In the folded fork structure shown in the inset, the lagging-strand DNA polymerase remains tied to the leading-strand DNA polymerase. This allows the lagging-strand polymerase to remain at the fork after it finishes the synthesis of each Okazaki fragment. As a result, this polymerase can be used over and over again to synthesize the large number of Okazaki fragments that are needed to produce a new DNA chain on the lagging strand.

In addition to the above group of core proteins, other proteins (not shown) are needed for DNA replication. These include a set of initiator proteins to begin each new replication fork at a replication origin, an RNAseH enzyme to remove the RNA primers from the Okazaki fragments, and a DNA ligase to seal the adjacent Okazaki fragments together to form a continuous DNA strand.



which the new daughter strand is being elongated in the 5′ to 3′ chemical direction. On the other arm, the new daughter strand would need to be produced in the opposite, 3′ to 5′ chemical direction (Fig. 1a). So, whereas Watson and Crick's central predictions were confirmed at the end of the first decade of research that followed their landmark discovery, the details of the DNA replication process remained a mystery.

Reconstructing replication

The mystery was solved over the course of the next two decades, a period in which the proteins that constitute the central players in the DNA replication process were identified. Scientists used a variety of experimental approaches to identify an ever-growing set of gene products thought to be critical for DNA replication. For example, mutant organisms were identified in which DNA replication was defective, and genetic techniques could then be used to identify specific sets of genes required for the replication process[10–12]. With the aid of the proteins specified by these genes, 'cell-free' systems were established, where the process was re-created *in vitro* using purified components. Initially, proteins were tested in a 'partial replication reaction', where only a subset of the protein machinery required for the full replication process was present, and the DNA template was provided in a single-stranded form[13]. New proteins that were identified were added one at a time or in combination to test their effects on the catalytic activity of DNA polymerase. Further advances in understanding replication then depended on creating more complex *in vitro* systems, in which, through the addition of a larger set of purified proteins, double-stranded DNA could eventually be replicated[14–15].

Today, nearly every process inside cells — from DNA replication and recombination to membrane vesicle transport — is being studied in an *in vitro* system reconstructed from purified components. Although laborious to establish, such systems enable the precise control of both the concentration and the detailed structure of each component. Moreover, the 'noise' in the natural system caused by side reactions — because most molecules in a cell are engaged in more than one type of reaction — is avoided by eliminating the proteins that catalyse these other reactions. In essence, a small fraction of the cell can be re-created as a

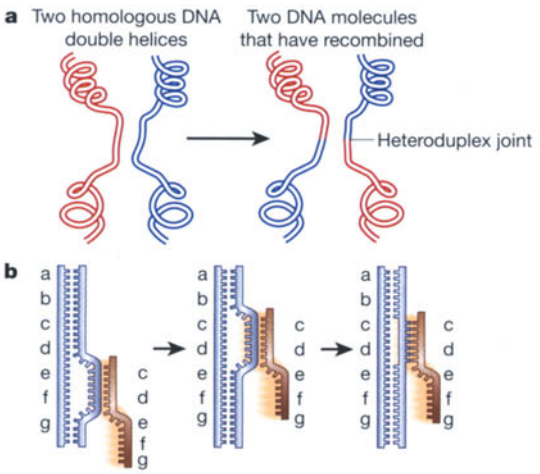
Box 2
DNA recombination

Homologous DNA recombination involves an exchange between two DNA double helices that causes a section of each helix to be exchanged with a section of the other, as illustrated schematically in panel **a** in the figure below (redrawn from ref. 27, with permission). Critical to the reaction is the formation of a heteroduplex joint at the point where the two double helices have been broken and then joined together. To form this joint, which glues two previously separate molecules together, a strand from one helix must form base pairs with a complementary strand from the second helix. This requires that the two DNA helices that recombine have a very similar sequence of nucleotides, that is, they must be homologous.

The DNA double helix poses a major problem for the DNA recombination process, because the bases that need to pair to form a heteroduplex joint are buried in the interior of the helix. How can two DNA helices recognize that they are homologous, in order to begin a recombination event, if their bases are not exposed?

The breakthrough came from the isolation and characterization of the RecA protein[17] from the bacterium *Escherichia coli*, which would turn out to be the prototype for a family of strand-exchange proteins that is present in all organisms, from bacteria to humans. The human equivalent of the RecA protein is the Rad51 protein. These proteins catalyse the central synapsis step of homologous DNA recombination — the process that brings two matching DNA helices together and causes them to exchange parts, resulting in either the reassortment or the repair of genetic information (panel **b** below). Powered by the energy generated from ATP hydrolysis, the RecA protein assembles into long filaments on a single-strand DNA molecule (brown strand). Because the RecA protein has a second DNA-binding site that recognizes a DNA double helix, a RecA-coated strand has the remarkable ability to scan for a complementary strand in any double helix (blue strand) that it encounters. Once found, the complementary strand is pulled from the helix to form a new 'hybrid helix' with the RecA-coated single strand, thereby initiating the formation of the heteroduplex joint needed for recombination, as illustrated schematically in panel **b** (RecA protein not shown).

DNA recombination makes it possible for a damaged chromosome to repair itself by using a second copy of the same genetic information as a guide. It also causes the extensive breakage and reunion of chromosomes that occurs during the development of eggs and sperm, which greatly increases the genetic variation produced by sexual reproduction. Many of the atomic details of the RecA protein reaction are still uncertain, remaining as a future challenge for scientists.



bounded set of chemical reactions, making it fully amenable to precise study using all of the tools of physics and chemistry.

By 1980, multiprotein *in vitro* systems had enabled a detailed characterization of the replication machinery and solved the problem of how DNA is synthesized on both sides of the replication fork (Fig. 1b). One daughter DNA strand is synthesized continuously by a DNA polymerase molecule moving along the 'leading strand', while a second DNA polymerase molecule on the 'lagging strand' produces a long series of fragments (called Okazaki fragments)[16] which are joined together by the enzyme DNA ligase to produce a continuous DNA strand. As might be expected, there is a difference in the proteins required for leading- and lagging-strand DNA synthesis (see Box 1). Remarkably, the replication forks formed in these artificial systems could be shown to move at the same rapid rates as the forks inside cells (500 to 1,000 nucleotides per second), and the DNA template was copied with incredibly high fidelity[15].

As more and more proteins were found to function at the replication fork, comparisons could be made between the replication machinery of different organisms. Studies of the replication machinery in viruses, bacteria and eukaryotes revealed that a common set of protein activities drives the replication forks in each organism (Box 1). Each system consists of: a leading- and a lagging-strand DNA polymerase molecule; a DNA primase to produce the RNA primers that start each Okazaki fragment; single-strand DNA binding proteins that coat the template DNA and hold it in position; a DNA helicase that unwinds the double helix; and additional polymerase accessory proteins the tie the polymerases to each other and to the DNA template. As one progresses from a simple virus to more complex organisms, such as yeasts or mammals, the number of subunits that make up each type of protein activity tends to increase. For example, the total number of polypeptide subunits that form the core of the replication apparatus increases from four and seven in bacteriophages T7 and T4, respectively, to 13 in the bacterium *E. coli*. And it expands to at least 27 in the yeast *Saccharomyces cerevisiae* and in mammals. Thus, as organisms with larger genomes evolved, the replication machinery added new protein subunits, without any change in the basic mechanisms[15,18–20].

While the work I have described on DNA replication was advancing, other groups of researchers were establishing *in vitro* systems in which homologous DNA recombination could be reconstructed. The central player in these reactions was the RecA type of protein[17], named after the bacterial mutant defective in recombination that led to its discovery (Box 2).

Protein machines

As for all other aspects of cell biochemistry, the DNA replication apparatus has evolved over billions of years through 'trial and error'— that is, by random variation followed by natural selection. With time, one protein after another could be added to the mix of proteins active at the replication fork, presumably because the new protein increased the speed, control or accuracy of the overall replication process. In addition, the structure of each protein was fine-tuned by mutations that altered its amino acid sequence so as to increase its effectiveness. The end results of this unusual engineering process are the replication systems that we observe today in different organisms. The mechanism of DNA replication might therefore be expected to be highly dependent on random past events. But did evolution select for whatever works, with no need for elegance?

For the first 30 years after Watson and Crick's discovery, most researchers seemed to hold the view that cell processes could be sloppy. This view was encouraged by knowledge of the tremendous speed of movements at the molecular level (for example, it was known that a typical protein collides with a second molecule present at a concentration of 1 mM about 10^6 times per second). The rapid rates of molecular movement were thought initially to allow a process like DNA replication to occur without any organization of the proteins involved in three-dimensional space.

Quite to the contrary, molecular biologists now recognize that evolution has selected for highly ordered systems. Thus, for example, not only are the parts of the replication machinery held together in precise alignments to optimize their mutual interactions, but energy-driven changes in protein conformations are used to generate coordinated movements. This ensures that each of the successive steps in a complex process like DNA replication is closely coordinated with the next one. The result is an assembly that can be viewed as a 'protein machine'. For example, the DNA polymerase molecule on the lagging side of the replication fork remains bound to the leading-strand DNA polymerase molecule to ensure that the same lagging-strand polymerase is used over and over again for efficient synthesis of Okazaki fragments[18,20,21] (Box 1). And DNA replication is by no means unique. We now believe that nearly every biological process is catalysed by a set of ten or more spatially positioned, interacting proteins that undergo highly ordered movements in a machine-like assembly[22].

Protein machines generally form at specific sites in response to particular signals, and this is particularly true for protein machines that act on DNA. The replication, repair and recombination of the DNA double helix are often considered as separate, isolated processes. But inside the cell, the same DNA molecule is able to undergo any one of these reactions. Moreover, specific combinations of the three types of reactions occur. For instance, DNA recombination is often linked directly to either DNA replication or DNA repair[23]. For the integrity of a chromosome to be properly maintained, each specific reaction must be carefully directed and controlled. This requires that sets of proteins be assembled on the DNA and activated only where and when they are needed. Although much remains to be learned about how these choices are made, it seems that different types of DNA structures are recognized explicitly by specialized proteins that serve as 'assembly factors'. Each assembly factor then serves to nucleate a cooperative assembly of the set of proteins that forms a particular protein machine, as needed for catalysing a reaction appropriate to that time and place in the cell.

A view of the future

It has become customary, both in textbooks and in the regular scientific literature, to explain molecular mechanisms through simple two-dimensional drawings or 'cartoons'. Such drawings are useful for consolidating large amounts of data into a simple scheme, as illustrated in this review. But a whole generation of biologists may have become lulled into believing that the essence of a biological mechanism has been captured, and the entire problem therefore solved, once a researcher has deciphered enough of the puzzle to be able to draw a meaningful cartoon of this type.

In the past few years, it has become abundantly clear that much more will be demanded of scientists before we can claim to fully understand a process such as DNA replication or DNA recombination. Recent genome sequencing projects, protein-interaction mapping efforts and studies in cell signalling have revealed many more components and molecular interactions than were previously realized. For example, according to one recent analysis, *S. cerevisiae*, a single-celled 'simple' eukaryotic organism (which has about 6,000 genes compared with 30,000 in humans), uses 88 genes for its DNA replication and 49 genes for its DNA recombination[24].

To focus on DNA replication, fully understanding the mechanism will require returning to where the studies of DNA first began — in the realms of chemistry and physics. Detailed atomic structures of all relevant proteins and nucleic acids will be needed, and spectacular progress is being made by structural biologists, owing to increasingly powerful X-ray crystallography and nuclear magnetic resonance techniques. But the ability to reconstruct biological processes in a test tube with molecules whose precise structures are known is not enough. The replication process is both very rapid and incredibly accurate, achieving a final error rate of about one nucleotide in a billion. Understanding how the reactions between the many different proteins and other molecules are coordinated to create this result will require that experimentalists determine all of the rate constants for the interactions between the various components, something that is rarely done by molecular biologists today. They can then use genetic engineering techniques to alter selected sets of these parameters, carefully monitoring the effect of these changes on the replication process.

Scientists will be able to claim that they truly understand a complex process such as DNA replication only when they can precisely predict the effect of changes in each of the various rate constants on the overall reaction. Because the range of experimental manipulations is enormous, we will need more powerful ways of deciding which such alterations are the most likely to increase our understanding. New approaches from the rapidly developing field of computational biology must therefore be developed — both to guide experimentation and to interpret the results.

The Watson–Crick model of DNA catalysed dramatic advances in our molecular understanding of biology. At the same time, its enormous success gave rise to the misleading view that many other complex aspects of biology might be similarly reduced to elegant simplicity through insightful theoretical analysis and model building. This view has been supplanted over subsequent decades, because most biological subsystems have turned out to be far too complex for their details to be predicted. We now know that nothing can substitute for rigorous experimental analyses. But traditional molecular and cell biology alone cannot bring a problem like DNA replication to closure. New types of approaches will be required, involving not only new computational tools, but also a greater integration of chemistry and physics[20,25]. For this reason, we urgently need to rethink the education that we are providing to the next generation of biological scientists[22,26]. □

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DNA damage and repair

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The aesthetic appeal of the DNA double helix initially hindered notions of DNA mutation and repair, which would necessarily interfere with its pristine state. But it has since been recognized that DNA is subject to continuous damage and the cell has an arsenal of ways of responding to such injury. Although mutations or deficiencies in repair can have catastrophic consequences, causing a range of human diseases, mutations are nonetheless fundamental to life and evolution.

"We totally missed the possible role of … [DNA] repair although … I later came to realise that DNA is so precious that probably many distinct repair mechanisms would exist." Francis Crick, writing in *Nature*, 26 April 1974 (ref. 1).

