REVIEW

CELL FUSION AND SOME SUBCELLULAR PROPERTIES OF HETEROKARYONS AND HYBRIDS

SAIMON GORDON

From the Genetics Laboratory, Department of Biochemistry, The University of Oxford, England, and The Rockefeller University, New York 10021

I. INTRODUCTION

The technique of somatic cell fusion has made it possible to study cell biology in an unusual and direct way. When cells are mixed in the presence of Sendai virus, their membranes coalesce, the cytoplasm becomes intermingled, and multinucleated homo- and heterokaryons are formed by fusion of similar or different cells, respectively (1, 2). By fusing cells which contrast in some important biologic property, it becomes possible to ask questions about dominance of control processes, nucleocytoplasmic interactions, and complementation in somatic cell heterokaryons. The multinucleate cell may divide and give rise to mononuclear cells containing chromosomes from both parental cells and become established as a hybrid cell line able to propagate indefinitely in vitro. The chromosome constitution of the hybrid cells can then remain relatively stable, as in intraspecific crosses, or may be unstable, as in man-rodent interspecific hybrids which tend to lose human chromosomes selectively, as first noted by Weiss and Green (3). This combination of cell mating and chromosome segregation makes it possible to perform genetic analysis on somatic cells, to assign human gene products to individual chromosomes, and to study the control of gene expression in animal cells.

Originally, cell hybridization was an uncontrolled, apparently spontaneous event which occurred rarely when different cells were simply mixed and co-cultivated. Barski et al. (4), and Ephrussi and Weiss (5) isolated and studied the first cell hybrids obtained by this method. The isolation of hybrid cells from such mixed cultures was greatly facilitated by the Szybalski et al. (6) and Littlefield (7, 8) adaptation of a selective medium containing hypoxanthine, aminopterin, and thymidine (HAT) to mammalian systems.

Further progress followed the use of Sendai virus by Harris and Watkins to increase the frequency of heterokaryon formation (9). These authors exploited the observation by Okada that UV irradiation could be used to inactivate Sendai virus without loss of fusion efficiency (10). It was therefore possible to eliminate the problem of virus replication in fused cells. Since many cells carry receptors for Sendai virus, including those of different species, it became possible to fuse a variety of cell types, including differentiated and nondividing cells.

The technique of cell fusion has already been applied to the study of many problems in the genetics and cellular biology of somatic cells. The purpose of this paper is to provide a selective review of experimental studies that are of special interest to the cell biologist, with emphasis on the principles and pitfalls underlying the use of this technique. Topics which have been extensively reviewed elsewhere will not be discussed in detail (11–14). These include linkage analysis and chromosome assignment (15), virus rescue by cell hybridization (12), and the application of cell hybridization to the study of malignancy (16).

II. METHODS

The genetic analysis of somatic cells by hybridization depends on the availability of efficient methods to fuse cells and purify hybrids. In this section, we deal with the use of viral and other agents to increase the frequency of heterokaryon formation, and we review our understanding of the mechanism of fusion. We also examine the use of mutant cells in devising suitable hybrid selection systems and consider the application of new methods of cell enucleation to fusion of subcellular fractions.

A. Cell Fusion

Sendai virus is the agent used most frequently to promote cell fusion. Like other paramyxoviruses it consists of a ribonucleoprotein core and a lipoprotein envelope (17). Virus particles mature by budding from the cell surface, and the envelope contains virus-determined proteins and lipids incorporated from the host cell. Several biological properties of paramyxoviruses have recently been associated with particular envelope proteins (18–20). Viral hemagglutinin and neuraminidase activity are found in a spike glycoprotein (HN) of mol wt 69,000. Cell-fusing activity seems to be associated with the presence of a 53,000 mol wt glycoprotein (F) which can be generated from a larger precursor molecule (F_o) by limited proteolytic cleavage in vivo or in vitro. Sendai virus grown in different host cells can differ in the content of this glycoprotein, which may also play a role in virus-induced hemolysis and the initiation of infection. However, the ability to fuse cells may also depend on the presence of other components of the viral envelope (21).

