CHAPTER 11

Cell Hybridisation

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A. Introduction

It has been suggested that cell fusion could be an early event in the neoplastic process (Miller 1974). In this chapter we examine the evidence for the occurrence of cell fusion in vivo, and explore the possibility that drug resistance can derive from mutations that accumulate in the enlarged gene pool of the hybrid cell. Since there is little direct information concerning the expression of drug resistance in hybrid cells in vivo, we shall discuss the implications for chemotherapy of the lessons learnt from the study of drug resistance in hybrid cells in vitro. The fusion of two cells is but one means of increasing cell ploidy (review, Brodsky and Uryvaeva 1977), so much of this discussion may be applicable to polyploid cells in general. Emphasis is placed on resistance to antitumour agents which exclusively involve nuclear genes. Mutants deficient in mitochondrial protein synthesis and respiratory functions coded by both nuclear and mitochondrial DNA are providing interesting insights into nuclear/cytoplasmic relationships, but to date none of these have involved antitumour drug resistance and will not be discussed. The interested reader can find information on this topic reviewed by Wright et al. (1980) and background reading on somatic-cell genetics in Harris (1970), Ephrussi (1972), Davidson and de la Cruz (1974), Ringertz and Savage (1976) and Shows and Sakaguchi (1980).

The initial product of cell fusion (Fig. 1) is a bi- or multinucleate cell that is either a heterokaryon if the parental cells were genetically different (Fig. 2) or a homokaryon if they were similar. In vivo, cells resulting from genetically programmed fusions often remain as multinucleate homokaryons. In vitro, the fused cells will be in cycle and will attempt to divide. At mitosis chromosomes from different nuclei come to share a common spindle apparatus and are drawn to the poles at anaphase to form two hybrid nuclei at telophase (Fig. 3). Clonogenic hybrids usually result from the fusion of two or three cells, since fusions of four or more cells run into mechanical difficulties at mitosis or are liable to contain a mixture of mitotic and interphase nuclei that are subject to premature chromosome condensation (Sect. D.V). The genetic redundancy in a hybrid nucleus permits the cell to accumulate recessive mutations and to undergo chromosomal rearrangements and loss of chromosomes without loss of viability. If these changes are beneficial, permitting the cell to divide faster or allowing it to occupy a new niche, then they will be selected for and persist. We now examine the evidence that supports the idea that drug resistance in vivo could result from such processes.

B. W. Fox et al. (eds.), *Antitumor Drug Resistance*

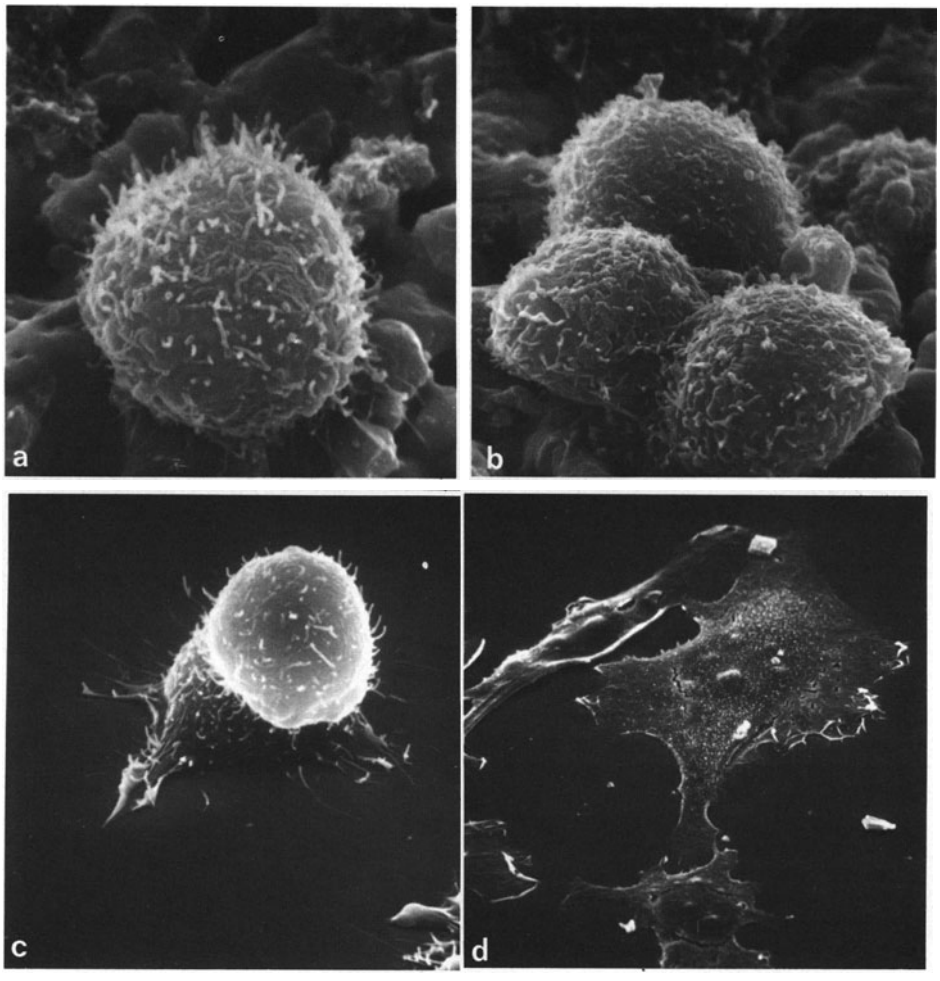

Fig. 1 a–d. In vitro fusion of Chinese hamster fibroblasts observed by scanning electron microscopy. **a** A Chinese hamster fibroblast observed prior to fusion showing a typical spherical morphology and microvillous topography of a cell in suspension. × 10,000. **b** Sendai virus, inactivated by treatment with β-propiolactone, has been added at 0 °C to a suspension of cells, causing agglutination. This clump of cells may be indicative of an early step in fusion with the microvilli of adjacent cells in intimate contact. × 7,000. **c** After adsorption of the virus the fusion mixture was incubated for 15 min at 37 °C before diluting into growth medium and incubating for a further 4 h. At this stage cells are resettling onto the substratum: the upper member of this pair still retains its spherical morphology. × 5,000. **d** 24 h postfusion, all cells are now attached to the substratum. The large cell, *upper right,* is approximately twice the size of the adjacent cells indicative of a heterokaryon. × 1,200. (P. J. Smith, T. D. Allen and J. M. Boyle, unpublished)

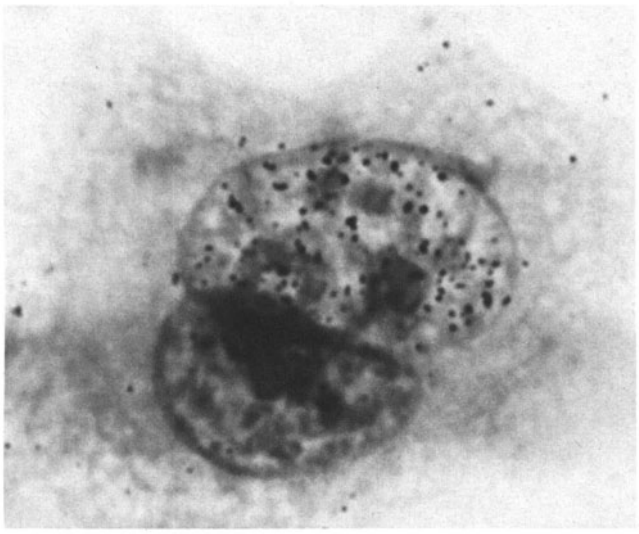

Fig. 2. A binucleate heterokaryon. Radioautogram of a heterokaryon containing one unlabelled and one labelled nucleus, resulting from fusion of two populations of Chinese hamster fibroblasts, one of which had been grown in the presence of [^{3}H]thymidine. (J. M. Boyle, unpublished)

B. Cell Fusion In Vivo

I. Occurrence of Multinucleate Cells

Bi- and multinucleate cells have been observed in a wide range of normal and pathological tissues (Table 1, and review by CHAMBERS 1978). In principle the presence of more than one nucleus could result from a mitotic division without cytokinesis. In many cases, however, multinucleation results from cell fusions that are a genetically programmed part of differentiation and can be observed to occur in tissue cultures. This is particularly important in haemopoietic tissues where macrophage-like cells fuse to form multinucleated giant cells and osteoclasts (TESTA et al. 1981). In vivo fusion of mononuclear cells to form giant cells may be in response to the presence of foreign bodies (SILVERMAN and SHORTER 1963) and occur in tuberculous lesions as Langhans cells (W. H. LEWIS 1927) and as frequent constituents of solid tumours (EVANS 1956). Fusions of macrophages to form multinucleated giant cells (M. R. LEWIS and W. H. LEWIS 1926; SUTTON and WEISS 1966), myoblasts (HOLTZER et al. 1958; KONIGSBERG et al. 1960; KONIGSBERG 1963; CAPERS 1960; YAFFE and FELDMAN 1965) and trophoblasts (PRIEST et al. 1980) have all been observed in vitro. In most cases where fusion is part of differentiation and the expression of specialised functions, there is little evidence that such cells divide and multiply (STOCKDALE and HOLTZER 1961), although synchronous DNA synthesis (RYAN and SPECTOR 1970) and both synchronous and asynchronous mitoses of multinucleated macrophages have been observed in vivo (MARIANO and SPECTOR 1974).