This retrospective reflection by Francis Crick, penned two decades after he and James Watson reported the structure of DNA, hints at the early perception of DNA as a highly stable macromolecular entity. This prevailing view at the time significantly delayed serious consideration of biochemical processes such as mutation and repair. It was once suggested by Frank Stahl that "the possibility that … genes were … subject to the hurly-burly of both insult and clumsy efforts to reverse the insults, was unthinkable."[2]

But subsequent work on three 'R's' of DNA metabolism — replication (copying of DNA prior to each cell division), recombination (exchanges between different DNA molecules in a cell) and repair (restoration of altered DNA to its normal state) — revealed the dynamic state of DNA. It became apparent that DNA in all living organisms continually incurs a myriad of types of damage, and that cells have devised ingenious mechanisms for tolerating and repairing the damage. Failure of these mechanisms can lead to serious disease consequences, as well illustrated in the human hereditary diseases xeroderma pigmentosum (XP), hereditary non-polyposis colon cancer (HNPCC) and some forms of breast cancer. XP is characterized by about a 10,000-fold increased risk of skin cancer associated with sunlight exposure; individuals with HNPCC manifest an increased hereditary predisposition to colon (and other) cancer.

The roots of repair

The early work on DNA damage and repair in the 1930s was stimulated by a small but prominent group of physicists[3]. As recounted by the geneticist Guido Pontecorvo, "in the years immediately preceding World War II something quite new happened: the introduction of ideas (not techniques) from the realm of physics into the realm of genetics, particularly as applied to the problems of size, mutability, and self-replication of genes"[4]. Seminal to this coalition between physics and biology in pre-war Germany was the collaboration between German physicists Karl Zimmer and Max Delbrück and the Russian geneticist Nikolai Timoféeff-Ressovsky[5]. Their partnership was stimulated by the work of Hermann Muller, a geneticist working on the fruitfly *Drosophila* who first demonstrated that external agents, such as ionizing radiation, can cause mutations in living organisms[6].

Timoféeff-Ressovsky and Zimmer were interested primarily in how such small amounts of energy in the form of ionizing radiation (formally equivalent to no more than the amount of energy absorbed as heat by drinking a cup of hot tea) could have such profound biological effects[3]. Delbrück and Muller, on the other hand, were intrigued by whether such mutations could reveal insight into the physical nature of the gene.

In retrospect, it was inevitable that the deployment of physical (and later chemical) tools, such as ionizing and ultraviolet (UV) radiation, to study genes would in due course lead to questions as to how these agents damaged DNA[3]. And, once it was recognized that these interactions promoted deleterious effects on the structure and function of genes, to questions concerning how cells cope with damaged DNA. Zimmer wrote, "one cannot use radiations for elucidating the normal state of affairs without considering the mechanisms of their actions, nor can one find out much about radiation induced changes without being interested in the normal state of the material under investigation."[7]

Hints of the ability of living cells to recover from the lethal effect of UV radiation emerged as early as the mid-1930s[8]. But the discovery of a DNA-repair mechanism had to wait until the end of the 1940s, through the independent, serendipitous observations of Albert Kelner[9] working in Milislav Demerec's group at the Cold Spring Harbor Laboratory, and Renato Dulbecco[10] in Salvador Luria's laboratory at The University of Indiana. Neither Kelner nor Dulbecco set out to study damage to DNA or its repair. They were both using UV radiation as an experimental tool, but observed anomalous survival rates when cells or bacteriophage (bacteria-infecting viruses) were inadvertently exposed to long-wavelength light, either as sunlight or fluorescent light in their respective laboratories[9,10]. Their efforts to explain these confounding observations led to the discovery of the phenomenon now known as photoreacti-

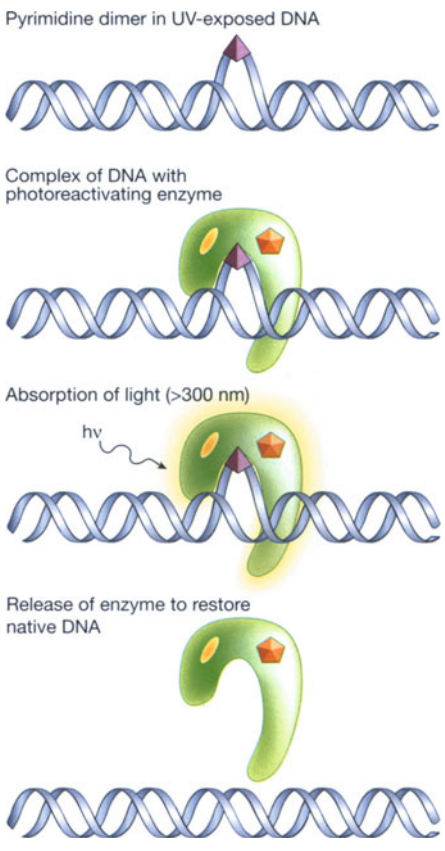

Figure 1 Photoreactivation reverses DNA damage. DNA exposed to ultraviolet (UV) radiation results in covalent dimerization of adjacent pyrimidines, typically thymine residues (thymine dimers), illustrated here as a purple triangle. These lesions are recognized by a photoreactivating enzyme, which absorbs light at wavelengths >300nm (such as fluorescent light or sunlight) and facilitates a series of photochemical reactions that monomerize the dimerized pyrimidines, restoring them to their native conformation.

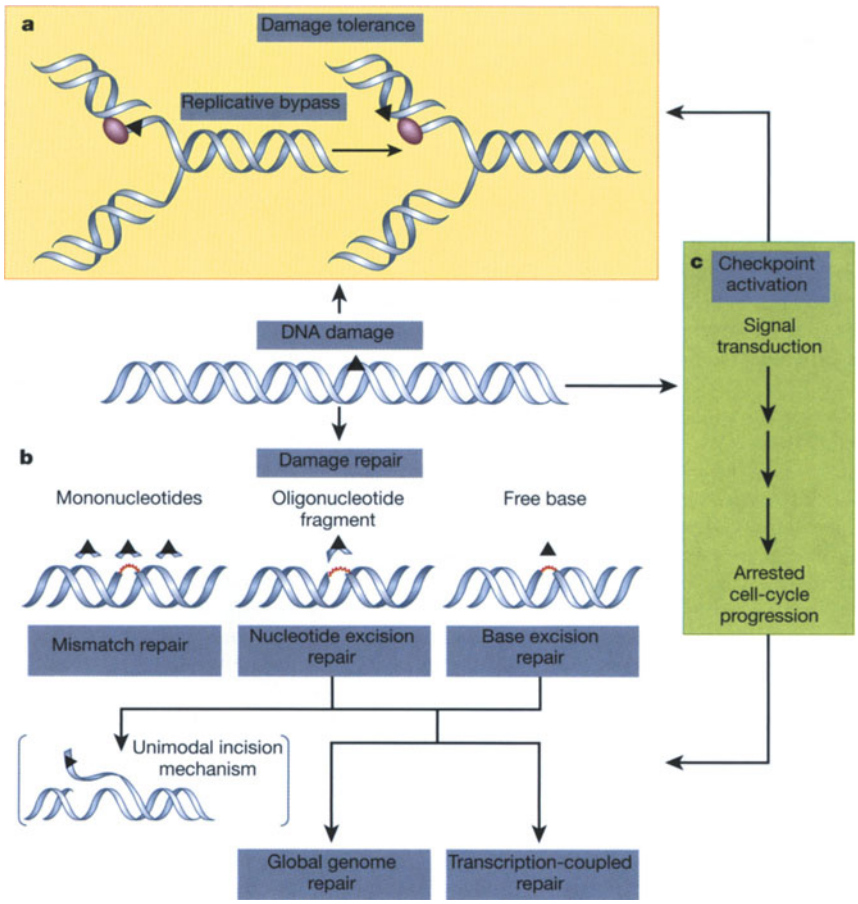

Figure 2 Responses to DNA damage. DNA damage (illustrated as a black triangle) results in either repair or tolerance. **a**, During damage tolerance, damaged sites are recognized by the replication machinery before they can be repaired, resulting in an arrest that can be relieved by replicative bypass (translesion DNA synthesis) (see Fig. 3). **b**, DNA repair involves the excision of bases and DNA synthesis (red wavy lines), which requires double-stranded DNA. Mispaired bases, usually generated by mistakes during DNA replication, are excised as single nucleotides during mismatch repair. A damaged base is excised as a single free base (base excision repair) or as an oligonucleotide fragment (nucleotide excision repair). Such fragments are generated by incisions flanking either side of the damaged base. Nucleotide excision repair can also transpire in some organisms by a distinct biochemical mechanism involving only a single incision next to a site of damage (unimodal incision). **c**, The cell has a network of complex signalling pathways that arrest the cell cycle and may ultimately lead to programmed cell death.

vation, whereby the DNA damage incurred by exposure to UV light is repaired by a light-dependent enzyme reaction[11] (Fig. 1).

Curiously, even with the elucidation of the structure of DNA only four years away, neither Dulbecco nor Watson — who was a graduate student in Luria's laboratory when Dulbecco stumbled on photoreactivation, and had himself examined the effects of ionizing radiation for his doctoral thesis[2] — thought about DNA repair. However, shortly after Watson and Crick reported on the DNA double helical structure, they noted the implications of the base-pairing rules for mutagenesis, stating "spontaneous mutation may be due to a base occasionally occurring in one of its less likely tautomeric forms"[12].

Tautomerism is the property of a compound that allows it to exist in two interconvertible chemical states; in the case of DNA bases, as either keto or enol forms. Watson and Crick had initially overlooked the complications of tautomerism and were trying unsuccessfully to construct their DNA model with the rare enol form of bases. It was only after Jerry Donohue, a former graduate student of Linus Pauling, pointed out to Watson that he should be using the more common keto form that the problem of how bases could stably pair was solved[13].

But no consideration was then given to the fact that the chemical lability of DNA implicit in tautomerism might have wider implications for the stability of genes. Indeed, the field gave little thought to the precise nature of DNA damage and its possible biological consequences. One must recall, however, that even at the time the DNA double helix was unveiled, its 'pathology' and the biological consequences thereof were far less compelling problems than deciphering the genetic code or understanding the essential features of DNA replication. Even mutagenesis — put to extensive use as a tool for determining the function of genes and their polypeptide products, and for defining the genetic code — was not widely considered in mechanistic terms until much later[11]. This is despite the fact that the repair phenomenon of photoreactivation was known before the discovery of the structure of DNA.

A DNA duplex for redundancy

Watson and Crick noted, with infamous prophetic understatement, "it has not escaped our notice that the specific [base] pairing we have postulated immediately suggests a possible copying mechanism for the genetic material"[14]. However, it was not intuitively obvious that a double-stranded molecule should be required for DNA replication. In principle, a single-stranded chain could just as easily do. But the significance of the duplex DNA structure soon became apparent. It was shown that DNA replicates in a semi-conservative fashion, whereby each strand of the double helix pairs with a new strand generated by replication. This enables errors introduced during DNA replication to be corrected by a mechanism known as excision repair, which relies on the redundancy inherent in having two complementary strands of the genetic code. If the nucleotides on one strand are damaged, they can be excised and the intact opposite strand used as a template to direct repair synthesis of DNA[15] (Fig. 2).

Many paths to mutation and repair

The elucidation of the DNA structure provided the essential foundation for defining the different types of mutations arising from both spontaneous and environmental DNA damage that affect all living cells[12]. Once again, the insights of physicists featured prominently[3], including among others, Richard Setlow who identified thymine dimers as stable and naturally occurring DNA lesions arising in cells exposed to sunlight (UV radiation). Such lesions comprise a covalent joining of two adjacent thymine residues in the same DNA chain. They generate considerable distortion of the normal structure of DNA and seriously impede DNA transactions such as replication and transcription. The repair of these lesions could be monitored

experimentally, and promoted the discovery by Setlow[16] and others[2] of excision repair in bacteria and higher organisms[17].

As the profusion of alterations in DNA became more widely recognized, scientists came to appreciate that the identification of any new type of naturally occurring base damage would, if one searched diligently enough, almost certainly lead to the discovery of one or more mechanisms for its repair or tolerance[2,18]. Such has indeed been the case. DNA repair now embraces not only the direct reversal of some types of damage (such as the enzymatic photoreactivation of thymine dimers), but also multiple distinct mechanisms for excising damaged bases, termed nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR)[11] (Fig 2). The principle of all three mechanisms of repair involves splicing out the damaged region and inserting new bases to fill the gap, followed by ligation of the pieces.

The process of NER is biochemically complicated, involving as many as 30 distinct proteins in human cells that function as a large complex called the nucleotide excision repairosome. This 'repair machine' facilitates the excision of damaged nucleotides by generating bimodal incisions in the flanking regions and removing a fragment about 30 nucleotides in length[11] (Fig. 2). Damaged bases that are not recognized by the NER machinery are corrected by BER, whereby the bases are excised from the genome as free bases by a different set of repair enzymes. In MMR, incorrect bases incorporated as a result of mistakes during DNA replication are excised as single nucleotides by yet a third group of repair proteins (Fig. 2). Both NER and BER transpire by somewhat different mechanisms depending on whether the DNA damage is located in regions of the genome that are undergoing active gene expression (transcription-coupled repair) or are transcriptionally silent (global genome repair)[11,19].

In addition to the various modes of excision repair that evolved to cope with damaged bases or mistakes during replication, cells frequently suffer breakage of one or both chains of the DNA duplex[11]. Naturally occurring reactive oxygen molecules and ionizing radiation are prevalent sources of such damage[11]. Strand breaks must be repaired in order to maintain genomic integrity. In particular, double-strand breaks (DSBs) sever the chromosomes and are lethal unless repaired[11].

Several mechanisms for the repair of DSBs have been elucidated (Fig. 3). One of these involves swapping equivalent regions of DNA between homologous chromosomes — a process called recombination[11]. This type of exchange occurs naturally during meiosis, the special type of cell division that generates the germ cells (sperm and ova). It can also be used to repair a damaged site on a DNA strand by using information located on the undamaged homologous chromosome. This process requires an extensive region of sequence homology between the damaged and template strands. Multiple proteins are required for DSB repair by recombination and deficiencies in this repair mechanism can cause cancer. For example, mutation of at least one of these repair proteins (called BRCA1) causes hereditary breast cancer. An alternative mechanism for the repair of DSBs, called non-homologous end joining, also requires a multi-protein complex, and essentially joins broken chromosome ends in a manner that does not depend on sequence homology and may not be error free (Fig. 3).