For the purpose of cell fusion, UV light (22) or β-propriolactone (23) is used to inactivate the virus, most of which is subsequently degraded within phagocytic vacuoles of the cell. When used in moderate concentrations, the virus has no obvious deleterious effect on the fused cell. A relatively high concentration of inactivated virus fuses cells "from without" by direct interaction with the plasma membrane, but cells can also be fused "from within" after replication in the cell. A measure of control of fusion efficiency is possible by promoting cell contact, either in suspension or on a surface, and by varying the proportion of each cell type used. The susceptibility of different cells to fusion varies a great deal, presumably depending on the presence of virus receptors and on the chemical composition of the plasma membrane of the cell (17). Other viruses that promote cell fusion, such as Newcastle disease virus, have been little used for the purpose of cell hybridization.

A chemical fusion procedure would be desirable, to avoid using a virus as a reagent and to fuse cells refractory to virus (24). Lysolecithin and other lipids have had limited use hitherto, partly because they often cause extensive cell lysis (25). Fusion by microsurgery perhaps offers more promise as an alternative (26, 27). Preselected cells, synchronized in telophase, can be aligned and induced to fuse by micromanipulation. The bicellular hybrids can then be isolated without the use of selection media. Although this method may be more cumbersome than mass fusion, it offers a unique opportunity for control and direct observation of early clone evolution. It should also facilitate the fusion of cells which differ in ploidy, so that the influence of dosage on subsequent gene expression could be studied to advantage. Microsurgical enucleation and fusion may cause less cell damage than cytochalasin B and Sendai virus in particularly delicate renucleation experiments (28, 29).

Finally, freshly prepared plant protoplasts can be induced to fuse by intimate cell contact (30, 31), and powerful "fusogens" of protoplasts such as polyethylene glycol may also be effective in hybridization of mammalian cells.[1]

THE MECHANISM OF FUSION

INTRODUCTION: Since membrane fusion plays an important role in many cellular functions including endocytosis and secretion, cell fusion could provide a useful model system for studying the biochemical and ultrastructural aspects of the fusion process. In addition to viruses and lipids, a variety of other methods including high temperature (32), Ca^{++} at pH 10.5 (33), or phospholipase C (34) can initiate fusion. These systems offer the advantage of synchronized, massive fusion which can be readily monitored by phase-contrast microscopy. Mammalian erythrocytes (35), nucleated avian erythrocytes, and various other cell types have been used for these studies.

VIRUS-INDUCED FUSION: The possible role of

[1] Pontecorvo, G. Production of indefinitely multiplying mammalian somatic cell hybrids by polyethylene glycol (PEG) treatment. Manuscript submitted for publication.

specific viral components in cell fusion has already been mentioned. Viral hemagglutinin and neuraminidase may contribute an ancillary function in promoting cell contact and modification of the plasma membrane. The virus binds to sialylglycoprotein receptors on the cell surface and can elute from the cell by hydrolysis of terminal neuraminic acid residues (36). Studies with liposomes suggest that gangliosides also serve as binding sites (37), in line with earlier observations that a high ganglioside to phosphatidyl ethanolamine ratio of cellular lipids is associated with enhanced fusibility (17). Cell receptors for paramyxoviruses may differ with cell type, e.g., T vs. B lymphocytes (38), and can even be absent, as in horse erythrocytes (39). Virus can be bound to such cells by concanavalin A and will induce hemolysis, but cell fusion has not yet been demonstrated in this instance.

Several stages can be distinguished in the fusion process (40). The first step of viral binding to the cell surface is independent of ions and occurs readily in the cold. Subsequent fusion is temperature dependent. Membrane between closely adjacent cells disappears, and membrane continuity is established. Cells in suspension often become spherical at this stage. Okada (40) suggested that membrane interruption precedes a repair process, and others have also noted a close relationship between cell lysis and fusion. Changes in cell permeability without lysis have also been described (41).

Fusion is independent of cellular protein synthesis, RNA synthesis, or DNA synthesis (40) and is not accompanied by significant turnover of phospholipids or other membrane components (42). Virus-induced fusion is optimal at pH 7.6–8.0. Divalent cations such as Ca^{++} and Mn^{++} are critical for fusion in most cases and may play a role in membrane stabilization or in a repair process since cell lysis ensues in their absence. Fusion also depends on a source of energy, and combined treatment with dinitrophenol and virus induces cell lysis (43). Oxidative phosphorylation or glycolysis can apparently drive the fusion reaction (44), but human erythrocytes and their ghosts have been fused in the absence of ATP (33). Much remains to be learned about the source and role of cellular energy in fusion.