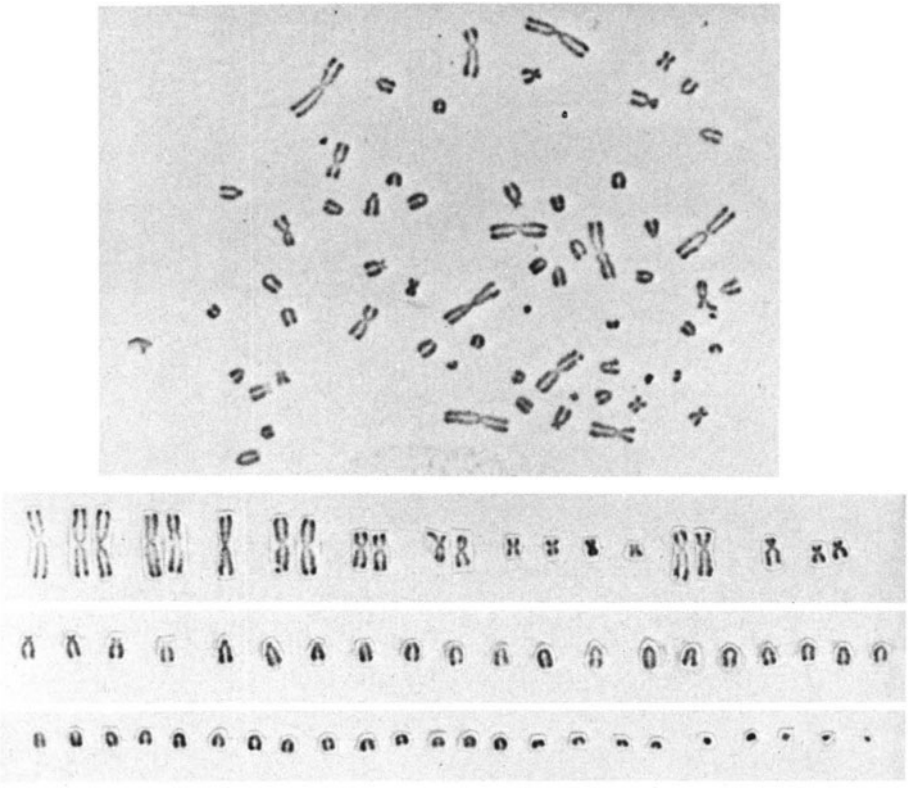

Fig. 3. Karyotype of a Chinese hamster V79 × mouse L1210 hybrid. *Upper,* metaphase figure stained with aceto-orcein. *Lower,* chromosomes of some metaphase chromosomes arranged in order of size, *top line* containing meta- and submetacentric chromosomes typical of Chinese hamster, *bottom line* containing telocentric chromosomes typical of mouse. (L. G. Durrant and J. M. Boyle, unpublished)

Two groups of viruses that frequently produce lesions containing fused cells are herpesvirus, which causes syncytia in skin lesions of patients with varicella (TYZZER 1906) and the paramyxoviruses, which include mumps, measles, respiratory syncytial virus and Sendai or haemagglutinating virus of Japan (HJV). Sendai virus is of interest as the agent used to produce the first intespecies hybrid cells between human and mouse (HARRIS and WATKINS 1965). ROIZMAN (1962a) discussed evidence that polykaryocytosis occurred mainly following low-level infection insufficient to allow virus multiplication and was enhanced by viral antibodies that prevented the spread of free viral particles. Viruses from other taxonomic groups (Table 1) have also been observed to cause fusion of cell cultures. This does not necessarily mean that such viruses cause fusion in vivo, since CASCARDO and KARZON (1965) showed that, although many epithelial cell lines were susceptible to fusion by measles virus, neither diploid human fibroblasts nor primary human epithelial cultures were susceptible. The Togavirus causing Western equine encephalomyelitis, apparently caused the production of hybrid erythrocytes when injected into chimeric chickens (KARAKOZ et al. 1969), al-

Table 1. Multinucleate cells in vivo

Occurrence	Reference
A. Normal tissues	
Binucleate cells of liver	LE BOUTON (1976)
Multinucleate cells of bladder epithelium	MARTIN (1972)
Myoblast fusion to give myotubules	MINTZ and BAKER (1967)
Megakaryocyte formation:	
Foreign body giant cells	CHAMBERS (1978)
Langhans giant cells	MARIANO and SPECTOR (1974)
Formation of osteoclasts	HAM (1974)
	TESTA et al. (1981)
Syncytiotrophoblast fusion during implantation	ENDERS and SCHLAFKE (1971)
Erythroid cell fusion in chimeric cattle	STONE et al. (1964)
Leucocyte fusion following transfusion *in utero*	TURNER et al. (1973)
B. Infected tissues	
Giant cells in tuberculous lesions	LEWIS (1927)
Viral infections:	
a) Paramyxoviruses	
Mumps	HENLE et al. (1954)
Measles	WARTHIN (1931)
Respiratory syncytial virus	MORRIS et al. (1956)
Sendai virus (HVJ)	OKADA (1962)
Newcastle disease virus	KOHN (1965)
b) Herpesviruses	
Varicella/zoster	TYZZER (1906)
	WELLER et al. (1958)
Herpes simplex	HOGGAN and ROIZMAN (1959)
	SCHERER (1953)
c) Leukoviruses	
Rous sarcoma virus	MOSES and KOHN (1963)
Visna virus	HARTER and CHOPPIN (1967)
d) Poxviruses	
Vaccinia	MCCLAIN (1965)
e) Togaviruses	
Dengue	SUITER and PAUL (1969)
Western equine encephalomyelitis	KARAKOZ et al. (1969)
f) Bungaviruses	
Germiston	
Wesselsbron	DJINAWI and OLSEN (1973)
g) Coronavirus	
Avian infectious bronchitis virus	AKERS and CUNNINGHAM (1968)
C. Malignant tissues	
Giant cells in malignant tissues	LUDFORD and SMILES (1952)
Warren sarcoma	LEWIS (1927)
Reed-Sternberg binucleate cells in Hodgkins lymphoma	JACKSON and PARKER (1944)

though other cases of hybrid blood cells did not involve (known) viruses (STONE et al. 1964; TURNER et al. 1973).

Multinucleate cells are frequently observed in tumours (ROIZMAN 1962 b), and CHAMBERS (1978) distinguished between multinucleated tumour cells capable of

division and "tumour-associated giant cells" that are rarely mitotic and have nuclei that are uniform in size and shape. These cells resemble foreign body giant cells and are thought to have a similar stem-cell origin. Examples cited are giant-cell tumour of soft parts (villonodular tenosynovitis), malignant giant-cell tumour of soft parts, osteoclastoma and malignant fibrous histiocytoma. Multinucleated tumour cells also occur. SHEEHY et al. (1974) found the frequency of bi- and multinucleated cells among malignant cells of ovarian carcinoma to be 7% and 65% respectively. Both DNA synthesis and mitosis were observed occurring asynchronously among nuclei of a single cell. Since multinucleate tumour cells are cycling, they will generate mononucleate hybrid cells that will contribute to the polyploidy of tumours. Some examples from an abundant literature on the incidence of polyploidy in tumours will illustrate the principles that the incidence and degree of polyploidy vary widely with the type of cancer and the stage of malignancy. MORARU and FADEI (1974) distinguished giant and multinucleated cells in eight cases of ovarian papillary adenocarcinomas. Frequencies varied from 0%–31% for giant cells to 0%–24% for multinucleated cells, the most invasive tumours having the highest proportion of both cell types. Mean chromosome numbers of the eight tumours showed variations from near diploid to hypotetraploid. In one tumour approximately 6% of metaphases had over 100 chromosomes. Comparison of tumour morphology and karyotype distinguished three stages of malignancy: stage I, mainly diploid, papilliform; stage II, mainly diploid with some heteroploid, proliferating malignant epithelium, covering papilli; stage III, heteroploid with marker chromosomes, invasive malignant epithelium. Ploidy can be more rapidly determined by microdensitometry of the DNA content of Feulgen-stained cells. A study of 23 ovarian carcinomas showed a good correlation between the DNA content measured in this way and the ploidy determined by chromosome counting (ATKIN 1971). A large study of the DNA contents of 1,465 malignant tumours of many different types was made by ATKIN and KAY (1979). Ploidy values were analysed in terms of 2.5-year survival data, and it was shown that most tumours except those of testis showed both low (near diploid) and high (triploid-tetraploid) groups, with prognosis being better for patients in the low group for all sites except carcinoma of the cervix uteri.