Damage tolerance

Although insights into DNA repair have progressed at an impressive pace, especially in the past decade, an understanding of the mechanisms of mutagenesis — a phenomenon that, as mentioned earlier, was demonstrated experimentally before discovery of the structure of DNA — has lagged. A breakthrough came from the experimental demonstration that some mutations arise as a consequence of a cell's efforts to tolerate damage. In this situation, the base damage and/or strand breaks in DNA persist in the genome, but their potential for interfering with DNA replication and transcription is somehow mitigated.

One such damage-tolerance mechanism, called translesion DNA synthesis, involves the replication machinery bypassing sites of base damage, allowing normal DNA replication and gene expression to proceed downstream of the (unrepaired) damage[20] (Fig. 4). It involves specialized low-fidelity ('sloppy') DNA polymerases that are able to bypass DNA lesions that typically stall the high-fidelity polymerases required for DNA replication. To overcome the block, these 'sloppy copiers' add nucleotides to the replicating strand opposing the DNA lesion, thus allowing replication to continue, but nevertheless introducing mutations into the newly synthesized sequence[20].

Cell suicide

Recent years have witnessed the recognition that biological responsiveness to genetic insult embraces more than the repair and

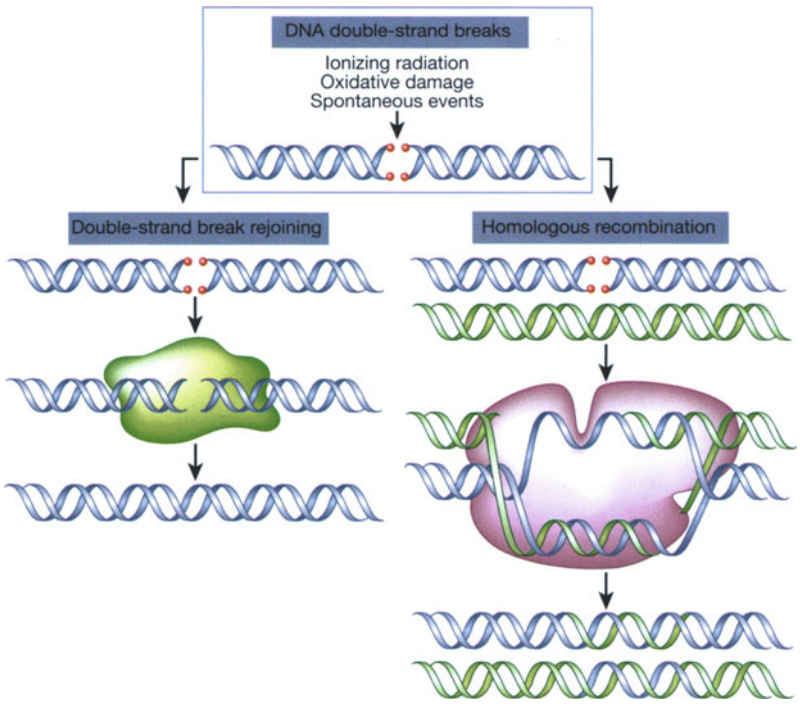

Figure 3 The repair of double-strand breaks in DNA. Double-strand breaks can result from exposure to ionizing radiation, oxidative damage and the spontaneous cleavage of the sugar-phosphate backbone of the DNA molecule. Their repair can be effected by either rejoining the broken ends (left) or by homologous recombination with a sister DNA molecule (right). Both processes involve different multi-protein complexes.

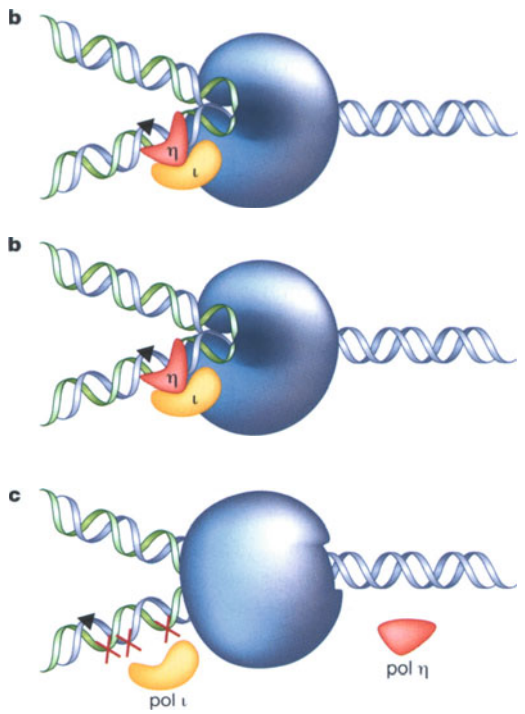

Figure 4 'Sloppy copiers' overcome blocks in replication caused by a DNA lesion (a process called translesion synthesis). **a**, The DNA replicative machinery (blue) stalls immediately behind a site of base damage (black triangle). Two specialized 'sloppy copier' polymerases (polη and polι) bind to the arrested replication complex. **b**, This interaction promotes a conformational change in the arrested replication machinery, placing polη in direct proximity to the site of base damage where it synthesizes across the lesion. **c**, Polη may then dissociate and allow polι to complete the process of replicative bypass by incorporating several more nucleotides (red crosses). Once the lesion has been completely bypassed, the replication machinery resumes DNA replication. As a result of this process, mutations to the DNA sequence are now incorporated into one strand.

tolerance of DNA damage. The exposure of cells to many DNA-damaging agents results in the transcriptional upregulation of a large number of genes, the precise function(s) of many of which remains to be established. Additionally, cells have evolved complex signalling pathways to arrest the progression of the cell cycle in the presence of DNA damage, thereby providing increased time for repair and tolerance mechanisms to operate[21] (Fig. 2c). Finally, when the burden of genomic insult is simply too large to be effectively met by the various responses discussed, cells are able to initiate programmed cell death (apoptosis), thereby eliminating themselves from a population that otherwise might suffer serious pathological consequences[22].

DNA damage and cancer

The 'somatic mutation hypothesis' of cancer embraces the notion that neoplastic transformation arises from mutations that alter the function of specific genes (now called oncogenes and tumour-suppressor genes) that are critical for cell division. This theory has its roots in correlations between chromosomal abnormalities and cancer first observed by the developmental biologist Theodore Boveri[23], who at the beginning of the twentieth century reported abnormal numbers of chromosomes (aneuploidy) in cancerous somatic cells.

The discovery of the structure of DNA progressed our understanding of tumorigenesis at several levels. Watson and Crick predicted from their DNA model that complementary base pairing had implications for recombination (the exchange of genetic material between chromosome pairs): "the pairing between homologous chromosomes at meiosis may depend on pairing between specific bases". The genetic basis of many cancers is now known to arise from abnormal recombination events, such as chromosomal translocations, where a region of one chromosome is juxtaposed to another chromosome. Watson himself developed an early and ardent interest in cancer biology when he recognized that the experimentally tractable genomes of oncogenic viruses could provide important insights into the pathogenesis of cancer. Mutagenesis is now documented as a fundamental cornerstone of the molecular basis of all forms of cancer[24].

Arguably the most definitive validation of the somatic mutation hypothesis derives from the discovery that defective responses to DNA damage and the accumulation of mutations underlies two distinct types of hereditary cancer; skin cancer associated with defective NER and colorectal cancer associated with defective MMR[11]. In both instances, credit belongs to scholars of DNA repair.

In the late 1960s, James Cleaver providentially noted an article in the *San Francisco Chronicle* that reported the extreme proneness to skin cancer in individuals with XP, a rare sun-sensitive hereditary disease[2]. Cleaver was then searching for mammalian cell lines that were defective in excision repair, and his intuitive notion that XP individuals might be sunlight-sensitive and prone to cancer because they were genetically defective in excision repair proved to be correct[25].

The subsequent elucidation of the genes defective in XP patients[26], and their role in NER of damaged bases in human cells[11,27,28], represents a triumph of modern genetics and its application to molecular biology. The additional discovery that the process of NER in eukaryotes requires elements of the basic transcription apparatus[11] has yielded insights into the complex relationships between deficient DNA repair, defective transcription and hereditary human diseases[11].

A fascinating denouement to the skin-cancer predisposition in XP patients derives from the recent solution of the 'XP variant problem'. A significant fraction of XP individuals who are clinically indistinguishable from those defective in NER were found to be proficient in this repair process[11]. It was shown that DNA polymerase-η (polη), one of the specialized DNA polymerases capable of overcoming replication blocks at DNA lesions, is mutated in all XP-variant patients so far examined[29]. Not only does polη replicate past thymine dimers in DNA, but — unlike the other specialized DNA polymerases — it also correctly inserts adenine residues[29], thereby preventing mutations at sites of thymine dimers. Therefore, even in XP patients with functional NER, in the absence of polη one or more other bypass polymerases attempts to cope with arrested replication at thymine dimers, but does so inaccurately[29]. Thus, cancer predisposition in XP essentially derives from an excessive mutational burden in skin cells associated with exposure to sunlight. These mutations accumulate either because thymine dimers are not excised (owing to defective NER) or because in the absence of polη, dimers are inaccurately bypassed by other DNA polymerases[29].

The association between HNPCC and defective MMR was determined more-or-less simultaneously by a number of investigators. Paul Modrich[2] surmised that the instability of repeated sequences in DNA associated with defective MMR in bacteria[30] might be causally related to the DNA sequence instability observed in patients with HNPCC[31]. This led to the formal demonstration of defective MMR in this human hereditary disease and formed another persuasive validation of the somatic mutation theory of cancer[32–34].

A look to the future

The study of biological responsiveness to DNA damage embraces DNA repair, mutagenesis, damage tolerance, cell-cycle checkpoint control, programmed cell death, and other cellular responses to

genomic insult. This integrated field is now deciphering the complex regulatory pathways transduced by signalling mechanisms that detect DNA damage and/or arrested DNA replication. As these pathways become better understood, parallel technological gains in gene therapy and therapeutic intervention by rational drug design will offer new strategies for blocking the unwanted consequences of DNA damage, especially cancer.

We must remember, however, that while evolution could not have transpired without robust cellular mechanisms to ameliorate the most serious consequences of spontaneous and environmental DNA damage, the process of evolution mandates that the genetic diversification on which Darwinian selection operates be maintained constantly. Thus, life is necessarily a delicate balance between genomic stability and instability — and of mutation and repair. □

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The double helix and immunology

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The immune system can recognize and produce antibodies to virtually any molecule in the Universe. This enormous diversity arises from the ingenious reshuffling of DNA sequences encoding components of the immune system. Immunology is an example of a field completely transformed during the past 50 years by the discovery of the structure of DNA and the emergence of DNA technologies that followed.

"This short history of research in one area, lymphocyte receptors, is yet another witness to the power of DNA technology, and to the ability of this approach not only to explain known biological phenomena, but also to contribute to the discovery of new biological systems."
Susumu Tonegawa, Nobel lecture, 8 December 1987.

The double helix is all about biological information: how it is encoded, stored, replicated and used when required. Immunology, too, is about information. What genetic processes control the vast array of synthetic potential within an immune system capable of reacting specifically to virtually any microbe or foreign molecule? The secret lies in unique DNA processing that occurs during the development of lymphocyte cells, which are responsible for the specific immune response to a foreign agent (antigen). B lymphocytes produce antibodies (on their surface as well as secreted) and T lymphocytes mount cellular attacks on pathogenic infiltrators.

As lymphocytes develop, an array of short genes are rearranged and assembled together at the DNA level to form genes whose products recognize distinct antigens. As the process is mostly random, each lymphocyte makes different choices and thus the result is a vast repertoire of lymphocytes reactive to different antigens. This process has implications for antibody formation, cell-mediated immunity and malignancies of the immune system.

One B cell produces one antibody

At the beginning of the last century, Paul Ehrlich[1] recognized that the specificity of antibodies lay in the complementarity of their shapes to the antigen(s) on the microbe being recognized. He saw antibodies as cellular 'side chains', which budded out from the cell surface as what today we would term receptors. Karl Landsteiner[2] then demonstrated the exquisite specificity of antibodies, showing that animals could make antibodies to almost anything, including small synthetic organic molecules that had never previously existed in nature. Moreover, tiny structural changes in the antigen could lead to the production of a different antibody. It beggared belief that there could be so many different side chains. When antibodies were shown to be proteins, it seemed natural to conclude that a specific antibody molecule was shaped in close proximity to an antigen molecule much as plastic or sheet metal is moulded against a template. This 'direct template' hypothesis[3] held sway for several decades.

In 1955, Niels Jerne[4] published his natural selection theory of antibody formation, which postulated the random synthesis of a million or more different sorts of antibodies. When an antigen enters the body, it unites with an antibody that just happens to fit it, the antigen–antibody complex is taken up by a cell and the antibody

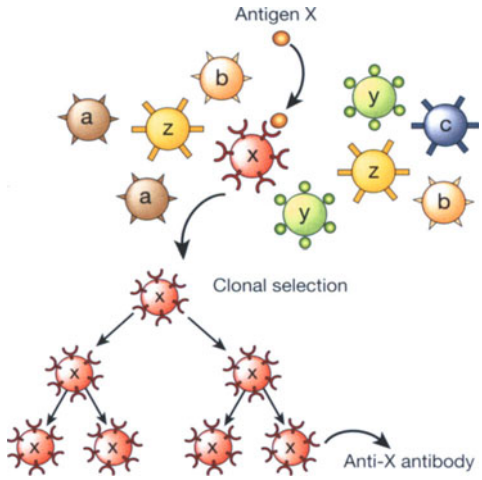

Figure 1 The clonal selection theory of antibody formation. Each B lymphocyte produces only one type of antibody receptor on its surface. An antigen recognizes one B lymphocyte out of a large repertoire. This triggers the rapid division and differentiation of the B cell to become a 'plasma cell', producing and secreting antibodies specific to the original antigen.

somehow acts as the template for the formation of more of itself. David Talmage[5] and Macfarlane Burnet[6] recognized that this theory would make more sense if the postulated natural antibodies were located on the surface of what we now call B lymphocyte cells. If each cell were endowed with only one sort of antibody specificity, then the antigen could select one lymphocyte out of a repertoire, cause its clonal division and stimulate antibody production and secretion (Fig. 1). In 1958, Joshua Lederberg[7] and I provided the first evidence for the clonal selection theory, namely that one B cell always produces only one antibody.

