Bacterial Chromatin
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Editors

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The birth and the development of molecular biology and, subsequently, of genetic engineering and biotechnology cannot be separated from the advancements in our knowledge of the genetics, biochemistry and physiology of bacteria and bacteriophages. Also most of the tools employed nowadays by biotechnologists are of bacterial (or bacteriophage) origin and the playground for most of the DNA manipulations still remains within bacteria. The relative simplicity of the bacterial cell, the short generation times, the well defined and inexpensive culturing conditions which characterize bacteria and the auto-catalytic process whereby a wealth of in-depth information has been accumulated throughout the years have significantly contributed to generate a large number of knowledge-based, reliable and exploitable biological systems.

The subtle relationships between phages and their hosts have produced a large amount of information and allowed the identification and characterization of a number of components which play essential roles in fundamental biological processes such as DNA duplication, recombination, transcription and translation. For instance, to remain within the topic of this book, two important players in the organization of the nucleoid, FIS and IHF, have been discovered in this way. Indeed, it is difficult to find a single fundamental biological process whose structural and functional aspects are better known than in bacteria.

However, a notable exception is represented by the physical and functional organization of the bacterial genome. Although some bacteria contain more than one chromosome and some chromosomes are known to be linear, the majority of bacterial cells contain a single circular chromosome. The chromosome of *Escherichia coli* consists of about 4.6 million bp corresponding to a fully extended circumference of about 1.6 mm and rapidly growing bacteria may contain up to almost four genomic equivalents. Thus, the need for compaction of this genetic material to fit within an approximately 500-fold smaller volume is obvious; likewise, also clear is the need for a dynamic “chromatin” structure capable of undergoing rapidly all kinds of vital transactions to respond promptly to different types of environmental cues, changes and stresses with focused and/or global reprogramming of gene expression. All this happens within one or a few ill-defined structures called “nucleoids” where the cellular DNA is localized.

The bacterial nucleoid is enclosed by the cytoplasm, likely separated from it by a physical chemical effect known as “molecular crowding” but not compartmentalized
by a nuclear envelope like that existing in eukaryotes. For many years, this circum-
stance, the size of the nucleoids, at the limits of resolution of the traditional detection
methods of cell biology, and the elusiveness of their morphology and composition
have made it particularly difficult to answer basic questions about the behavior and
the structural and functional organization of the bacterial chromosome.

About 30 years ago, when I started being interested in the organization of the
nucleoid and, more particularly, in the chemical nature, role and expression of the
proteins associated with the bacterial chromosome, studies on this subject were at
their infancy.

Indeed, a huge gap existed between the morphological information obtained
through the pioneering studies of electron microscopists such as the late Professor
Eduard Kellenberger and his colleagues and the almost non-existent biochemical
characterization of the nucleoid and of its protein components. In 1977, Varshavsky
had detected by SDS-PAGE the presence of two “histone-like” proteins within a
purified *E. coli* deoxyribonucleoprotein preparation. He named the proteins B1 and
B2 but, aside from their molecular weights, no other property was given, so that our
present belief that these two proteins corresponded to HU and H-NS cannot be sup-
ported by any evidence. In fact, most scientists at that time considered the bacterial
DNA to be “naked”, neutralized by mono- and divalent cations and polyamines
and, given the absence of eukaryotic-type histones, they questioned the mere exis-
tence of DNA-associated architectural proteins.

Nuclease treatment of nucleoids obtained from gently lysed cells had already
shown the existence of topologically independent domains of supercoiling as well
as an “organizing” central core of RNA. While the latter turned out to be a prepara-
tion artifact, the existence of the topologically independent negatively supercoiled
loops was later confirmed, initially by tri-methyl psoralen crosslinking and then by
elegant site-specific recombination experiments and by accurate EM observations.
The use of site-specific recombination between directly repeated *res* sites mediated
by *gd* resolvase engineered to have different half-lives within the cell and the use
of supercoiling sensitive reporter genes revealed the existence of approximately
450 domains of supercoiling per genome having a mean size of 11 kb and randomly
located barriers. Further studies have also shown how the transcriptional activity of
the chromosome may contribute to shaping the nucleoid and how rapidly disas-
sembled nucleoid components can reassemble.