II. Experimental Production of Hybrids In Vivo

Support for the inference that cell fusion is involved in the production of polyploidy among tumour cells comes from studies with animal tumour-cell lines. There is strong evidence for fusion occurring in vivo between different tumour cell lines (JANZEN et al. 1971) and between tumour cells and normals cells of the host animal (Table 2). Definitive proof of hybridisation requires the demonstration that a cell possesses chromosomes, antigenic determinants or isozymes characteristic of both cell types involved in the fusion. To facilitate these measurements biopsy material is cultured in vitro to obtain hybrid clones, a necessity which led to the criticism that the hybrid clones isolated could have arisen from fusions occurring during culture in vitro. To overcome the objection as far as possible, BER et al. (1978) used tumour-cell lines of the universal fuser type (Sect. C), which allowed biopsy material to be cultured in a selective medium that rapidly killed both the tumour-cell line and normal host cells, but permitted the growth of hybrid cells.

Table 2. Hybridisation involving tumour cells in vivo

Tumour cells	Host	Reference
A. Tumour × tumour hybrids		
Mouse L5178Y lymphoma × mouse sarcoma 180	C3H mice	JANZEN et al. (1971)
B. Tumour × host hybrids		
Human gastric stem-cell lymphoma	Golden hamster (*Mesocricetus auratus*)	GOLDENBERG et al. (1971, 1974)
Mouse cell lines:		
A9HT	C3H × CBA mice C3H × C57B1 C3H × ACA CBA T6T6	
B82HT	C3H × CBA C3H × C57B1 C3H × ACA	WIENER et al. (1972)
SEWA (polyoma-induced sarcoma, ascitic form)	$(A \times A.SW)F_1$; $(A \times DBA/2)F_1$ CBA/H T_6	FENYO et al. (1973)
SEYF (polyoma-induced fibrosarcoma)	$(A \times A.BY)F_1$; $(A.BY \times C3H)F_1$ $(A.BY \times A.SW)F_1$	
MSWBS (methylcholanthrene-induced ascites sarcoma)	$(A.SW \times C3H)F_1$	
TA3Ha (ascitic form, spontaneous mammary adenocarcinoma)	$(C3H \times C57B1)F_1$ $(C3H \times DBA/2)F_1$	WIENER et al. (1974a)
A9HT(*HGPRT⁻ OUA^r*)	C3H; C3H × C57B1 C3H × A.SW: C3H × A CA	
501-1(*HGPRT⁻ OUA^r CAP^r*)	C3H × A.CA: C3H × C57B1	BER et al. (1978)
Ehrlich ascites	CBA/H T_6 mice	LALA et al. (1980)
Ehrlich ascites	Swiss mice	AGNISH and FEDEROFF (1968)
C1.1D (L cell)	C3H C3H × DBA/2 mice	AVILES et al. (1977)
PAZG (non-pigmented 8 AzG^r P/51 melanoma derived from B16 melanoma in C57B1/6 mice)	C57B1/6 mice	HU and PASZTOR (1975)
Cloudman (*HGPRT⁻*) melanoma	C57B1 DBA/2 mice	HALABAN et al. (1980)

The same group found that hybridisation in vivo occurs within 24 h of tumour-cell injection, a conclusion shared by LALA et al. (1980) for Ehrlich ascites cells grown subcutaneously in CBA/H T_6 mice. In the latter study the proportion of cells carrying the host marker chromosome T_6 increased to about 18% after 60 weekly passages of the tumour and subsequently stayed at this level. However, after only 16 weekly passages, all cells that had the sum of Ehrlich ascites plus host-cell chromosome numbers carried the T_6 marker. Examination of tumour smears showed that up to 5.6% of the cells of old tumours (13 days after injection) were multinucleate of which the majority were tumour × tumour homokaryons. The remainder were tumour × host heterokaryons in which the host nuclei had the morphology of macrophages or lymphocytes but not granulocytes, and

host × host homokaryons. WIENER et al. (1974b) also obtained evidence that ascites tumour cells fuse in vivo with haemopoietic cells. Tumour cells were injected into radiation chimeras that had been repopulated with donor cells carrying T_6 chromosomal and H-2 antigenic markers. Ascites tumours formed hybrids with the repopulating haemopoietic cells whereas solid tumours fused with cells of the irradiated host. Subsequent experiments established that the host-cell component of ascites tumour hybrids was non-thymus derived (WIENER et al. 1976).

III. Modified Phenotypes of Hybrids Induced In Vivo

Apart from the use of $HGPRT^-$ (hypoxanthine guanine phosphoribosyl transferase) and ouabain resistance in the selection of hybrids, there appear to have been no studies of drug resistance in hybrids formed in vivo. However, some observations have been made on factors that may affect the fitness of hybrid cells to grow in vivo, and thereby contribute indirectly to drug resistance.

There appear to be no clear rules about the expression of malignancy in hybrids made in vivo. Malignancy was increased in hybrids of human tumours grown in golden hamsters (GOLDENBERG et al. 1971, 1974), was similar to that of the tumour-cell line (WIENER et al. 1974c; AVILES et al. 1977; HALABAN et al. 1980), or was suppressed (HU and PASZTOR 1975). When Ehrlich ascites tumour cells were injected subcutaneously into mice, their malignancy was related to the haplotype expressed, which changed according to the strain of mouse in which the tumour was passed (LALA et al. 1980). The authors interpreted this observation as the result of tumour × host fusion followed by extensive chromosome loss giving rise to "isoantigenic variants" (WIENER et al. 1974a) that were insensitive to immune attack at the subcutaneous site of injection. The malignancy of Ehrlich cells passed as ascites showed no such haplotype dependency.

Occasionally an unexpected phenotype has been observed in hybrids produced in vivo. Thus hybrids between host cells and B16 (HU and PASZTOR 1975) or Cloudman (HALABAN et al. 1980) melanomas showed increased melanogenesis in contrast to the usual observation that melanin synthesis is a differentiated function that is extinguished in hybrids produced in vitro (DAVIDSON et al. 1966; SILAGI 1967). It was suggested that this difference might result from gene dosage, or from differences in the cell type with which the melanomas fused in vitro and in vivo.

C. Use of Drug Resistance for the Selection of Hybrid Clones In Vitro

Table 3 gives a selected list of cytotoxic drugs which have been used in cell hybridisation studies. Not all are antitumour agents, but their cellular targets include those important for antitumour agents, and therefore information on the expression of resistance to these agents in hybrid cells should provide insights into the principle modes of expression of antitumour drug resistance in hybrids.

The selection of resistance in many instances is multistep through a series of increasing drug concentrations, in line with clinical experience. Many of the

Table 3 Selectable drug resistance markers used in cell hybridisation studies

Marker designation	Drug selection	Molecular target	Expression in hybrids	Reference
AzaG^r	8-Azaguanine, single-step	HGPRT (hypoxanthine guanine phosphoribosyl transferase)	Recessive	SZYBALSKI and SMITH (1959) LITTLEFIELD (1963)
TG^r	6-Thioguanine, single-step	HGPRT	Recessive	STUTTS and BROCKMAN (1963)
AzaA^r	8-Azaadenine, multistep	APRT (adenine phosphoribosyl transferase)	Recessive	JONES and SERGENT (1974)
FA^r	2-Fluoroadenine, multistep, 0.05–40 $\mu g\,ml^{-1}$	APRT	Recessive	BENNETT et al. (1966)
DAP^r	2,6-Diaminopurine, multistep, 2.5–100 $\mu g\,ml^{-1}$	APRT	Recessive	LIEBERMAN and OVE (1960) HARRIS and RUDDLE (1961) ATKINS and GARTLER (1968) CHASIN (1974)
FAR^r	2-Fluoroadenosine, multistep, up to 10 $\mu g\,ml^{-1}$	ADK (adenosine kinase)	Recessive	CHAN et al. (1978)
Toy^r	Toyocamycin, single-step, 0.02–0.10 $\mu g\,ml^{-1}$	ADK	Recessive	GUPTA and SIMINOVITCH (1978 d)
Tub^r	Tubericidin single-step, 0.1–1.0 $\mu g\,ml^{-1}$			
MeMPR	6-Methyl thiopurine ribonucleoside, single-step, 2–20 $\mu g\,ml^{-1}$			
AraC^r	Cytosine arabinoside, multistep, 0.5 (low) to 5 $\mu g\,ml^{-1}$ (high)	Complex dCTP pools (low^r) deoxycytidine kinase (high)	Codominant recessive	DE SAINT VINCENT and BUTTIN (1979) DESCHAMPS et al. (1974)
	0.2–2.4 $\mu g\,ml^{-1}$ plus 0.2 $\mu g\,ml^{-1}$ thymidine	Ribonucleotide reductase	Codominant (*araC*^{r}, *TdR*^{r}) TdR auxotrophy, recessive	MEUTH et al. (1979)
BudR^r	Bromodeoxyuridine, multistep	TK (thymidine kinase)	Recessive	KIT et al. (1963) CLIVE et al. (1972)
IUdR^r	Iododeoxyuridine, multistep	TK	Recessive	FOX (1971)

Table 3 (continued)