DNA shuffling in antibody formation

Antibodies are multichain proteins that come in different forms. The most abundant, immunoglobulin-γ (IgG), consists of two identical light (L) chains and two identical heavy (H) chains[8] (Fig. 2a). The carboxy-terminal halves of the two light chains are identical to each other, but the amino-terminal halves differ in more than 50 residues, called the 'variable' region'[9]. The heavy chains, too, consist of a variable (V) part and a constant (C) part.

In 1965, William J. Dreyer and J. Claude Bennett[10] bucked the dogma at the time that 'one gene makes one protein', and put forward the revolutionary concept that the carboxy-terminal C region of the L chain was always encoded by a single gene, but that the amino-terminal V half could be encoded by multiple separate genes, perhaps as many as 100,000 in number. It followed that a chosen V gene must then somehow become associated with a C gene by a DNA rearrangement event in each lymphocyte cell, because only when the V–C regions were spliced together could a functional protein be expressed.

At that time, there was no way to interrogate the genome directly to test this concept, and for a decade debate and controversy raged. One school, led by Leroy Hood, favoured the idea that a large array of germline-encoded V genes for the L and H chains underwent rearrangement. At the other extreme were proponents of a single, very highly mutable V gene that was extensively mutated in emerging B cells. In the middle were those who favoured the idea of a handful of V genes that were subject to extensive recombination in somatic cells (the cells of the body, excluding the sex cells). This compromise was supported by luminaries such as Oliver Smithies and Gerald Edelman. Francis Crick was quite taken with the idea that just two V genes undergo rearrangement in the germ line, with further mutation in somatic cells.

Before arriving at the solution brought by advances in molecular biology, one more fact is worthy of note. Elvin Kabat astutely pointed out that in the V regions of both heavy and light chains there were also three short stretches of amino acids where variation was considerably greater than elsewhere in the molecules, and these so-called hypervariable regions were deemed likely to be sites of union with the antigen[11]. Could it be that there was actually an assembly of several, rather than just two, genes encoding each chain?

The new tools for manipulating and sequencing DNA came to the rescue. In 1976, Nobumichi Hozumi and Susumu Tonegawa[12] conducted a landmark experiment. They used a DNA cutting enzyme known as a restriction endonuclease to digest the DNA extracted from a mouse embryo and from an antibody-secreting tumour. The resulting DNA fragments were then separated on the basis of size, and

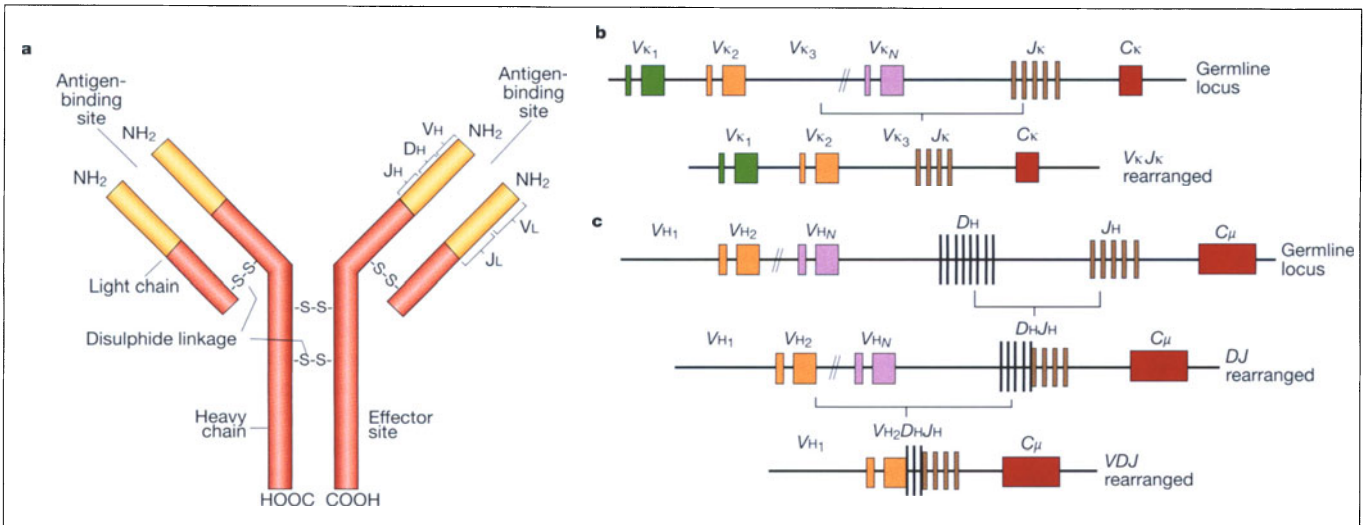

Figure 2 Antibody formation. **a**, Structure of an antibody (immunoglobulin). Two identical heavy chains are connected by disulphide linkages. The antigen-recognizing site is composed of the variable regions (yellow) of the heavy and light chains, whereas the effector site (which determines its function) is determined by the amino-acid sequence of the heavy chain constant region (red). **b**, Assembly of the light (κ)- and heavy (H)-chain genes of antibodies by somatic recombination during B-lymphocyte development. The L chain is encoded by variable (V), joining (J) and constant (C) genes. While the developing B cell is still maturing in the bone marrow, one of the 30–40 V genes combine with one of the five J genes and is juxtaposed to a C gene. The recombining process involves deletion of the intervening DNA between the selected genes. **c**, The H chain is encoded by V, D, J and C genes. The assembly of the H chain gene occurs in two stages: one of the D genes joins with a J gene, then one of the V genes joins with that DJ assembly.

were reacted with radioactive probes, one corresponding to the whole L chain, the other to just the C portion. In the tumour, both probes lit up the identical fragment, whereas in the embryonic extract, two different fragments hybridized to the full-length probe, but only one of them to the C-region probe. Both fragments from the embryo sample were different in size from the single hybridizing fragment in the tumour sample. The experiment strongly argued for the V and C genes being some distance from each other in the embryo, but having been rearranged and assembled during development of antibody-forming cells in adults to form a continuous DNA sequence constituting the full L-chain gene.

Generating more diversity

Definitive elucidation of immunoglobulin gene structure depended on molecular cloning and subsequent sequencing of the genes themselves[13]. Here came another surprise, and one that could really not have been anticipated. V-region genes in the germline were found to be significantly shorter than is required to code for the V region of the L chain. It turned out that there is a series of 'minigenes' known as 'joining' (J) genes, which code for about 13 amino acids of the L chain. Thus, the full L chain is actually encoded by V, J and C genes (Fig. 2b). For the H chain, it is still more complicated, as there exists a series of 'diversity' (D) genes that encode up to eight amino acids that lie between the V and J regions. Thus, the H chain is encoded by V, D, J and C genes (Fig. 2c). The assembly of a complete H-chain V region occurs in two separate steps: first, one of the D regions joins with one of the J regions, then one of many V regions joins with that DJ assembly (Fig.2c). The joining process is followed by deletion of the intervening DNA between the chosen minigenes. This is the first example of a somatic cell possessing a different genome from its fellow cells.

This minigene assembly process has important implications for antibody diversity. In humans, here are two types of L chains, κ and λ, each with its own sets of V and J genes. For the κ light chain, there are 40 functional V genes and 5 functional J genes; for the λ chain, there are 31 and 4, respectively. There is only one kind of variable region for the H chain, encoded by 51 V genes, 25 D genes and 6 J genes. To a first approximation, therefore, there are $(40 \times 5) + (31 \times 4) = 324$ different possible assemblies of L chains, and $51 \times 25 \times 6 = 7{,}650$ combinations for H chains. Thus, together, there are potentially 2,478,600 different types of germline-encoded antibodies.

But this is a considerable underestimate for two reasons. Recombination junctions can occur at different positions and this junctional diversity increases variability. Furthermore, a few extra nucleotides, called N regions, can be inserted between D–J junctions and V–D junctions in many H chains, and in a smaller percentage of L-chain V–J junctions. These nucleotides are not present in the germline and add to antibody diversity.

Yet further diversity can be generated by DNA mutations in dividing B cells. B cells expressing newly assembled immunoglobulin genes, each with its own unique specificity, constitute the 'primary repertoire'. When an antigen stimulates a chosen B cell to divide (Fig. 1), a proportion of the progeny migrate into the vicinity of antigen-capturing follicular dendritic cells (FDCs) and gradually form a 'germinal centre'. FDCs retain antigen on their surface for long periods and stimulate further rounds of division. Within the germinal centre the B cells display an extraordinarily high rate of somatic mutation in V genes, estimated at 10^{-3} per nucleotide per division[14]. As antibody production accumulates, only those B cells with heightened affinity for the antigen gain access to FDC-bound antigen and thus are further stimulated to divide. As a result, B-cell clones secreting higher affinity antibody are selected in an iterative manner.

The 'memory' B cells that emerge from the germinal centre constitute the 'secondary repertoire', which is even more diverse than the primary one. Twenty mutations per chain are not uncommon; nor are thousand-fold increases in affinity. Thus, as it turned out, two early theories of antibody diversification proved to be correct: rearrangement of germline genes gives the naive B-cell repertoire, and somatic mutation ensures further diversification during memory B-cell development.

Box 1
Tolerating self

How does the immune system distinguish foreign molecules from the cells of its own body? The failure to form antibodies against self components confounded scientists until Macfarlane Burnet proposed that an antigen introduced during embryonic development would 'trick' the immune system into regarding it as self, so that even in later life it could not react against that antigen[22]. Peter Medawar's group proved the prediction correct by inducing immunological tolerance to transplantation antigens. Injecting foreign cells from a donor into late-stage embryo mice within the uterus led to a situation where, when these mice had grown up, they could not reject skin grafts from the donor strain[23].

What happens if the antibody generated by a newly created B cell is reactive with a self component? The cell either dies immediately, if the stimulus is one capable of strongly cross-linking the cell-surface immunoglobulin receptors, or else is given a negative signal which impedes its full function[24]. This negative selection contributes to immunological tolerance.

For a T cell, this is more of a juggling act. Although it must recognize the self-molecule major histocompatibility complex (MHC), it must avoid mounting an abnormal immune response to the host's own cells. For the T-cell repertoire to be fashioned correctly within the thymus, two sorts of selection are required. If the T cell, having assembled its receptor genes, has the capacity to recognize self MHC, it is allowed to mature. This step is known as positive selection. But the T cell must not leave the thymus if it has strong potential to cause autoimmunity. This would be the case if there were too high an affinity of binding with self MHC, or with a peptide from a normal cellular component bound to the MHC groove. Such cells undergo programmed cell death within the thymus, the process of negative selection.

Defects in the immune system's ability to recognize self are the basis of many autoimmune diseases, such as juvenile diabetes and multiple sclerosis.

Switching function

There are several different classes of antibodies, all of which have distinct roles that are also produced by rearrangements at the DNA level. There are eight different genes for the C region of the H chain, which specify different antibody functions. Each B cell first links the chosen VDJ assembly to a C gene known as μ, creating an antibody class called IgM. If that cell is propelled into a pathway favouring the production of an antibody prominent in mucus secretions (such as in the gastrointestinal tract), the VDJ section is switched over to a C region encoded by C gene α, and the cell produces IgA. If, on the other hand, the antigen is of parasite origin, or an allergen such as a pollen grain, the cell may be stimulated to produce IgE, in which case the VDJ region associates with the product of the C gene ε. All of this occurs without any change in the specificity of the antibody being secreted. Although the detailed molecular mechanisms are still being investigated, the class switching again involves sequential excision of portions of the genetic material. Cytidine deaminase induced by B-cell-specific activation may be significant in both class switching and somatic hypermutation.

Assembly of T-cell receptors

Whereas B cells make antibodies against antigens, the thymus-derived or T cells also respond to foreign agents, specializing in a more localized form of combat. Cytotoxic T cells are capable of killing virus-infected cells or cells displaying cancer-specific antigens. Other

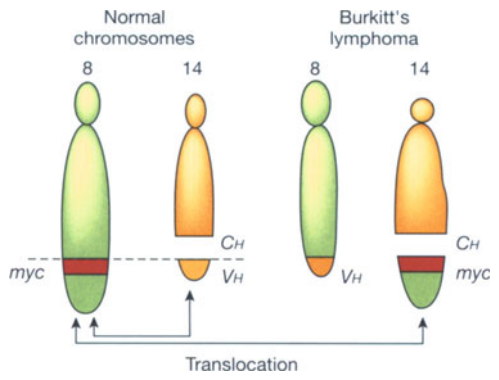

Figure 3 Reciprocal chromosomal translocations in Burkitt's lymphoma, a solid tumour of B lymphocytes. The genes for making the heavy chains of antibodies (C_H) are located on chromosomes 14, whereas those for making the light chains are on chromosomes 2 and 22. These genes are expressed exclusively in B lymphocytes, because only these cells have the necessary transcription factors to switch on their expression. In most (over 90%) of Burkitt's lymphoma cases, a reciprocal translocation moves the proto-oncogene c-*myc* from its normal position on chromosome 8 to a location close to the antibody heavy-chain genes on chromosome 14 (ref. 18). In other cases, c-*myc* is translocated close to the antibody genes on chromosome 2 or 22. In every case, c-*myc* now finds itself in a region of active gene transcription, and it may simply be the overproduction of the c-*myc* product (a transcription factor essential for cell division) that propels the lymphocyte down the pathway towards cancer.

T cells secrete powerful stimulatory and inflammatory molecules, most of which act in a strictly localized context. T cells can also help guide B cells down appropriate pathways of differentiation.