The separation of the chromosome into independent, negatively supercoiled
loops, half of which are plectonemic, turns out to be of paramount importance not
only as one of the mechanisms responsible for bacterial chromosome compaction
within the nucleoid, but also for preventing the loss of DNA superhelicity. In fact,
the existence of non-restrained negative supercoiling is required for a plethora of
DNA functions and well known are the adverse, often lethal effects caused by both
hyper- and hypo-supercoiling.

In addition to the aforementioned macro-molecular crowding and DNA super-
coiling, an important role in DNA condensation is played by nucleoid-associated
proteins which in the meantime have been identified and rigorously characterized.
In fact, following a shaky and uncertain beginning which characterized the 1970s
and the first half of the 1980s, when several articles appeared reporting conflicting properties of ill-defined proteins supposedly associated with the chromosome and to which various names had been attributed, the major components of the nucleoid were finally thoroughly purified and their precise biochemical and genetic identities established. In this way, it was possible to discover that *E. coli* HU in reality consisted of two different polypeptide chains (HUα and HUβ) whose amino acid sequences were promptly determined. Shortly thereafter, also the structural genes encoding these two proteins (hupB and hupA) were identified, mapped and sequenced and a close similarity between the two HU subunits and the two subunits of IHF (IHF-A and IHF-B) was detected. Likewise, the amino acid sequence of H-NS and the nucleotide sequence of its structural gene *hns* were determined. In turn, these data led to the detection of a close similarity between H-NS and StpA, a less abundant, yet probably not less important, nucleic acid binding protein. In the same period, two additional proteins (FIS and Lrp), which later turned out to be important components of the nucleoid, were also isolated and characterized.

It is now well established that these proteins are nucleoid structuring proteins which bind curved DNA, recognizing short, more or less degenerate consensus sequences, bend DNA and influence DNA supercoiling. In addition to contributing, through different mechanisms, to DNA compaction, at least some of these proteins participate in forming the dynamic barriers separating the topologically independent domains of supercoiling. Furthermore, it is also clear that the NAPs, in addition to being architectural proteins of the nucleoid, play other roles in the cell. In fact, several lines of evidence, including the highly pleiotropic effects displayed by mutations in their structural genes, indicate that the NAPs participate in DNA transactions such as recombination, repair and replication. Of particular relevance, in this connection, is the fact that all the NAPs, alone or in combination through synergistic or antagonistic mechanisms, have profound effects on the transcriptional activity of the cell.

The level of expression of the genes encoding NAPs is not constant during the growth cycle so that the intracellular concentration of these proteins varies as a function of the metabolic state of the cell and/or as a consequence of environmental changes. Since several promoters have been found to possess multiple, sometimes partially overlapping binding sites for these proteins, it is possible to envisage the existence of an intricate pattern of cross talks between the NAPs (e.g. the antagonistic effects of H-NS and FIS and HU and H-NS on the activity of some promoters) and the cyclic establishment or loss of integrated regulatory networks controlling global responses to environmental changes.

Taken together, all the data accumulated so far underlie the tight link existing between nucleoid architecture and nucleoid function and the close relationship between two apparently conflicting needs, namely that of condensing DNA and that of ensuring its accessibility through dynamic movements of the nucleoid and of its components.

Recent years have witnessed the development of new, powerful techniques to investigate the structure and functional organization of the bacterial nucleoid which have led to a renewed flourishing of the studies on this subject. Aside from the
aforementioned site-specific recombination, new microscopic techniques (e.g. confocal microscopy and AFM) and the manipulation of single and dual DNA molecules have contributed to giving a sharper image of the mechanisms by which the bacterial chromosome is condensed, made accessible and segregated. The picture that emerges is that of an analogic “machine” for which the most appropriate definition would be that of deterministic and organized chaos.

After studying the various chapters of this book, written by excellent scientists working at the forefront of this important aspect of molecular microbiology, the reader will certainly appreciate how much light has been shed on the bacterial nucleoid since the time it was considered stochastic chaos and bacterial DNA was regarded as “naked”. However, aside from realizing the extent of progress made in the last few years in understanding the nucleoid, the attentive reader will also perceive how much more remains to be learned.

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