Marker designation	Drug selection	Molecular target	Expression in hybrids	Reference
FUdR^r	Fluorodeoxyuridine, multistep	TK	Recessive	MORSE and POTTER (1965) SLACK et al. (1976)
emt^r	Emetine, multistep *emt*^{rI}, 0.1–0.2 μ*M* *emt*^{rII}, 3–5 μ*M*	40S ribosome subunit	Recessive emt^{rII} recessive to emt^{rI}	GUPTA and SIMINOVITCH (1978 a)
tri^r	Trichodermin, single-step $10^{-6}M$	60S ribosome subunit	Recessive	GUPTA and SIMINOVITCH (1978 b)
oua^r	Ouabain, single step $10^{-6}M$ (human) $3 \times 10^{-3}M$ (rodent)	Na/K ATPase transport system	Codominant	BAKER et al. (1974) CORSARO and MIGEON (1978)
CH^r	Colchicine, multistep 0.1–5 μg ml^{-1}	High mol. wt. membrane glycoprotein P	Codominant	LING and BAKER (1978)
Dip^r	Diphtheria toxin, single-step 0.01–20 flocullating units	I. membrane receptor II a. elongation factor 2 II b. unidentified protein synthesis factor	Recessive	MOEHRING and MOEHRING (1977) GUPTA and SIMINOVITCH (1978 c) GUPTA and SIMINOVITCH (1980)
Dex^r	Dexamethasone, single-step $10^{-6}M$	Steroid receptor	Recessive	VENETIANER et al. (1978) PFAHL and BOURGEOIS (1980)
Polyene, antibiotic resistance				
Amphotericin B		Sterols	Dominant	FISHER et al. (1979)
Amphotericin B methyl ester				GOLDSTEIN and FISHER (1978)
Fungizone				
Filipin				FISHER et al. (1979)
Primaricin				
Nystatin methyl ester				FISHER et al. (1978)
CL^r	Cyclo-leucine, single-step 4–8 mg ml^{-1}	MAT (methionine adenosine transferase)	Codominant	CABOCHET and MULSANT (1978)

Table 4. Systems for the selection of hybrid clones

Acronym	Selective components	Phenotype selected	Reference
HAT	Hypoxanthine, aminopterin (methotrexate), thymidine	*HGPRT*$^+$ *TK*$^+$	SZYBALSKI et al. (1962) LITTLEFIELD (1964)
HAM	Hypoxanthine, aminopterin (methotrexate), 5-methydeoxycytidine	*Deoxycytidine deaminase*$^+$ *HGPRT*$^+$ *TK*$^+$	CHAN et al. (1975)
HAT dC	Hypoxanthine, aminopterin, thymidine, deoxycytidine	*Deoxycytidine kinase*$^+$ *HGPRT*$^+$ *TK*$^+$	DESCHAMPS et al. (1974)
AA	Alanosine, adenine	*APRT*$^+$	KUSANO et al. (1971)
GAMA	Guanine, adenine, mycophenolic acid, azaserine	*HGPRT*$^+$ *APRT*$^+$	LISKAY and PATTERSON (1979)
AAU	Alanosine, adenosine, uridine	*ADK*$^+$	CHAN et al. (1978)
ArUr	Adenosine, uridine	*Uridine kinase*$^+$	MEDRANO and GREEN (1974)
Systems for use with "universal fusers"			
HOT	HAT + ouabain	*HGPRT*$^+$ *oua*^{r} *or TK*$^+$ *oua*^{r}	CORSARO and MIGEON (1978) WEISSMAN and STANBRIDGE (1980)
–	HAT polyene antibiotics	*HGPRT*$^+$ *antibiotic*^{r} *or TK*$^+$ *antibitiotic*^{r}	FISHER et al. (1978, 1979) GOLDSTEIN and FISHER (1978)

markers involve the purine and pyrimidine salvage pathways. Mutants can be selected that are resistant to toxic analogues because they are deficient in target enzymes required for the incorporation of exogeneously supplied purines and pyrimidines into DNA and RNA. If such mutants are grown in the presence of methotrexate (amethopterin) then de novo synthesis of purine and pyrimidines is prevented and the mutants die. Complementation between different mutants can be demonstrated by the rescue of hybrid clones from methotrexate toxicity by salvage of normal nucleotide precursors. Thus LITTLEFIELD (1964) demonstrated intergenic complementation between mutants resistant to 8-azaguanine ($HGPRT^- TK^+$) and bromodeoxyuridine ($HGPRT^+ TK^-$) by selection in medium containing hypoxanthine, aminopterin and thymidine (HAT medium). A number of other selective systems have been devised based on this principle (Table 4). Cell lines called "universal fusers" have used in situations where it is desirable to produce hybrids with wild-type cell lines or primary cultures. Such cells carry two mutations, one recessive, the other dominant. On fusion with a wild-type cell, the hybrid phenotype expresses the dominant wild-type allele that complements the recessive mutation, as well as the dominant mutation of the universal fuser (Table 4).

The use of these selection systems has yielded information concerning gene expression in hybrids, which will now be discussed.

D. Expression of Drug Resistance in Hybrid Cells

I. Dominance and Complementation

The HAT system of Littlefield described above is an example of intergenic complementation in which one parent contributes an active enzyme for which the other parent is deficient. The active allele is thus dominant over the inactive, recessive allele. Recessive mutations may be due to deletion of a gene (no product) or alteration of the coding sequence resulting in an altered product that may be inactive or partially active. If the gene product is a protein consisting of several identical subunit polypeptides, then occasionally the subunits of two altered proteins may interact to produce an active protein. Such intragenic complementation has been observed in rare *HGPRT* mutants having altered electrophoretic mobilities (CHASIN and URLAUB 1976). Since it requires the interaction of two different mutants of the same gene, intragenic complementation is unlikely to occur in vivo. Enzyme deficiency in mutants might also result from repression of mRNA synthesis, and KADOURI et al. (1978) produced evidence for a transacting dominant repressor of HGPRT. Such a mechanism is clearly important since it would permit the spread of TG^r in tumour-host hybrids formed in vivo. However, we have been unable to confirm this observation (BOYLE and FOX 1980).

Dominant gene expression implies the production of an altered gene product or an increase in the amount of gene product. In hybrids, wild-type and resistant alleles may be expressed together and result in resistance intermediate between that of the wild-type (sensitive) parent and the resistant mutant. This incomplete dominance, or codominance, is observed for resistance to ouabain (BAKER et al. 1974; ROBBINS and BAKER 1977) due to modification of the membrane-bound

Na^+/K^+-dependent ATPase; to colchicine (LING and BAKER 1978) due to increased production of a high molecular weight membrane glycoprotein; and to cycloleucine (CABOCHET and MULSANT 1978) due to increased levels of methionine adenosyl transferase. Resistance to several polyene macrolide antibiotics which affect sterol binding has also been shown to be dominant, but the membrane target of this resistance has not been defined (FISHER et al. 1979). Other chemotherapeutic agents to which resistance is codominant are α-amanitin (LOBBAN and SIMINOVITCH 1975), hydroxyurea (LEWIS and WRIGHT 1979) and some classes of methotrexate resistance (FLINTOFF et al. 1976).

The selection of mutants resistant to different concentrations of a drug can result in a series of mutants whose alleles show markedly different expression in hybrids. Thus Chinese hamster cells selected in 0.5 μg/ml araC (class I) were cross-resistant to excess thymidine (TdR) and had an expanded pool of deoxycytidine-5′-triphosphate (dCTP). When selected in 5 μg/ml araC only class II mutants were obtained which exhibited resistance to high levels of araC (50 μg/ml) and were deficient in deoxycytidine kinase. In hybrids with wild-type, araC-sensitive cells, class I mutants were recessive and class II mutants were codominant (DE SAINT VINCENT and BUTTIN 1979). A third class of *araC*^{r} mutant has been described (MEUTH et al. 1979) which is resistant to 0.2–2.4 μg/ml araC when grown in the presence of 0.2 μg/ml TdR. These mutants require TdR for growth, and in hybrids TdR auxotrophy is recessive whereas resistance to araC and TdR is dominantly expressed. Reversion to TdR prototrophy is accompanied by reversion to araC and TdR sensitivity, suggesting that a single mutation controls both auxotrophy and resistance and the authors favour an altered ribonucleotide reductase.

Low and high levels of resistance to emetine, which is a potent inhibitor of protein synthesis through its action on 40S ribosome subunits, are expressed by mutant classes *rI* and *rII* that are both recessive to emetine sensitivity. High-level *rII* mutants are also recessive to *rI* in hybrids (GUPTA and SIMINOVITCH 1978 a). Although hybrid cells produce both *em*^{s} and *em*^{r} 40S subunits the dominance of sensitivity in this case is due to the mode of action of emetine which blocks the passage of ribosomes along the mRNA (GUPTA and SIMINOVITCH 1978 b).