In common with B cells, T cells also have one-receptor specificity and, for simplicity, I shall mention only the αβ T-cell receptor (TCR), a heterodimer consisting of two subunits, the α- and β-chains, joined by disulphide bonds[15,16]. The strategy for generating T cells with different receptors is strikingly similar to that used by B cells to produce different antibodies and, in fact, the TCR binding surface looks much like that of an antibody. The β-chain of the TCR is assembled in somatic cells from V, D, J and C genes; the α-chain from V, J and C genes. There are additions of N-region nucleotides between V and D, as well as between D and J on the β-chain; and between V and J on the α-chain. A similar rearrangement also takes place for the γδ TCR.

But something peculiar about T-cell recognition was noted by Rolf Zinkernagel and Peter Doherty[17], who demonstrated that cytotoxic T cells could recognize viral antigens only if a specific 'self' molecule were also present on the target cell (see Box 1). The key part of the T-cell recognition puzzle fell into place when it was discovered that the TCR recognized short antigenic peptides bound to the groove of a self molecule known as the major histocompatibility complex (MHC), as well as surrounding portions of the MHC molecule itself. Cells have special mechanisms for fragmenting proteins into peptides of 8–24 amino acids in length, attaching these to MHC molecules and transporting the entire complex to the cell surface. TCRs then 'see' these short linear portions of antigens, be these of viral, bacterial or parasitic origin, or even portions of normal intracellular components. Such a system can help to control infections where the pathogen goes 'underground' inside a cell, and can also eliminate cells with mutated self antigens, such as cancer cells.

Lymphocytes and cancer

Lymphocytes have been a favourite tool in cancer research. A notable example of DNA science applied in this way relates to the B-cell tumour of humans known as Burkitt's lymphoma. Occasionally DNA strands break and are incorrectly repaired. Thus, a piece of a chromosome becomes attached to the broken end of another one, and vice versa, in a process known as reciprocal translocation (Fig. 3). In the case of Burkitt's lymphoma, a tumour-promoting gene or oncogene called *myc* is translocated from its normal position on chromosome 8 right into the middle of the IgH chain locus on chromosome 14 (ref. 18). In this highly active transcriptional environment, *myc* expression is switched on, and eventually cancer develops.

It has been possible to create lymphoma-prone transgenic mice, which express *myc* in aberrantly high amounts. Because cancer is typically a multistage process, if further oncogenes are expressed simultaneously in transgenic mice, the onset of cancer can be dramatically accelerated. One such example involves the gene *bcl-2*. When this gene is expressed, it stops cells from undergoing natural programmed death (apoptosis)[19]. Mice expressing *myc* and *bcl-2* showed very rapid development of tumours. An enormous amount of literature has accumulated related to the expanding family of *bcl-2*-related genes and their roles in the regulation of programmed cell death. Models derived from lymphocytes and their malignancies have led to insights with implications well beyond immunology.

DNA vaccines

DNA research has been of immense value to vaccine research. Through gene cloning and expression, candidate antigens can be identified and tested. In an era of rapid nucleotide sequencing, the whole genome of a pathogen can be determined, and computer programs can search for sequences likely to encode outer membrane proteins, which can be assessed as candidate vaccine molecules (see, for example, the series of papers published recently in *Nature* (**419,** 489–542, 2002) on the genomics of the malaria parasite).

Amazingly, DNA itself can serve as a vaccine. DNA vaccines work on the principle that the gene sequence for one or more candidate antigens is introduced into an animal or person via a delivery vehicle known as a vector, together with a strong promoter that can switch on its expression in mammalian cells. Cells that take up the injected DNA transcribe and translate the gene and release the relevant antigen protein, which the body can in turn manufacture antibodies against. Thus, the body itself becomes a vaccine factory[20]. Unfortunately, so far this approach has worked better in mice than in humans, but many avenues are being pursued to improve this situation.

To strengthen the immune response to a vaccine, it may be necessary to use an adjuvant substance. Here, DNA may also be of potential use. Scavenger cells, which capture antigens, have evolutionarily conserved receptors, known as Toll-like receptors (TLRs), which recognize antigens common to many pathogens. One such receptor is TLR-9, which recognizes unmethylated CpG motifs commonly found in bacterial but not mammalian DNA. Accordingly, unmethylated CpG-rich DNA sequences represent a promising new category of adjuvant[21].

Future directions

The solutions to the puzzle of antibody diversity and mystery of T-cell recognition of antigenic peptides are among the brightest chapters of biology in the last quarter of the twentieth century. The future of immunology will be all about how the system is regulated and how it makes decisions: whether to respond or not; whether to direct efforts towards antibody formation or cell-mediated immunity; and, if the latter, whether more towards cytokine-secreting T cells or cytotoxic T cells.

As in the past, the future will be about information and thus about DNA science. All the complex signalling pathways, the feedback loops, and the intricate rules governing cell division on the one hand or programmed cell death on the other, will be progressively revealed. As this happens, the possibilities for applied research and development will be immense. In particular, new therapeutic targets will be identified. The 'miracle' drug for chronic myelogenous leukaemia, Glivec, was made possible after the characterization of the extraordinary cancerous potential of the chimaeric oncogene *bcr–abl*. This will surely be only the first of a plethora of more intelligently designed

anti-cancer drugs. Potent cytokines and monoclonal antibodies directed against cell surface-associated structures are already prominent within a radically revised pharmaceutical armamentarium in areas including cancer, autoimmunity, allergy and transplantation. DNA research is therefore crucial to a new generation of immunologists, from those striving towards the development of novel vaccines to those seeking to understand and control autoimmune diseases, allergy and transplant tolerance. □

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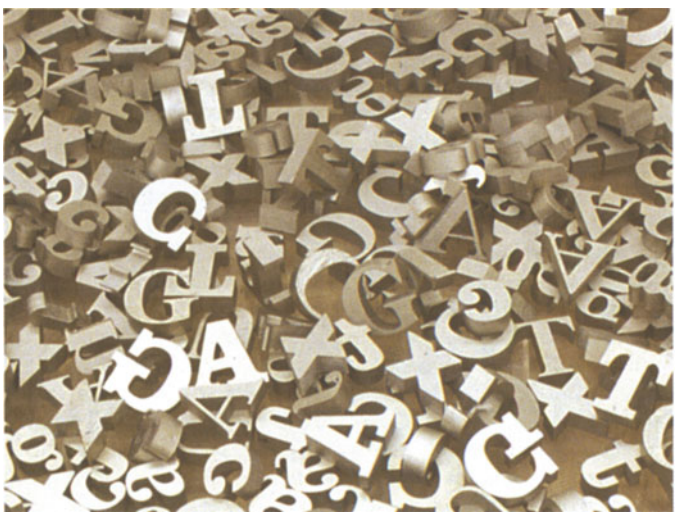

DIFFERENCE AND REPETITION (DETAIL) BY SUZANNE ANKER (2000)
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The digital code of DNA

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The discovery of the structure of DNA transformed biology profoundly, catalysing the sequencing of the human genome and engendering a new view of biology as an information science. Two features of DNA structure account for much of its remarkable impact on science: its digital nature and its complementarity, whereby one strand of the helix binds perfectly with its partner. DNA has two types of digital information — the genes that encode proteins, which are the molecular machines of life, and the gene regulatory networks that specify the behaviour of the genes.

"Any living cell carries with it the experiences of a billion years of experimentation by its ancestors." Max Delbruck, 1949.

The discovery of the double helix in 1953 immediately raised questions about how biological information is encoded in DNA[1]. A remarkable feature of the structure is that DNA can accommodate almost any sequence of base pairs — any combination of the bases adenine (A), cytosine (C), guanine (G) and thymine (T) — and, hence any digital message or information. During the following decade it was discovered that each gene encodes a complementary RNA transcript, called messenger RNA (mRNA)[2], made up of A, C, G and uracil (U), instead of T. The four bases of the DNA and RNA alphabets are related to the 20 amino acids of the protein alphabet by a triplet code — each three letters (or 'codons') in a gene encodes one amino acid[3]. For example, AGT encodes the amino acid serine. The dictionary of DNA letters that make up the amino acids is called the genetic code[4]. There are 64 different triplets or codons, 61 of which encode an amino acid (different triplets can encode the same amino acid), and three of which are used for 'punctuation' in that they signal the termination of the growing protein chain.

The molecular complementary of the double helix — whereby each base on one strand of DNA pairs with its complementary base on the partner strand (A with T, and C with G) — has profound implications for biology. As implied by James Watson and Francis Crick in their landmark paper[1], base pairing suggests a template-copying mechanism that accounts for the fidelity in copying of genetic material during DNA replication (see accompanying article by Alberts, page 117). It also underpins the synthesis of mRNA from the DNA template, as well as processes of repairing damaged DNA (discussed by Friedberg, page 122).

Tools to modify DNA

The enzymes that function in cells to copy, cut and join DNA molecules were also exploited as key tools for revolutionary new techniques in molecular biology, including the cloning of genes and expression of their proteins, and mapping the location of genes on chromosomes. The ability to recreate the process of DNA replication artificially in the laboratory led to the development of two techniques

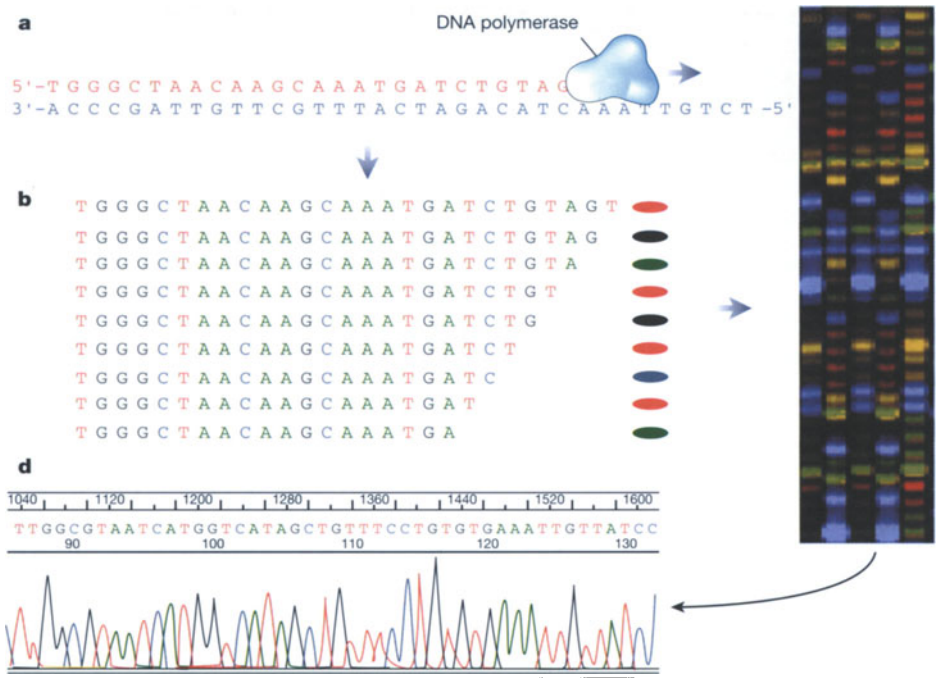

Figure 1 How to sequence DNA. **a**, DNA polymerase copies a strand of DNA. **b**, The insertion of a terminator base into the growing strand halts the copying process. This is a random event that results in a series of fragments of different lengths, depending on the base at which the copying stopped. The fragments are separated by size by running them through a gel matrix, with the shortest fragments at the bottom and largest at the top. **c**, The terminators are labelled with different fluorescent dyes, so each fragment will fluoresce a particular colour depending on whether it ends with an A, C, G, or T base. **d**, The sequence is 'read' by a computer. It generates a 'sequence trace', as shown here, with the coloured peaks corresponding to fluorescent bands read from the bottom to the top of one lane of the gel. The computer translates these fluorescent signals to DNA sequence, as illustrated across the top of the plot. Image adapted from ref. 20.

that transformed biology: a manual DNA sequencing method in 1975 and, in 1985, the discovery of the polymerase chain reaction (PCR), whereby DNA sequences could be amplified a millionfold or more[5].

Although sequencing and PCR transformed the science of biology, they also had wide applications for medicine and forensics. The detection of variation in DNA sequence from one individual to the next — so-called 'polymorphisms' — forms the basis of DNA 'finger-printing' of individuals. Forensics uses these fingerprints to deal with paternity disputes, as well as criminal cases such as rape. The finding that many specific DNA polymorphisms are associated with disease or disease susceptibility has brought DNA diagnostics to medicine and opened the pathway to truly predictive medicine, where the risks of disease can be identified in advance of symptoms (see accompanying article by Bell, page 100).

Automated DNA sequencing

The first efforts to sequence DNA, pioneered by Walter Gilbert[6] and Fred Sanger[7] in the 1970s, decoded stretches of DNA a few hundred bases long. When the first complete genome was sequenced over a period of about one year in 1977–78 — that of a viral genome of about 5,000 bases[8] — it became clear that DNA sequence data could provide unique insights into the structure and function of genes, as well as genome organization. It was this potential to generate vast amounts of information about an organism from its genetic code that inspired efforts towards the automation of DNA sequencing (Fig. 1).

The combination of technical wizardry and intensive automation in the decade that followed launched the 'genomic era'. A series of new instruments enabled novel approaches to biological analysis[9–11]. The first sequencing machine — invented by Leroy Hood, Lloyd Smith and Mike Hunkapiller in 1986 (ref. 12) — was automated in data acquisition, but still required substantial manual attention and the sequencing rate was low, roughly 250 bases per day. Over the next ten years, the development of automated DNA sequencing accelerated, rapidly passing through three distinct stages: the prototype sequencing machine (1986); a robust instrument that could be used routinely in a standard laboratory (1989); and finally, a machine that formed part of an integrated factory-like production line where DNA sample preparation and sequencing were all fully automated (1998). The advances in sequencing capacity have been striking — the latest sequencing machines are able to decode approximately 1.5 million bases over 24 hours — 6,000 times the throughput of the prototype.