II. Gene Dosage and Functional Hemizygosity

When resistance results from a recessive mutation of an autosome-linked gene, mutation of only one homologue often results in partial resistance. Such is the case with resistance to pyrimidine analogues (CLIVE et al. 1972), purine analogues (RAPPAPORT and DEMARS 1973; JONES and SERGENT 1974) and dexamethasone (BOURGEOIS and NEWBY 1977; PFAHL et al. 1978; PFAHL and BOURGEOIS 1980). The phenomenon arises because the cellular concentration of the target macromolecules is dependent on the number and proportion of recessive and dominant alleles that a cell inherits.

Gene dosage can also affect the phenotype of cells when resistance results from a dominant autosomal mutation, as with class IIb resistance to diphtheria toxin. Subunit B of the toxin binds to specific membrane receptors that are altered or deficient in class I mutants, whereas subunit A causes ADP ribosylation of elongation factor 2, thus interfering with translation of mRNA. Class IIa mu-

tants possess altered elongation factor 2 which is insensitive to ADP ribosylation. In hybrids class I mutations are recessive whereas class IIa mutations of Chinese hamster ovary (CHO)-K1 cells are codominant (MOEHRING and MOEHRING 1977), although apparently similar mutations in Chinese hamster V79 cells behave recessively (GUPTA and SIMINOVITCH 1980). A second class of mutant (IIb) with altered protein synthesis was described by GUPTA and SIMINOVITCH (1978c) which showed 50% inhibition of protein synthesis in the presence of toxin, implying that only one of a pair of homologous chromosomes carried the mutant allele, and that both wild-type and mutant alleles were being expressed equally. In these mutants the resistant allele (*R*) was codominant with the wild-type allele (*S*). However, when the mutant (*R*/*S*) was fused with a wild-type cell (*S*/*S*) the resulting hybrids were sensitive, indicating that three sensitive alleles were dominant over one resistant allele. Class IIb mutants are apparently affected in an unidentified protein synthesis factor since these mutants complement those of class IIa (GUPTA and SIMINOVITCH 1980).

The interpretation of gene dose relationships is clearly dependent on a knowledge of the number of gene copies per cell, their distribution among chromosomes and what proportion are functional. The question of functional ploidy was raised by DEAVAN and PETERSON (1973) and reviewed by SIMINOVITCH (1976), who argued that the relatively high frequently with which recessive mutations arise in CHO cells might be due to the production of functionally hemizygous portions of chromosomes as a result of extensive chromosomal rearrangements. This idea was substantiated by GUPTA (1980), who showed that hemizygosity was not restricted to one or a few chromosomal regions. SICILIANO et al. (1978), who examined the isoenzyme patterns of electrophoretic shift variants from 11 different loci and found that the majority expressed both wild-type and mutant isozymes, concluded that CHO cells are only as functionally hemizygous as would be expected of a slightly hypodiploid cell line.

III. Multifunctional Enzymes

During the past 5 years evidence has been accumulating that, as in prokaryotes, one mechanism of coordinate regulation in mammalian cells is the association of related enzymic activities in a single multifunctional structure (reviewed KIRSCHNER and BISSWANGER 1976). Such complex enzymes are known for tetrahydrofolate metabolism (PAUKERT et al. 1976; TAN et al. 1977) where formyltetrahydrofolate synthetase, methenyltetrahydrofolate cyclohydrolase and methylene tetrahydrofolate dehydrogenase reside in a protein of one (pig liver) or two (sheep liver) identical subunits. Pig liver also contains formiminoglutamate-tetrahydrofolate formiminotransferase and formiminotetrahydrofolate cyclodeaminase activities, in a protein of probably eight identical polypeptides (DRURY et al. 1975).

The elegant analyses of pyrimidine biosynthesis by groups led by PATTERSON and STARK have revealed three complementation groups, urd A, B and C (Fig. 4). *urd* A^- mutants selected as uridine auxotrophs simultaneously lose carbamyl phosphatase, aspartate transcarbamylase and dihydroorotase activites, which are regained simultaneously in revertants (PATTERSON and CARNRIGHT 1977). These observations are consistent with an active enzyme of molecular weight 600,000

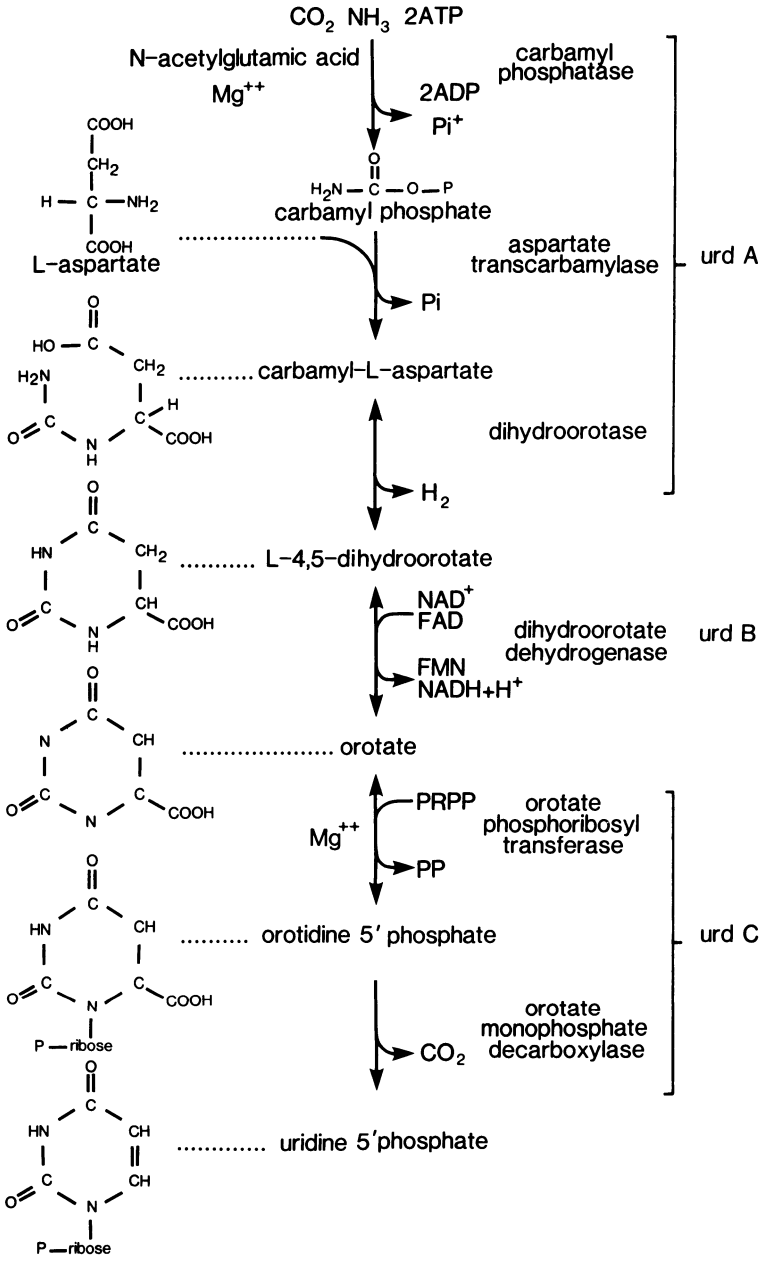

Fig. 4. Pathway of de novo synthesis of pyrimidines, showing the relationships between the biochemical pathway, genetic complementation groups urd A, B and C, and the steps catalysed by multifunctional enzymes (*bracketed*)

consisting of a trimer of identical polypeptides (COLEMAN et al. 1977; DAVIDSON et al. 1979). All three activites are overproduced in mutants resistant to *N*-(phosphonacetyl)-L-aspartate (PALA), which inhibits aspartate transcarbamylase (KEMPE et al. 1976). *urd* B^- is represented by a mutant of the fourth en-

zyme in the pathway, dihydro-orotate dehydrogenase (STAMATO and PATTERSON 1979). Unlike all the other enzymes of pyrimidine synthesis, which are found in the cytosol, this one is located in mitochondria although coded by the nucleus (CHEN and JONES 1976). *urd* C^- mutants show a simultaneous deficiency in the last two enzymes of pyrimidine synthesis, orotate phosphoribosyltransferase (OPRT) and orotate monophosphate decarboxylase (ODCase). In CHO cells which lack uracil phosphoribosyl transferase, the conversion of 5-fluorouracil (FU) to the nucleotide is dependent on OPRT, and hence OPRT-deficient mutants can be isolated that are resistant to the toxic effects of FU (PATTERSON 1980). Patients with orotic acid uria are similarly deficient in both OPRT and ODCase (KROOTH 1964). In order to grow most *urd* C^- mutants need to salvage uridine. However, some do not and these are most probably partially defective in OPRT and ODCase since there is no complementation between uridine-requiring and non-requiring mutants (PATTERSON 1980). Mutants of all three complementation groups have mutations that are recessive to wild-type alleles, and since *urd* A^- and *urd* C^- mutants complement each other, the multifunctional activites of these two classes exist on separate polypeptides.