The goals of high-throughput biological instrumentation are to increase throughput, enhance the quality of the data, and greatly reduce the cost of per unit information acquired. To reach these goals in the future, the miniaturization, automation, parallelization and integration of successive procedures will propel DNA sequencing technology into the realm of microfluidics and microelectronics, and eventually into the area of nanotechnology. With single-DNA-molecule sequencing, we foresee a time when the entire genome of an individual could be sequenced in a single day at a cost of less than $US10,000 (compared with the US$50 million or more it would cost today). This will readily enable the decoding of the genomic sequence of virtually any organism on the planet and provide unparalleled access to the foundations of biology and the study of human genetic variability.

The Human Genome Project

The breathtaking speed at which automated DNA sequencing developed was largely stimulated by the throughput demands of the Human Genome Project (HGP), which officially started in 1990 following discussions and studies on feasibility and technology that began in earnest in 1985. The objectives of the HGP were to generate a finished sequence in 15 years[13], but a draft of the human genome sequence was available in 2001. Two versions of the draft were generated and published in 2001, one by the publicly funded International Human Genome Sequencing Consortium[14], and another by the biotechnology company Celera[15] (Box 1). In the process of developing the tools and methodology to be able to sequence and assemble the 3 billion bases of the human genome, a range of plant, animal and microbial genomes was sequenced and many more are currently being decoded. As genome sequences become available, different areas of biology are being transformed — for example, the discipline of microbiology has changed significantly with the completion of more than 100 bacterial genome sequences over the past decade.

The HGP profoundly influenced biology in two respects. First, it illustrated the concept of 'discovery science' — the idea that all the elements of the system (that is, the complete genome sequence and

the entire RNA and protein output encoded by the genome) can be defined, archived in a database, and made available to facilitate hypothesis-driven science and global analyses. Second, to succeed, the HGP pushed the development of efficient large-scale DNA sequencing and, simultaneously, drove the creation of high-throughput tools (for example, DNA arrays and mass spectrometry) for the analysis of other types of related biological information, such as mRNAs, proteins and molecular interactions.

The digital nature of biological information

The value of having an entire genome sequence is that one can initiate the study of a biological system with a precisely definable digital core of information for that organism — a fully delineated genetic source code. The challenge, then, is in deciphering what information is encoded within the digital code. The genome encodes two main types of digital information — the genes that encode the protein and RNA molecular machines of life, and the regulatory networks that specify how these genes are expressed in time, space and amplitude.

It is the evolution of the regulatory networks and not the genes themselves that play the critical role in making organisms different from one another. The digital information in genomes operates across three diverse time spans: evolution (tens to millions of years), development (hours to tens of years), and physiology (milliseconds to weeks). Development is the elaboration of an organism from a single cell (the fertilized egg) to an adult (for humans this is 10^{14} cells of thousands of different types). Physiology is the triggering of specific functional programmes (for example, the immune response) by environmental cues. Regulatory networks are crucial in each of these aspects of biology.

Regulatory networks are composed of two main types of components: transcription factors and the DNA sites to which they bind in the control regions of genes, such as promoters, enhancers and silencers. The control regions of individual genes serve as information processors to integrate the information inherent in the concentrations of different transcription factors into signals that mediate gene expression. The collection of the transcription factors and their cognate DNA-binding sites in the control regions of genes that carry out a particular developmental or physiological function constitute these regulatory networks (Fig. 2).

Because most 'higher' organisms or eukaryotes (organisms that contain their DNA in a cellular compartment called the nucleus), such as yeast, flies and humans, have predominantly the same families of genes, it is the reorganization of DNA-binding sites in the control regions of genes that mediate the changes in the developmental programmes that distinguish one species from another. Thus, the regulatory networks are uniquely specified by their DNA-binding sites and, accordingly, are basically digital in nature.

One thing that is striking about digital regulatory networks is that they can change significantly in short periods of evolutionary time. This is reflected, for example, in the huge diversity of the body plans, controlled by gene regulatory networks, that emerged over perhaps 10–30 million years during the Cambrian explosion of metazoan organisms (about 550 million years ago). Likewise, remarkable changes occurred to the regulatory networks driving the development of the human brain during its divergence from its common ancestor with chimpanzees about 6 million years ago.

Biology has evolved several different types of informational hierarchies. First, a regulatory hierarchy is a gene network that defines the relationships of a set of transcription factors, their DNA-binding sites and the downstream peripheral genes that collectively control a particular aspect of development. A model of development in the sea urchin represents a striking example[16] (Fig. 2). Second, an evolutionary hierarchy defines an order set of relationships, arising from DNA duplication. For example, a single gene may be duplicated to generate a multi-gene family, and a multi-gene family may be duplicated to create a supergene family. Third, molecular machines may be assembled into structural hierarchies by an ordered assembly process. One example of this is the basic transcription apparatus that involves the step-by-step recruitment of factors and enzymes that will ultimately drive the specific expression of a given gene. A second example is provided by the ribosome, the complex that translates RNA into protein, which is assembled from more than 50 different proteins and a few RNA molecules. Finally, an informational hierarchy depicts the flow of information from a gene to environment: gene → RNA → protein → protein interactions → protein complexes → networks of protein complexes in a cell → tissues or organs → individual organisms → populations → ecosystems. At each successively higher level in the informational hierarchy, information can be added or altered for any given element (for example, by alternative RNA splicing or protein modification).

Box 1

Sequencing the human genome

The first complete drafts of the human genome sequence were published in 2001 by the International Human Genome Sequencing Consortium (IHGSC), a publicly funded effort, and Celera, a biotechnology company, using different approaches. Both efforts used a random or shotgun approach where the original DNA to be sequenced was randomly broken into overlapping fragments that were then cloned, and 500 base pairs (bp) were 'read' from one or both ends of the clones.

For the draft genome sequences, each base was read six to ten times to optimize the accuracy of the sequence. The stretches of DNA sequence were read by a computer and assembled into a complete sequence. The IHGSC effort randomly cleaved DNA into ~200,000-bp fragments and generated a map of these fragments across the 24 different human chromosomes; it then used the shotgun approach to sequence the pre-ordered fragments clone by clone. In contrast, Celera randomly fragmented the entire genome into three sizes of fragments (approximately 2,000, 10,000 and 200,000 bp), sequenced both ends of the clones and then used the end sequences to assemble the entire genome sequence, without the aid of a map.

Celera's 1998 announcement that it would sequence the human genome within three years was greeted with considerable scepticism, but it succeeded in producing a draft sequence and considerably accelerating the public effort. The efforts of both groups benefited science by producing draft genome sequences considerably earlier than expected.

Although minor differences were noted between the two drafts, the overall conclusions concerning gene numbers, repeated sequences and chromosomal organization were remarkably similar. For example, both groups identified 30,000–35,000 genes, far fewer than the 100,000 expected from an earlier (admittedly 'back of the envelope') calculation.

Systems approaches to biology

Humans start life as a single cell — the fertilized egg — and develop into an adult with trillions of cells and thousands of cell types. This process uses two types of biological information: the digital information of the genome, and environmental information, such as metabolite concentrations, secreted or cell-surface signals from other cells or chemical gradients. Environmental information is of two distinct types: deterministic information where the consequences of the signals are essentially predetermined, and stochastic information where chance dictates the outcome.

Random, or stochastic, signals can generate significant noise in biological systems, but it is only in special cases that noise is converted into signals. For example, stochastic events govern many of the genetic mechanisms responsible for generating antibody diversity. In the immune response, those B cells that produce antibodies that bind

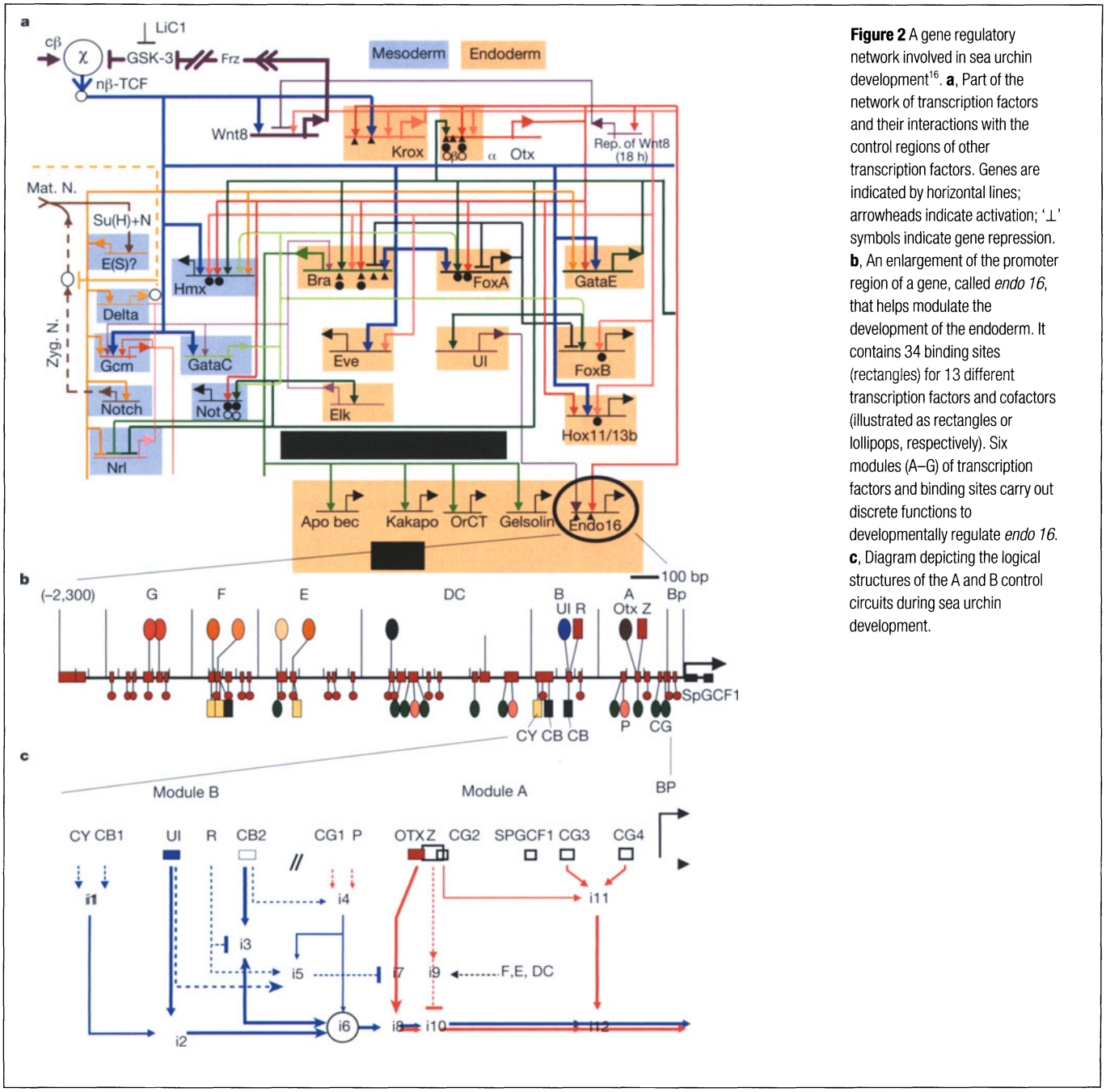

Figure 2 A gene regulatory network involved in sea urchin development[16]. **a**, Part of the network of transcription factors and their interactions with the control regions of other transcription factors. Genes are indicated by horizontal lines; arrowheads indicate activation; '⊥' symbols indicate gene repression. **b**, An enlargement of the promoter region of a gene, called *endo 16*, that helps modulate the development of the endoderm. It contains 34 binding sites (rectangles) for 13 different transcription factors and cofactors (illustrated as rectangles or lollipops, respectively). Six modules (A–G) of transcription factors and binding sites carry out discrete functions to developmentally regulate *endo 16*. **c**, Diagram depicting the logical structures of the A and B control circuits during sea urchin development.

tightly to the antigen (that is, those having high affinities) undergo an expansion in number that is proportional to the strength of the antibody affinity (see accompanying article by Nossal, page 126). Hence, the signal (high affinity) is distinguished from the noise (low affinity). Moreover, high levels of mutation in the B cells causes specific diversification of antibody genes in the presence of antigen and permits the affinity to increase even more. The cells carrying the higher-affinity antibody genes are then preferentially selected for survival and proliferation.

The key question is what and how much signal emerges from the noise. Analysis of stochastic events and the differentiation between signal and noise will be a future challenge for contemporary biology. The immune response has been studied for more than 100 years, yet we still have only a partial understanding of its systems properties, such as the immune response and tolerance (the unresponsiveness to one's own cells). This is because until recently immunologists have been able to study this complex system only one gene or one protein at a time.

The systems approach permits the study of all elements in a system in response to genetic (digital) or environmental perturbations. Global quantitative analyses of biological information from different levels each provide new insights into the operation of the system; hence, information at as many levels as possible must be captured, integrated, and ultimately, modelled mathematically. The model should explain the properties of the system and establish a framework that allows us to redesign the system in a rational way to generate new emergent properties.

Several systems have been explored successfully. The utilization of the sugar galactose in yeast has been analysed using genetic perturbations (inactivation of genes) and four levels of information were gathered — RNA and protein concentrations as well as protein–protein and protein–DNA interactions[17]. Using an iterative and integrative systems approach, new insights into the regulation of galactose use were gained. Moreover, the relationships of the galactose regulatory network to other modules in the yeast cell were also delineated. Likewise, systems approaches to early embryonic

development in the sea urchin have delineated a regulatory network that has significant predictive power[16] (Fig. 2). Finally, systems approaches to metabolism in an archaeal halobacterium (an organism thriving in up to five-molar salt solutions, such as the Dead Sea) have revealed new insights into the inter-relationships among several modules controlling energy production in the cell[18].