IV. Steroid Resistance and Enzymic Induction by Hormones

Many lymphoid cell lines are killed by high concentrations of glucocorticoid sterols. This results from a chain of events starting with the binding of glucocorticoid to a specific membrane-bound receptor that is activated and transports the glucocorticoid to the nucleus (nuclear transfer). Here the hormone-receptor complex binds to DNA and initiates an as yet poorly understood mechanism which finally leads to cell death. Mutants resistant to high concentrations of dexamethasone have been classified into four classes distinguishable biochemically and genetically as being affected at different stages of hormone interaction (GEHRING 1980). Mutants resistant to killing can either lack the receptor (r^-) or have normal receptors but fail to perform nuclear transfer (nt^-) of the receptor-hormone complex. In a study of dexamethasone resistance in mouse S49 lymphoma and WEHI 7 thymoma PFAHL and BOURGEOIS (1980) found no complementation between r^- and nt^- mutants, supporting the idea that both r^- and nt^- are alleles of r^+. Fusions between wild-type Dex^s (r^+) and Dex^r (r^-) cells resulted in Dex^s hybrid cells, indicating dominance of r^+, accompanied by the synthesis of widely differing numbers of receptor sites in different hybrid clones.

A third phenotype (nt^i) of S49 lymphoma cells, reported by YAMAMOTO et al. (1976), was characterised by increased affinity of the receptor-steroid complex for DNA that was due to abonormal nt^i receptors of molecular weight 50,000 compared with molecular weight 90,000 for wild-type receptors. In wild-type $\times$ nt^i hybrids synthesis of both types of receptors occurred (codominance), and sensitivity of dexamethasone was codominant when cloning ability was measured: $nt^i \times r^-$ failed to complement. Thus in each case involving mutant receptors sensitivity to dexamethasone was dominant.

A fourth class of Dex^r mutant has been described in mouse L cells which apparently has normal steroid receptors (VENETIANER et al. 1978). Most Dex^r (r^+)

mutants were cross-resistant to the glucocorticoids dexamethasone (9-α-fluoro-16-α-methyl prednisolone), prednisolone, cortisolone, corticosterone, aldosterone and also non-glucocorticoids 17-α-methyl testosterone, progesterone and 17-β-esteradiol when inhibition of [^{3}H]-thymidine uptake was the end point measured. Fusions of $Dex^r(r^+) \times Dex^s(r^+)$ fibroblasts showed sensitivity to be dominant, as was also observed in hybrids between $Dex^r(r^+)$ mouse lymphoma and $Dex^s(r^+)$ mouse myeloma (GEHRING et al. 1972). Other reports of $Dex^r(r^+)$ mutants have been made in other murine and human cell lines (YAMAMOTO et al. 1976; LIPPMANN et al. 1974) although HUET (1979) reported that only $Dex^r(r^-)$ or $Dex^r(nt^-)$ mutants could be isolated from WEHI 7 thymoma after a variety of treatments inducing point mutations, deletions, chromosome rearrangements and chromosome loss. YAMAMOTO et al. (1976) coined the term "deathless" (d^-) for their mutants, implying that resistance was due to some defect in cell killing after nuclear transfer had occurred. There has been no study of complementation between d^- mutants and the other three classes (GEHRING 1980).

In contrast to the dominant lethal effects of glucocorticoids in lymphoid cells the ability of glucocorticoids to induce enzymes in hepatoma cells is a recessive phenotype. Thus in rat hepatoma cells tyrosine aminotransferase (TAT) and alanine amino transferase can be modulated from low constitutive levels to high induced levels by exposure of cells to dexamethasone (SCHNEIDER and WEISS 1971; WEISS and CHAPLAIN 1971; SPARKES and WEISS 1973), a property that is lost (extinguished) upon hybridisation with mouse fibroblasts or rat epithelial cells. Subclones isolated some time after hybridisation show loss of some chromosomes (Sect. D.V) and the reappearance of inducibility. In some subclones inducibility of alanine aminotransferase was re-expressed in the absence of inducibility of tyrosine aminotransferase, indicating that steroid induction of these enzymes may have some steps that are independent. Rat hepatoma hybrids also show extinction of enzymes that are not inducible (BERTOLOTTI and WEISS 1972 a, b). On the other hand, inducible enzymes are not always extinguished. Thus BENEDICT et al. (1972) made hybrids between mouse 3T3 fibroblasts, which have arylhydrocarbon hydroxylase inducible by benz(*a*)anthracene, and rat hepatoma cells with TAT inducible by dexamethasone. The hybrids produced inducible hydroxlase at levels the same as, to 20-fold greater than, the 3T3 parent, while TAT was not inducible, even though most hybrids contained nearly complete sets of chromosomes from both parents. Hydroxlase induction is therefore a dominant trait in hybrids and involves a mechanism that is different to induction of TAT.

V. Segregation of Resistance

The conversion of polykaryons into hybrid cells occurs at mitosis when two or more genomes become aligned on a common spindle apparatus, and is followed by the subsequent formation of hybrid nuclei at telophase. The probability that a fusion event will give rise to a viable hybrid cell capable of producing a clone is very low. Even under optimal experimental conditions where more than 50% of nuclei are in heterokaryons, the frequency of hybrid clones is rarely more than one per hundred cells fused (BOYLE and FOX 1980; BOYLE et al. 1977; RECHSTEINER and PARSONS 1976; DAVIDSON 1969). By the use of interspecies fusions involving

cells with morphologically distinct nuclei, or by labelling the nuclei of one parent with [^{3}H]-thymidine prior to fusion, it is possible to follow the fate of parental chromosomes during the first four divisions after fusion. In human × mouse fusions (RECHSTEINER and PARSONS 1976) and human × rat kangaroo (*Potorous tridactylis*) (PETERSON and BERNS 1979) the chromosomes from different nuclei tended to remain separate during the initial mitosis after fusion, mingling in subsequent mitoses, although separation of human and mouse chromosomes was still seen in some hybrid colonies containing eight cells. In half the human × rat kangaroo metaphases, chromosomes were left at the metaphase plate at anaphase and became trapped by the constricting mid-body during cytokinesis. In polykaryons containing nuclei of different ages, interphase nuclei that were in close proximity to mitotic nuclei were observed to go into mitosis before completion of the cell cycle (PETERSON and BERNS 1979). The interphase chromosomes went through a process of premature chromosome condensation (PCC, JOHNSON and RAO 1970; JOHNSON et al. 1970) and presented different morphologies depending on the phase of the cycle they were in before condensation. Prematurely condensed G1 and G2 chromosomes were entire and had one or two chromatids respectively, whilst condensed S-phase chromosomes appeared pulverised. RAO and JOHNSON (1972) demonstrated a correlation between PCC involving S-phase nuclei and a reduced chromosome complement in derived hybrid clones. PCC appears largely confined to the first mitosis after fusion (RECHSTEINER and PARSONS 1976), since the chromosomes in homokaryons rapidly become synchronised at mitosis and at the initiation of DNA synthesis (GRAVES 1972). Trapping of chromosomes and PCC provide two mechanisms that contribute to the phenomenon of chromosome loss in hybrid cells.

Loss of human chromosomes from human × rodent hybrids can be extensive, and the concordant loss of a biochemical phenotype with a specific chromosome has been a fruitful method for assigning genes to chromosomes (MIGEON and MILLER 1968; MATSUYA and GREEN 1969; KAO and PUCK 1970). (For recent reviews of human gene mapping the reader is directed to MCKUSICK and RUDDLE 1977 and HUMAN GENE MAPPING 1978, 1979). The method for assigning genes to chromosomes assumes that chromosome segregation occurs randomly *in a population*. However, it is worth pointing out that *within a cell* chromosome loss may not be entirely random, the loss of one chromsome influencing the loss of others, an observation that may result from the compartmentalisation of chromosomes at mitosis described above (MARIN and PUGLIATTI-CRIPPA 1972; RUSHTON 1976).

In general, intraspecies hybrids tend to be more stable than interspecies hybrids (SINISCALCO et al. 1969; NADLER et al. 1970; SOBEL et al. 1971; HANDMAKER 1973; SPURNA and NEBOLA 1973; WORTON et al. 1977) although some exceptions have been described (ENGEL et al. 1969 a, b, 1971). It is possible that this observation is more apparent than real, being governed by the rodent and human cell types available for fusion. If, instead of fusing primary human cells with heteroploid rodent cells, one fuses heteroploid human cells with primary rodent cells then rodent chromosomes are preferentially lost instead of human (MINNA and COON 1974; CROCE 1976). Although the factors controlling chromosome loss are obscure, there may be some relationship between these observations and those of

RUSSELL et al. (1979), who demonstrated an initially rapid segregation of chromosomes in mouse hybrids between heteroploid and euploid cells as compared with heteroploid × heteroploid hybrids or euploid × euploid human cells (MIGEON et al. 1974; HOEHN et al. 1975). Some progress towards understanding the genetic control of segregation, at least in rodent × human hybrids, has come from the intriguing observation that transcription of rRNA genes is suppressed from the chromosomes of the species that will show preferential chromosome loss in human × rodent hybrids (PERRY et al. 1979; DEV et al. 1979), whereas the rRNA genes of both parents are transcribed in rodent × rodent hybrids (MILLER et al. 1978; WEIDE et al. 1979). Mouse-human hybrids also appear to lose the mitochondrial DNA of the parent whose chromosomes are preferentially lost (ATTARDI and CROCE 1980). Because of the implications for gene mapping, attempts to influence the direction and extent of chromosome loss have also been made by the selective production of damage in the chromsomes whose loss was desired (PONTECORVO 1971, 1974; GOSS and HARRIS 1977; LAW and KAO 1978; GRAVES 1980) (see also Sect. E.II).