The study of cellular and organismal biology using the systems approach is at its very beginning. It will require integrated teams of scientists from across disciplines — biologists, chemists, computer scientists, engineers, mathematicians and physicists. New methods for acquiring and analysing high-throughput biological data are needed. A powerful computational infrastructure must be leveraged to generate more effective approaches to the capture, storage, analysis, integration, graphical display and mathematic formulation of biological complexity. New technologies must be integrated with each other. Finally, hypothesis-driven and discovery science must be integrated. In short, both new science and technology must emerge for the systems biology approach to realize its promise. A cultural shift in the biological sciences is needed, and the education and training of the next generation of biologists will require significant reform.

Gordon Moore, the founder of Intel, predicted that the number of transistors that could be placed on a computer chip would double every 18 months. It has for more than 30 years. This exponential growth has been a driver for the explosive growth of information technology. Likewise, the amount of DNA sequence information available to the scientific community is following a similar, perhaps even steeper, exponential increase. The critical issue is how sequence information can be converted into knowledge of the organism and how biology will change as a result. We believe that a systems approach to biology is the key. It is clear, however, that this approach poses significant challenges, both scientific and cultural[19]. The discovery of DNA structure started us on this journey, the end of which will be the grand unification of the biological sciences in the emerging, information-based view of biology. □

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Controlling the double helix

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Chromatin is the complex of DNA and proteins in which the genetic material is packaged inside the cells of organisms with nuclei. Chromatin structure is dynamic and exerts profound control over gene expression and other fundamental cellular processes. Changes in its structure can be inherited by the next generation, independent of the DNA sequence itself.

Genes were first shown to be made of DNA only nine years before the structure of DNA was discovered (ref. 1; and see accompanying article by McCarty, page 92). Although revolutionary, the idea that genetic information was protein-free ultimately proved too simple. DNA in organisms with nuclei is in fact coated with at least an equal mass of protein, forming a complex called chromatin, which controls gene activity and the inheritance of traits.

'Higher' organisms, such as yeast and humans, are eukaryotes; that is, they package their DNA inside cells in a separate compartment called the nucleus. In dividing cells, the chromatin complex of DNA and protein can be seen as individual compact chromosomes; in non-dividing cells, chromatin appears to be distributed throughout the nucleus and organized into 'condensed' regions (heterochromatin) and more open 'euchromatin' (see accompanying article by Ball, page 107). In contrast, prokaryotes, such as bacteria, lack nuclei.

The evolution of chromatin

The principal protein components of chromatin are proteins called histones (Fig. 1). Core histones are among the most highly conserved eukaryotic proteins known, suggesting that the fundamental structure of chromatin evolved in a common ancestor of eukaryotes. Moreover, histone equivalents and a simplified chromatin structure have also been found in single-cell organisms from the kingdom Archaeabacteria[2,3].

Because there is more DNA in a eukaryote than in a prokaryote, it was naturally first assumed that the purpose of histones was to compress the DNA to fit within the nucleus. But subsequent research has dramatically revised the view that histones emerged as an afterthought, forced on eukaryotic DNA as a consequence of large genome size and the constraints of the nucleus.

It was known that different genes are active in different tissues, and the distinction of heterochromatin and euchromatin suggested that differences in chromatin structure were associated with differences in gene expression. This led to the early supposition that the histones were also repressor proteins designed to shut off unwanted expression. The available evidence, although rudimentary, does indeed suggest that archaeal histones are not merely packaging factors, but function to regulate gene expression[2–5]. They

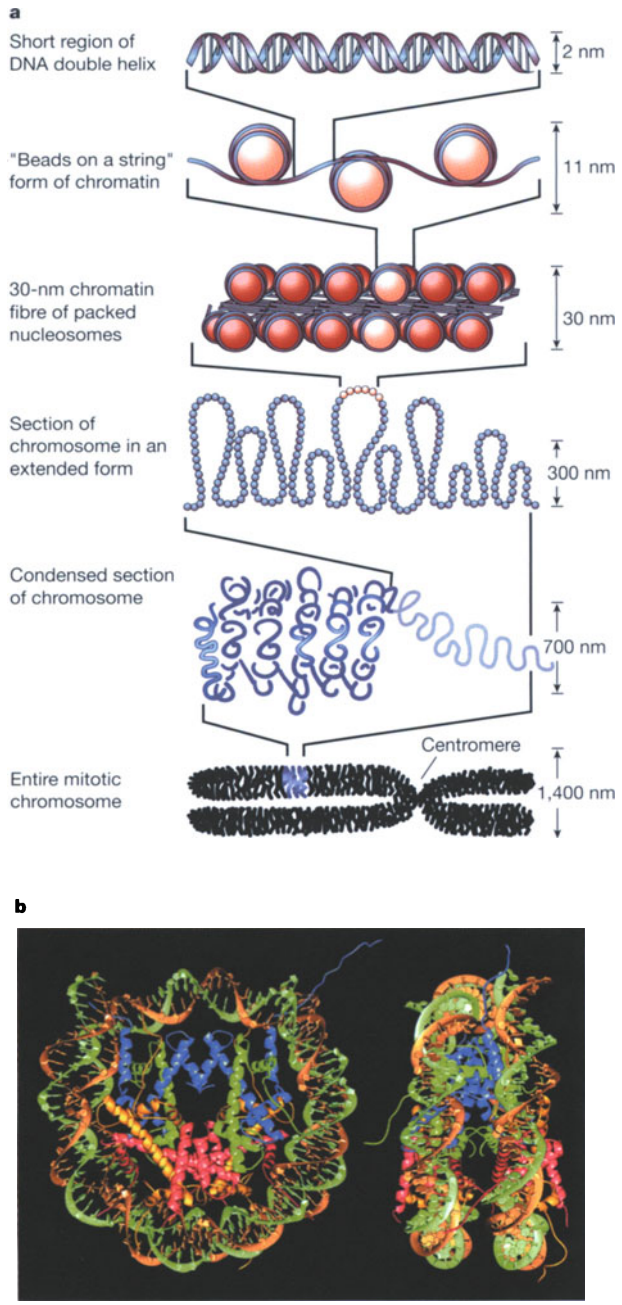

Figure 1 Packaging DNA. **a**, The organization of DNA within the chromatin structure. The lowest level of organization is the nucleosome, in which two superhelical turns of DNA (a total of 165 base pairs) are wound around the outside of a histone octamer. Nucleosomes are connected to one another by short stretches of linker DNA. At the next level of organization the string of nucleosomes is folded into a fibre about 30 nm in diameter, and these fibres are then further folded into higher-order structures. At levels of structure beyond the nucleosome the details of folding are still uncertain. (Redrawn from ref. 41, with permission). **b**, The structure of the nucleosome core particle was uncovered by X-ray diffraction, to a resolution of 2.8Å (ref. 42). It shows the DNA double helix wound around the central histone octamer. Hydrogen bonds and electrostatic interactions with the histones hold the DNA in place.

may facilitate gene activation, by promoting specific structural interactions between distal sequences, or repression, by occluding binding sites for transcriptional activators.

We suggest that the function of archaeal histones reflects their ancestral function, and therefore that chromatin evolved originally as an important mechanism for regulating gene expression. Its use in packaging DNA was an ancillary benefit that was recruited for the more complex nucleosome structure that subsequently evolved in the ancestors of modern eukaryotes, which had expanded genome sizes. Although their compactness might seem to suggest inertness, chromatin structures are in fact a centre for a range of biochemical activities that are vital to the control of gene expression, as well as DNA replication and repair.

Packaging DNA into chromatin

The fundamental subunit of chromatin is the nucleosome, which consists of approximately 165 base pairs (bp) of DNA wrapped in two superhelical turns around an octamer of core histones (two each of histones H2A, H2B, H3 and H4). This results in a five- to tenfold compaction of DNA[6]. The DNA wound around the surface of the histone octamer (Fig. 1) is partially accessible to regulatory proteins, but could become more available if the nucleosome could be moved out of the way, or if the DNA partly unwound from the octamer. The histone 'tails' (the amino-terminal ends of the histone protein chains) are also accessible, and enzymes can chemically modify these tails to promote nucleosome movement and unwinding, with profound local effects on the chromatin complex.

Each nucleosome is connected to its neighbours by a short segment of linker DNA (~10–80 bp in length) and this polynucleosome string is folded into a compact fibre with a diameter of ~30 nm, producing a net compaction of roughly 50-fold. The 30-nm fibre is stabilized by the binding of a fifth histone, H1, to each nucleosome and to its adjacent linker. There is still considerable debate about the finer points of nucleosome packing within the chromatin fibre, and even less is known about the way in which these fibres are further packed within the nucleus to form the highest-order structures.

Chromatin regulates gene expression

Regulatory signals entering the nucleus encounter chromatin, not DNA, and the rate-limiting biochemical response that leads to activation of gene expression in most cases involves alterations in chromatin structure. How are such alterations achieved?

The most compact form of chromatin is inaccessible and therefore provides a poor template for biochemical reactions such as transcription, in which the DNA duplex must serve as a template for RNA polymerase. Nucleosomes associated with active genes were shown to be more accessible to enzymes that attack DNA than those associated with inactive genes[7], which is consistent with the idea that activation of gene expression should involve selective disruption of the folded structure.

Clues as to how chromatin is unpacked came from the discovery that components of chromatin are subject to a wide range of modifications that are correlated with gene activity. Such modifications probably occur at every level of organization, but most attention has focused on the nucleosome itself. There are three general ways in which chromatin structure can be altered. First, nucleosome remodelling can be induced by complexes designed specifically for the task[8]; this typically requires that energy be expended by hydrolysis of ATP. Second, covalent modification of histones can occur within the nucleosome[9]. Third, histone variants may replace one or more of the core histones[10–12].

Some modifications affect nucleosome structure or lability directly, whereas others introduce chemical groups that are recognized by additional regulatory or structural proteins. Still others may be involved in disruption of higher-order structure. In some cases, the packaging of particular genes in chromatin is required for their expression[13]. Thus, chromatin can be involved in both activation and repression of gene expression.

Chromatin remodelling

Transcription factors regulate expression by binding to specific DNA control sequences in the neighbourhood of a gene. Although some DNA sequences are accessible either as an outward-facing segment on the nucleosome surface, or in linkers between nucleosomes, most

Box 1

Histone modifications

Many amino acids of histones, particularly those in the 'tails', are chemically modified[47]. These include lysine residues that may be acetylated, methylated or coupled to ubiquitin (a large polypeptide chain); arginine residues that may be methylated; and serine residues that are phosphorylated. All modifications can affect one another, and many are positively or negatively correlated with each other. Collectively, they constitute a set of markers of the local state of the genetic material, which has been called the 'histone code'[48].

Histone modification is a dynamic process. Chromatin in the neighbourhood of transcriptionally active genes is enriched in acetylated histones, and the enzymes responsible for both acetylation and deacetylation are often recruited to sites where gene expression is to be activated or repressed, respectively. Within the nucleus, local states of both acetylation and phosphorylation can change rapidly. Methylation at certain histone amino-acid residues may also be important for activation, whereas at other sites it is a signal for inactivation.

Many (perhaps all) of the histone modifications interact with each other in ways that are still not completely understood. For example, in mammals, histone H2B can be modified by ubiquitin at Lys 120 (123 in yeast), and this modification is necessary for methylation at Lys 4 and Lys 79 of histone H3, reactions that are controlled by two different methylating enzymes. Influences between nearby modification sites have also been observed, such that phosphorylation at one site can facilitate acetylation at another, methylation and phosphorylation at adjacent sites may interfere with one another, and methylation and acetylation cannot occur simultaneously on the same lysine residue.

Box 1 Figure Histone modifications. Each modification is colour coded as indicated and the position of the modified amino acid labelled[47,49–51].

are buried inside the nucleosome. Regulatory factors must therefore seek out their specific DNA-binding sites and gain access to them. They are aided by chromatin-remodelling complexes that continually shuffle the positions of individual nucleosomes so that sites are randomly exposed for a fraction of time[8,14].

A number of chromatin-remodelling complexes mobilize nucleosomes, causing the histone octamers to move short distances along the DNA[8]. Each complex carries a protein with ATPase activity, which provides the necessary energy. Many of these complexes are members of the so-called SWI/SNF family, which includes SWI/SNF in budding yeast and human, RSC in yeast, and Brahma in *Drosophila*. They have similar helicase-motif subunits, but varying co-factors within the complex. Another SWI/SNF subfamily is based on the helicase-domain protein ISWI, which combines with other proteins to form the complexes NURF, CHRAC and ACF in *Drosophila*, and RSF in humans. A third subfamily is based on the helicase motif protein Mi-2.

Remodelling complexes differ in the mechanisms by which they disrupt nucleosome structure, and they are associated with co-factors that allow them to interact selectively with other regulatory proteins that bind to specific DNA sequences. For example, only certain classes of transcription factors interact with the mammalian SWI/SNF remodelling complex. Thus remodelling complexes can be selective in the genes they modify, and transcription factors recruit these complexes as tools to gain access to chromatin.

Histone modification

Nucleosomes are not passive participants in this recognition process. They can accommodate chemical modifications — either on histone 'tails' that extend from the nucleosome surface, or within the body of the octamer — that serve as signals for the binding of specific proteins. A large number of modifications are already known, such as acetylation of amino acids in the histone tails, and new ones are being identified at a bewildering rate (Box 1). Many modifications are associated with distinct patterns of gene expression, DNA repair or replication, and it is likely that most or all modifications will ultimately be found to have distinct phenotypes.

In addition to histone modifications, nucleosomes can have core histones substituted by a variant, with functional consequences. Histone H2AZ, which is associated with reduced nucleosome stability, replaces H2A non-randomly at specific sites in the genome. Histone H2AX, which is distributed throughout the genome, is a target of phosphorylation accompanying repair of DNA breakage[11], and also seems to be involved in the *V(D)J* recombination events that lead to the assembly of immunoglobulin and T-cell-receptor genes. A histone H3 variant, H3.3, can be incorporated into chromatin in non-dividing cells, and seems to be associated with transcriptionally active genes[10]. Each of these histone substitutions is likely to be targeted by, and associated with, the binding of other proteins involved in gene activation; thus these proteins can be considered central to the formation of localized chromatin structures that are specific for gene activation or accessibility.