Chromosome segregation appears to be the main cause of re-expression of recessive alleles in hybrid cells, although recombination (WORTON et al. 1980) and epigenetic events (HARRIS 1975) have also been suggested as possible mechanisms. RUSSELL et al. (1977) inferred that segregation chiefly accounts for chromosome loss, while recombination, i.e. translocations, accounted for chromosome heterogeneity. Loss of the X chromosomes was correlated with segregation of X-linked markers from intraspecies hybrids of human (BENGTSSON et al. 1975), mouse (HASHMI and MILLER 1976) and Chinese hamster (FARRELL and WORTON 1977). The application of selective pressure can cause preferential loss of chromosomes from the parent complement carrying the allele selected against. Thus growth of Chinese hamster ($HGPRT^-$) × mouse (TK^-) hybrids with 6-thioguanine or bromodeoxyuridine resulted in loss of many mouse or hamster chromosomes respectively (MARIN and PUGLIATTI-CRIPPA 1972). Similarly chromosomal segregants can be selected by immune mechanisms (KNOWLES and SWIFT 1975; COLLINS et al. 1975) which may be significant in producing hemizygosity in vivo. Chromosome loss may also be important in the expression of recessive drug resistance mutations occuring in autosomes (see Sect. D.II), causing the "unmasking" of a recessive mutation by removal of the wild-type allele upon loss of the homologous chromosome (CHASIN and URLAUB 1975).

The frequency with which recessive alleles are re-expressed in hybrid populations can vary widely. In hamster × mouse hybrids the frequencies of resistance to 6-thioguanine and bromodeoxyuridine (BUdR) varied from $<10^{-5}$ to 3×10^{-2} and from $<10^{-5}$ to 7×10^{-3} respectively (MARIN and MANDUCA 1972). The greater stability of intraspecies hybrids may be reflected by lower segregation frequencies, as in mouse hybrids where segregation of 8-azaguanine (8-AZG) was 5×10^{-6}–5.2×10^{-4} (SPURNA and NEBOLA 1973). In quasi-tetraploid Chinese hamster hybrids the *rate* of segregation for membrane-defective *8-AZG*^{r}*ts* and phytohaemaglutinin resistance was 5×10^{-5} and 10^{-5} events/cell per generation, and these values increased 40- and 200-fold respectively in quasi-hexaploid hybrids in which the resistance alleles were present at twice the gene dosage ($2r:1s$) but decreased to 0.04×10^{-5} when the sensitive alleles were present at twice the

dosage (1*r*:2*s*) (HARRIS and WHITMORE 1977). Similarly PFAHL and BOURGEOIS (1980) found the frequency of *Dex*^{r} segregants from mouse hybrids (3*r*:1*s*) was about 10^3–10^4 times more frequent than from heterozygous diploid cells (1*r*:1*s*).

VI. Gene Activation

Occasionally hybrid clones are isolated that unexpectedly show a phenotype expressed by neither parent, as with fusion of rat hepatoma cells and mouse fibroblasts or lymphocytes which resulted in the production of mouse serum albumin (PETERSON and WEISS 1972; MALAWISTA and WEISS 1974). Fusion of rat × mouse cells also resulted in hybrids that were unexpectedly resistant to ionising radiations (LITTLE et al. 1972, see Sect. E.I). Such examples presumably reflect changes in regulatory control. Two further examples indicate that activation can be locus specific and can apparently operate against very strong repression mechanisms. Female cells are functionally hemizygous for the X chromosomes, one X chromosome becoming inactive early in embryogenesis (LYON 1971). In hybrid cells, active X chromosomes usually remain active and inactive chromosomes remain inactive (SINISCALCO et al. 1969; SILAGI et al. 1969; MIGEON et al. 1974). However, exceptional human × mouse hybrid clones have been isolated carrying an active gene on an otherwise inactive X chromosome (KAHAN and DEMARS 1975; HELLKUHL and GRZESCHIK 1978). Localised derepression occurred at a rate of 10^{-6} per inactive X chromosome per cell generation and was maintained in the absence of any other human chromosome (KAHAN and DEMARS 1980). There have also been a number of reports of *HGPRT*$^+$ human × *HGPRT*$^-$ rodent hybrids which expressed rodent HGPRT, despite the fact that the rodent parent cells were previously thought to contain HGPRT deletions due to the extremely low frequency or absence of reversion at this locus (WATSON et al. 1972; CROCE et al. 1973; BAKAY et al. 1973, 1978). In the light of KAHAN and DEMARS' observations it is possible that some HGPRT "deletions" may represent locally inactive regions on the X chromosome that can be occasionally reactivated by the conditions used for cell hybridisation (SHIN et al. 1973), through a mechanism involving DNA methylation.

The pattern of methylation of DNA at the 5-position carbon atom of cytosine appears to be an important epigenetic mechanism controlling tissue differentiation (review, RAZIN and RIGGS 1980), lack of methylation of critical cytosine residues being correlated with transcriptional activity. Hypomethylation can be induced experimentally by growing cells with 5-azacytidine, which replaces cytosine in DNA, but cannot be methylated because of a nitrogen atom at position 5. The pattern of methylation is inherited in daughter cells in the absence of more 5-azacytidine, supposedly because the critical sites are in palindromic sequences which are monitored by a maintenance methylase that methylates daughter strands in half-methylated palindromes. Using 5-azacytidine to induce hypomethylation in a human × mouse hybrid MOHANDAS et al. (1981) were able to reactivate the human *HGPRT* gene on an inactive X chromosome. BOEHM and DRAHOVSKY (1981) reported that MNU (1-methyl-1-nitrosourea) caused hypomethylation of the DNA of human Raji cells, thus suggesting the possibility of an epigenetic origin for some dominant "mutations" induced by alkylating agents. The converse, i.e. hypermethylation, might explain some forms of recessive "mutation".

E. Radiation Responses of Hybrid Cells

I. Sensitivity to Ionising Radiation and Ultraviolet Light

Studies of the radiation sensitivity of hybrid cells were stimulated by reports that mouse and rat hybrids were unexpectedly twofold more resistant to X-rays (ratio of Do values) than their parent cell lines (LITTLE et al. 1972) and were cross-resistant to α-particles (ROBERTSON and RAJU 1980) and also to actinomycin D and cordycepin, suggesting that X-ray resistance might involve some aspect of RNA metabolism (ROBERTSON et al. 1977). Resistance was not associated with enhancement of the repair of either sublethal or potentially lethal damage.

Later reports on a range of inter- and intraspecies hybrids showed that the resistance of rat × mouse hybrids was exceptional. The sensitivities of hybrids showed the same range as that previously observed with tetraploid cell lines (LIMBOSCH et al. 1974; ZAMPETTI-BOSSELER et al. 1976; BOYLE et al. 1979). From the fusion of a pair of cell lines, hybrid clones can be selected with sensitivities ranging from similar or intermediate to the parent cells, to marginally more resistant. Resistance appears to be a dominant phenotype and is unstable, its loss being associated with loss of chromosomes in some but not all cases (BOYLE et al. 1979; ROBERTSON and RAJU 1980).

Similar results have been obtained for hybrids exposed to ultraviolet light. Hybrid sensitivity was similar to (ROMMELAERE and ERRERA 1972; ROBERTSON et al. 1977) or less than (BOYLE et al. 1979; ZAMPETTI-BOSSELER et al. 1980) the parental cell lines, and the resistant phenotype was unstable (PETROVA 1977; BOYLE et al. 1979).

The variable sensitivity of hybrid cells probably reflects the polygenic control of DNA repair and cellular recovery mechanisms. These observations may be significant for adjuvent therapy and perhaps give a clue to the likely responses of hybrids to chemotherapeutic agents that damage DNA.

Mouse L5178Y lymphoma cells (LS) are relatively radiation sensitive with reported Do values of 40–60 rads (ZAMPETTI-BOSSELER et al. 1976; DALE 1979) compared with 100–200 rads for the majority of mammalian cells. Complementation of the sensitive phenotype was achieved after hybridisation with a radiation-resistant L5178Y variant (LR) or with Chinese hamster fibroblasts and mouse L cells (DALE 1979; ZAMPETTI-BOSSELER et al. 1976). FOX (1979) showed that resistance to UV and ethyl methane sulphate (EMS) was also dominant in LS × LR hybrids. The sensitivity of LS cells reflects their lymphocyte origin; hence these results suggest that hybridisation in vivo of lymphoid tumours with cells of other tissues could markedly affect the response of the tumour to DNA-damaging agents.