Interdependence of histone modifications

An interplay exists between histone modification and chromatin remodelling. For example, expression of a gene may require disruption of nucleosomes positioned at the promoter by a chromatin-remodelling complex before an enzyme required for histone

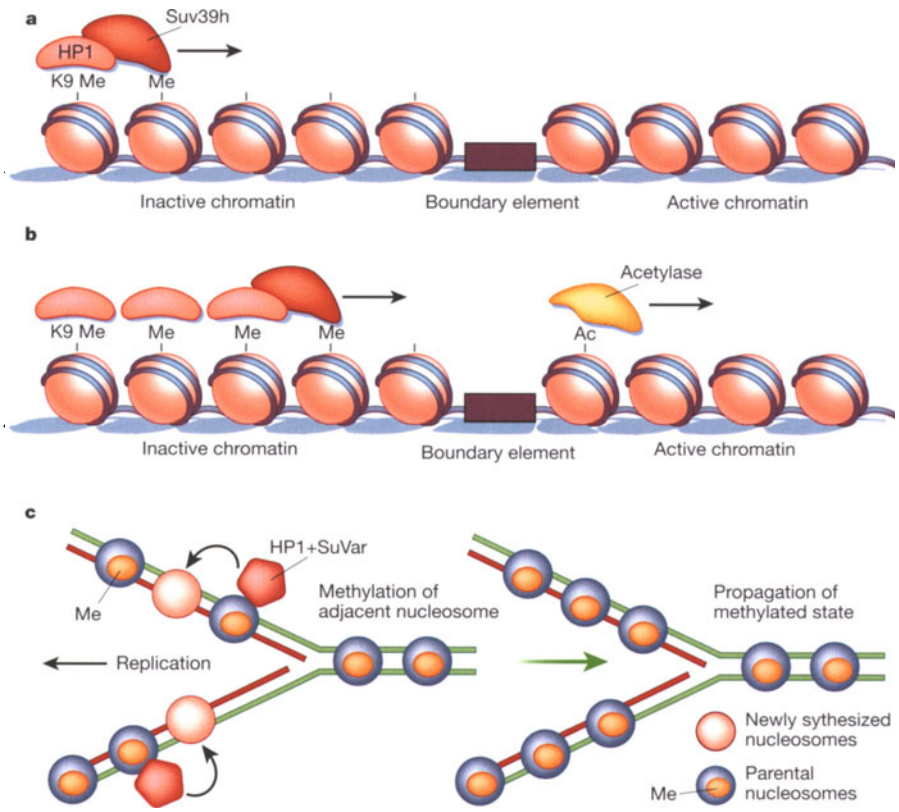

Figure 2 Propagation of inactive ('condensed') and active chromatin states (adapted from ref. 43). **a**, Nucleosomes methylated at H3 Lys 9 are a mark of inactive chromatin and are bound by the heterochromatin protein HP1. HP1 in turn recruits a histone methyltransferase enzyme, Suv39h, that specifically methylates H3 Lys9, allowing methylation and HP1 binding to extend to successive nucleosomes in a self-propagating fashion[43–45]. Some DNA sequence elements (purple rectangle) and their associated proteins may serve as barriers between different chromatin regions, perhaps by blocking the propagation of histone modifications and/or the binding of heterochromatin proteins, thus helping to establish well-defined domains[46]. **b**, A similar propagation mechanism may be constructed for activation by histone acetylation (right). Here, acetylated lysines are recognized by an acetylase enzyme, resulting in acetylation of the adjacent nucleosome. **c**, A proposed model for epigenetic inheritance of methylation. During replication, parental nucleosomes carrying H3 with Lys 9 methylation (blue) are distributed randomly to both sides of the replication fork. Nucleosomes containing newly synthesized histones (pink) are deposited between the old ones, and are methylated by a mechanism similar to that described above. The daughter-cell chromatin then carries the same modification as the parent.

acetylation can be recruited[15]. In contrast, expression of a different gene may require that histone-acetylating enzymes and even RNA polymerase bind to the promoter prior to recruitment of the chromatin-remodelling complex[16]. There is no common series of steps that underlies all or even most processes of gene activation. For any given gene, however, the order of recruitment of chromatin-modifying factors may be crucial for the appropriate timing of expression.

Aside from activating gene expression, histone modifications and chromatin remodelling can also silence genes. Specific histone modifications and chromatin-remodelling complexes, such as the NuRD complex, have been implicated in silencing at some loci[8]. Even SWI/SNF complexes, which are strongly correlated with gene activation, also seem to silence a number of genes.

Specialized chromatin structures

Some regions of the genome are packaged in chromatin with distinct structural features. Three of the most studied such regions are centromeres (important for chromosomal organization during mitosis), telomeres (at the ends of chromosomes) and the inactive X chromosome in mammals. In each case, specific chromosomal structures are defined both by histones modified or substituted in specific patterns, and by the association of additional non-histone proteins or even by regulatory RNA molecules, which increasingly are implicated in chromatin organization[17–19].

Inactive X chromosomes in mammals are enriched for the histone variant macroH2A[20], which is almost three times as large as H2A itself. At vertebrate centromeres, one of the core histones, H3, is replaced by a variant, CENP-A; a similar replacement occurs in centromeres of the fruitfly *Drosophila*, indicating that this is an ancient evolutionary adaptation at centromeres. CENP-A in turn forms a complex with the centromere proteins CENP-B and -C, which mediates the formation of phased arrays of CENP-A-containing nucleosomes. In turn, additional proteins are recruited during cell division to enable the orderly separation of the two chromatids that make up each chromosome. After DNA replication, the sister chromatids are held together initially by a multisubunit complex called cohesin, while a second complex, condensin, helps to compact the chromosomes[21]. These complexes recognize distinct centromere structures, and a specialized nucleosome-remodelling complex associates with cohesin to help it gain access to the chromosomes[22].

In the budding yeast *Saccharomyces cerevisiae*, gene silencing at the ends of chromosomes is mediated by a complex that assembles at telomeres. The complex is stabilized by the binding of the protein RAP1 to the telomere repeat sequences. Additional components, including the silent information regulator (SIR) proteins, then bind inward from the telomere ends, partly through interactions with local nucleosomes[23]. One of the SIR proteins is a histone deacetylase and is thought to repress gene expression at this site. Some components of these unique complexes are evolutionarily conserved, suggesting that these unusual chromatin structures may be found in organisms other than yeast.

The silencing of genes in the vicinity of centromeres in the fission yeast *Schizosaccharomyces pombe* has been shown recently[17–19] to depend on a set of RNA-processing enzymes involved in RNA interference, a process by which double-stranded RNA directs sequence-specific degradation of messenger RNA. One of these enzymes, Dicer, generates RNA fragments about 23 nucleotides long from transcripts of centromeric regions, which then seem in some way to be targeted back to the centromere to initiate the histone-dependent silencing mechanism. Moreover, non-coding RNA transcripts have been identified on the inactive X chromosome and elsewhere in the genome, and may have related roles at those loci[24].

Epigenetic inheritance

An epigenetic trait is one that is transmitted independently of the DNA sequence itself. This can occur at the level of cell division — for example, daughter cells may inherit a pattern of gene expression from parental cells (so-called cellular memory) — or at the generational level, when an offspring inherits a trait from its parents.

The classic example of epigenetic inheritance is the phenomenon of imprinting, in which the expression status of a gene depends upon

the parent from which it is derived. In mammals, for example, the *Igf2* gene (encoding insulin-like growth factor-2) is expressed only from the paternal copy of the gene, whereas the *H19* gene is expressed solely from the maternal allele. The mechanism by which this pattern of inheritance is accomplished involves (in part) DNA methylation on the paternal allele. This causes dissociation of a chromatin protein known as CTCF, which normally blocks a downstream enhancer; consequently, the enhancer is then free to activate *Igf2* expression[25,26].

The methylation state of an allele is linked inextricably with patterns of histone modification[27]. Methylated CpG (guanine–cytosine) dinucleotide sites near a gene recruit specific DNA-binding proteins, which in turn recruit histone deacetylases, resulting in loss of histone acetylation and silencing of gene expression. But if histone deacetylation occurs first, it is possible to replace the acetyl group at histone H3 lysine 9 (Lys 9) with one to three methyl groups. It has been shown in turn in the fungus *Neurospora* that the ability to methylate histone Lys 9 is essential for DNA methylation[28], suggesting that local methylation at Lys 9 may provide a signal for methylation of the underlying DNA. Furthermore, in a different reaction pathway, maintenance of histone acetylation at promoters can lead to inhibition of DNA methylation[29].

Epigenetic inheritance involves the maintenance of patterns of histone modification and/or of association of chromosomal proteins correlated with specific expression states. The same mechanisms for propagating permissive or repressive chromatin structure could preserve the pattern of histone modification during replication, when old nucleosomes are distributed randomly on both sides of the fork, with the newly synthesized histones interspersed (Fig. 2).

The maintenance of repressed or activated transcription states represents an efficient mechanism for progressive cellular differentiation[30]. In such a model, fundamental decisions regarding the turning on or off of genes or groups of genes need to be made only once. This principle is perhaps most clearly illustrated by the example of Polycomb-group (PcG)-mediated gene repression in *Drosophila*[31]. At a specific time during development, a complex of proteins, encoded by a collective of PcG genes, binds to sequences within some genes, but only in cells where the genes are silent. At subsequent stages of development, the repressed state is maintained by the PcG complex in the absence of the original negative signals. Activated expression states can be similarly maintained, again in the absence of the original transcriptional activators, by a complex of proteins encoded by genes collectively termed the trithorax group[31]. In both cases, the maintenance of gene-expression patterns is associated with specific histone modification and chromatin-remodelling activities[32–34].

Chromatin and nuclear self-organization

Although bacteria lack a true nucleus, a specific region of the cell, called the nucleoid, contains the chromosome, which in turn is organized into supercoiled domains or loops emanating from central nodes. The organization of the *Escherichia coli* genome into such domains is necessary to allow it to fit within the confines of the cell[2]. Extensions of the chromosome into the cytoplasm correlate with regions that are transcriptionally active. Upon inhibition of transcription, these extensions recede to the nucleoid to give it a more even, spherical shape. The localization of genomic sequences within a bacterial cell is thus determined by their association with the transcriptional/translational apparatus.

The organization of the genome in eukaryotic nuclei, while necessarily more complex than in bacteria, seems to follow the same model as *E. coli*. Individual chromosomes largely occupy distinct 'territories' within the nucleus. Within these territories, actively transcribed genes are on surfaces of channels within subchromosomal domains[35] where soluble transcription factors are presumably more likely to gain access to them.

There is, however, more to the story. The eukaryotic nucleus has distinct subcompartments within which specific nuclear proteins are enriched. For example, the nucleolus, where high-level transcription of ribosomal genes occurs, and splicing-factor compartments accumulate high local concentrations of certain proteins. In some cases there are attachment sites within the nucleus for the proteins. As a rudimentary example, one or more of the proteins associated with yeast telomeres is able to tether the telomeres in clusters to the nuclear periphery[36]. This clustering creates a high local concentration of binding sites for the SIR silencing proteins, which in turn results in a high local concentration of these proteins, and a high occupancy of even relatively weak binding sites. The effect is to increase the extent of telomeric silencing — SIR-dependent gene silencing can be accomplished just by artificially tethering a gene to the nuclear periphery[37].

What organizes the formation of nuclear subdomains? Although there is evidence for a proteinaceous nuclear matrix[38], the example provided by yeast telomeres suggests that the chromatin fibre itself may be the organizer. Many, and probably most, chromatin-binding proteins are in continuous flux between association with chromatin and the nucleoplasm[39,40]. Even such fundamental chromatin proteins as histone H1 have been found to bind for periods of only a few seconds, interspersed with periods of free diffusion. The notable exceptions to this rule are the core histones, the binding of which is much more stable — on the order of minutes for H2A/H2B, and hours for H3/H4. The on–off rates of proteins binding different regions of the genome may depend on the pattern of histone modifications, which in turn determines their relative enrichment in different regions of the nucleus. Thus, the genome as packaged with histones could determine the nature of nuclear subcompartments.

Future challenges

Chromatin proteins and DNA are partners in the control of the activities of the genetic material within cells. The rate-limiting step in activating gene expression typically involves alterations of chromatin structure. The chromosome is an intricately folded nucleoprotein complex with many domains, in which local chromatin structure is devoted to maintaining genes in an active or silenced configuration, to accommodating DNA replication, chromosome pairing and segregation, and to maintaining telomeric integrity. Recent results suggest strongly that in all of these cases the primary indicators of such specialization are carried on the histones. Thus, the regulatory signals that determine local properties, as well as epigenetic transmission of those properties, are likely to be on histones.

The already large catalogue of histone modifications continues to grow rapidly. Although in most cases the loss of the modification (for example, by mutating the responsible enzyme) has a detectable effect on phenotype, the function of many modifications has not yet been determined. While this will be the focus of future research, it presents significant problems because a given modification will occur at many sites in the genome, and mutations could have widespread effects, both direct and indirect. A second significant challenge arises from the potential redundancy of the 'histone code': it is possible that either of two distinct modifications could specify a single structural and functional state, or that the two modifications are always linked to one another. Significant effort will be necessary to determine the complexity of this code, that is, the number of distinct states that can be specified.

The most important immediate problem is to identify the initiating step in establishing a local chromatin state, which may also correspond to an epigenetic state. Silencing at centromeres and perhaps elsewhere seems to be initiated by small RNA transcripts from within the region to be silenced, but formation of other kinds of structures might be triggered directly by a specific histone modification. In the longer term it will be necessary to relate the reactions at individual nucleosomes to higher-order chromatin structures; this will depend in part on the development of higher-resolution methods for determining those structures, and their organization within the nucleus.

At its simplest level, chromatin should be viewed as a single entity, carrying within it the combined genetic and epigenetic codes.

Ultimately our understanding of the dynamic states of chromatin throughout the genome will be integrated with a detailed knowledge of patterns of regulation of all genes. □

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