II. Rescue of Genes from Lethally Irradiated Cells

As described earlier, hybrid clones resulting from the fusion of TG^r and $BUdR^r$ cell lines can be selected in HAT medium because the respective genotypes $HGPRT^- TK^+$ and $HGPRT^+ TK^-$ are complementary for the enzymes that salvage exogenous hypoxanthine and thymidine. HARRIS (1972) demonstrated that even if one of the fusion partners was lethally irradiated it was still capable of contributing the complementary allele for hybrid selection. These experiments paral-

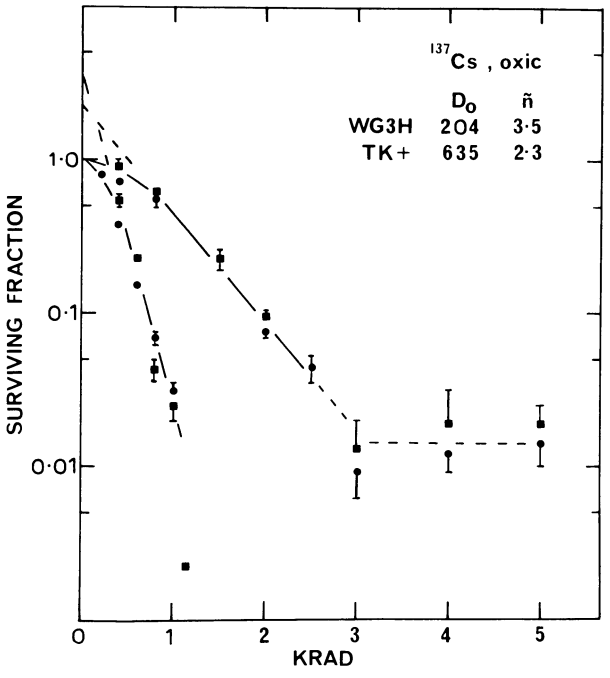

Fig. 5. Cell survival, and rescue of TK^+ by cell fusion following γ-irradiation. Chinese hamster wg3h cells (TK^+, $HGPRT^-$) were irradiated in oxic suspensions with the indicated doses of γ-radiation from a ^{137}Cs source and cell survival measured by colony formation in growth medium. Cell samples from each radiation treatment were also fused with unirradiated Chinese hamster a23 cells (TK^-HGPRT^+) and the frequency of hybrid clones expressing TK^+ derived from irradiated wg3h cells was measured by colony formation in HAT-selective medium. *Circles* and *squares* represent two separate experiments. *Left curve,* survival of wg3h; *right curve,* survival of TK^+ hybrids. [BOYLE (1979), reproduced by kind permission of Taylor and Francis Ltd]

leled those of PONTECORVO (1971, 1974), who demonstrated that irradiation of either mouse or Chinese hamster cells prior to fusion led to the preferential loss of the irradiated chromosomes from the resulting hybrids (Sect. D.V). However, irradiation of mouse cells prior to fusion with human cells did not reverse the genetically controlled preference for loss of human chromosomes.

Another way of expressing HARRIS' observations is that the unirradiated fusion partner is able to rescue a functional salvage enzyme gene from the irradiated genome. Since hybrid clones are produced in inverse proportion to the radiation dose, the Do value (^{1}/slope) is an index of the sensitivity of those processes leading to the rescue of the marker gene. When compared with the radiation sensitivity of the colony-forming ability of cells irradiated, marker rescue was approximately two to eight times more resistant for fusions involving different markers and different cell lines (KINSELLA et al. 1976; BOYLE et al. 1977; JULLIEN et al. 1978). For hybridisation between a given pair of cell lines the ratio Do marker rescue: Do cell survival was fairly constant between 3 and 4 for cells irradiated with X-rays, ^{137}Cs and 14-MeV neutrons, and in air or under hypoxic conditions (BOYLE 1979)

(Fig. 5). Marker rescue data from high-LET (linear energy transfer) radiations (14 MeV neutrons) have been analysed by target theory and resulted in a target volume that was equivalent to 0.54%–0.91% of the DNA of a Chinese hamster cell (BOYLE 1979). Since this is orders of magnitude larger than a gene, the implication is that genes are inactivated largely by events occurring outside their coding sequences.

At X-ray doses above 2–3 krad there is an abrupt change in slope of the marker rescue curve at about 1% survival, the curve now becoming infinitely more resistant to further radiation (JULLIEN et al. 1978; BOYLE 1979). Indeed, hybrids have been isolated even after doses to one partner of 20 krad (MEGUMI 1976). It has been suggested (BOYLE 1979) that for doses up to the inflexion point marker gene inactivation may result from loss of markers in acentric fragments produced by chromosome breaks occurring between the centromere and the marker. With increasing dose further breaks will reduce the acentric fragments to a size that can readily undergo recombination with intact chromosomes. At doses above the inflexion point further marker inactivation may be the result of damage sustained within the marker gene. One implication of this interpretation is that the frequency with which genes syntenic with the marker allele will be retained in hybrids will be largely dependent on their linkage to the selected marker. This has been born out in practice and developed into a radiological method of gene mapping by GOSS and HARRIS (1977).

Marker rescue has also been demonstrated after exposure of one parent to other agents that damage DNA, e.g. ultraviolet light (BOYLE et al. 1977) and methyl nitrosourea (J. M. Boyle, unpublished results), but not after exposure to acute thermal shock (HARRIS 1972).

When both partners were irradiated prior to fusion the dose response for hybrid colony formation was either intermediate between the two parents (JULLIEN et al. 1978) or marginally more resistant by virtue of an increased shoulder on the survival curve (BOYLE et al. 1977).

F. Conclusions: Possible Therapeutic Implications of Cell Hybridisation

In considering the possible implications of these genetic studies we are concerned primarily with drug resistance in tumour cells. We have seen that in animals tumour cells can fuse with each other and with host cells to produce clonogenic mononucleate hybrid cells. The frequency with which this occurs is apparently high, since hybrids can be detected as early as 24 h after tumour inoculation (BER et al. 1978; LALA et al. 1980). An approximate estimate of the proportion of hybrids in tumours was 10^{-5}–10^{-6} (see discussion following WIENER et al. 1974b). If fusion occurs at similar frequencies in humans then hybrid clones may be well established by the time clinical diagnosis is made and therapy started. Because malignancy is a recessive character (HARRIS et al. 1969) tumour × tumour hybrids will be at an advantage over tumour × host hybrids until the latter have segregated the wild-type alleles that suppress malignancy. However, the animal studies clearly demonstrate that tumour × host hybrids do proliferate.

Within the hybrid populations drug-resistance mutations can accumulate prior to therapy, although presumably they will not usually confer any advantage on the cells possessing them until challenged by therapy. Recessive mutations will be masked by wild-type alleles and dominant mutations may be subject to gene dose effects which only allow partial expression. The enlarged gene pool may also favour the generation of dominant resistance by gene amplification (BIEDLER et al. 1974) and recessive resistance by gene inactivation which has been postulated to result from translocation of euchromatin adjacent to heterochromatin (MORROW 1977) or by changes to the pattern of DNA methylation (Sect. D.VI).

The full expression of resistance in hybrid cells may require the unmasking of the mutation by removal of the wild-type allele in cases where resistance is autosomally linked. Segregation by loss of the homologous chromosome carrying the wild-type allele is probably the most important means whereby unmasking occurs and has been demonstrated in vitro for X-linked 6-thioguanine resistance in intraspecies Chinese hamster hybrids (CHASIN and URLAUB 1975). The frequency of segregation of intraspecies hybrids in vitro is about 10^{-5} and is affected by a number of variables including the ratio of sensitive to resistant alleles and the cell types involved (Sect. D.V). The segregation rate in vivo is unknown. Presumably it may be at least as high as in vitro and could be higher due to immune selection of segregants and the clastogenic effects of some drugs once therapy has started.

The frequent observation of tumours with karyotypes in the triploid-hypotetraploid range is consistent with segregation from a tetraploid origin. If the general concept that polyploidy, whether derived by hybridisation or not, is a means of harbouring mutations has any validity, then tumours from patients resistant to therapy might be expected to show higher ploidy than tumours from a similar group of patients before therapy. LEISTENSCHNEIDER and NAGEL (1979) reported such a situation in a group of 26 patients with prostatic carcinoma. Ten patients had received no treatment and had tumours with ploidies ranging from diploid to tetraploid. Sixteen patients had received treatment and their tumours were resistant to either cyproterone acetate (an antiandrogen) or estracyte (estramustine phosphate). Of these the karyotypes of three were mainly tetraploid, seven were 2n–6n and six were 2n–8n. The bases of resistance were not explored, nor is it known whether resistance to these agents is dominant or recessive; hence it is not possible to assess the importance of gene dosage in this context. Clearly it would be useful to have more data relating karyotype and drug resistance in tumours where polyploidy is a feature. The current use of combination therapy in humans makes experimental tumours in animals attractive, particularly where such studies can be augmented by the in vitro manipulation of cell lines with suitable genotype.

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