Local anesthetics such as dibucaine, drugs of the phenothiazine class (45), and cytochalasin B (41) inhibit fusion. These compounds act either directly on the plasma membrane or by an indirect mechanism.

Morphologic studies hitherto have not resolved conflicting hypotheses whether fusion occurs directly between cells or via an intermediate step of fusion with the viral envelope (46–48). Recent studies with newer methods are beginning to shed more light on the initial stages of virus-cell interaction. After brief periods of incubation of virus with nonendocytic cells such as erythrocytes, the pattern of distribution of viral components suggests that the viral envelope is able to fuse directly with the plasma membrane (49). Studies with ferritin-labeled antibodies show that viral antigens do not remain as clusters, but probably become dispersed within the plane of the membrane. The use of combined freeze-fracture and immunologic labeling techniques (50) indicates that intramembranous particles, which may contain the glycoprotein receptors for virus (51), become aggregated during the early stages of fusion. It is not yet clear how specific these changes in membrane structure are or what role viral neuraminidase may play at this stage. It would be of interest to compare membrane changes after exogenous virus treatment with membrane changes that may occur during fusion "from within", since viral antigens remain clustered in relation to underlying nucleoprotein cores during budding (17).

LIPID-INDUCED FUSION: Although it is not clear to what extent membrane fusion by viruses and lipids involves a common mechanism, the use of defined lipids makes it possible to study several aspects of fusion in a systematic fashion (52). Unsaturated fatty acids, such as oleic acid, and their esters as well as shorter-chain saturated fatty acids and retinol are all able to fuse avian erythrocytes, especially if dextran is added to control cell lysis (53). Lipid-induced erythrocyte fusion also requires Ca^{++} ions, but it occurs at a lower pH optimum (pH 5–6) than fusion by virus. Negative staining reveals new macromolecular assemblies when lecithin, sphingomyelin, and phosphatidylserine interact with "fusogenic" lipids, but the significance of these structural changes is unclear (54).

The fusion of negatively charged phospholipid vesicles in the presence of Ca^{++} has also been reported (55). Ultracentrifugation and gel filtration were used to demonstrate a transfer of ^{3}H-labeled lipid from small to larger vesicles and to exclude exchange diffusion or aggregation. Lipo-

somes prepared from phospholipids or by incorporating long-chain cationic molecules with a lytic lipid are also able to induce fusion of animal cells (56, 57). The incorporation of lipid vesicles into cells by fusion provides a potential method for introducing foreign materials directly into the plasma membrane or cytoplasm. Other mechanisms such as endocytosis, surface absorption, and molecular exchange could also play a part in these model systems.

Many of these observations have been interpreted in terms of the model of a mosaic membrane which contains liquid regions with a high degree of independent mobility (58). Lipids that are more fluid when measured by differential scanning calorimetry may be more effective in promoting fusion (55). The possible importance of micellar structures (50) and membrane ATPase (45) in membrane fusion has also been discussed.

B. Selection of Hybrids

Heterokaryons form a minority of the cell population in a complex fusion mixture, and only a small fraction of these (0.1–10%) is able to evolve into a permanently hybrid cell line. It is therefore necessary to provide the potential hybrid with a selective growth advantage over parental cells. Selection procedures are also used to recover subpopulations of hybrid cells, often by back selection against the trait originally used. In addition to selecting for the ability to grow in vitro, it is also possible to perform selection in vivo to recover malignant segregants from heterogeneous cell populations.

In the simplest case, differential adherence or growth can form a basis for selection, e.g., human peripheral blood lymphocytes grow in suspension and do not proliferate continuously in culture except after transformation with Epstein-Barr virus and are therefore readily eliminated during serial passage. However, in the case of established cell lines it is usually necessary to use for hybridization a strain which bears a stable conditional lethal marker. If such a trait is recessive, complementation can occur after fusion, and the hybrid cell survives selectively under nonpermissive conditions. Selection for or against a dominant or codominant trait, on the other hand, depends on its continued expression in the hybrid. Selection strategy may involve both parent cells or only one. Multiple markers make it possible to isolate hybrids without using the mutation under study.

Suitably marked diploid fibroblast and pseudodiploid lymphoid cell lines can be derived from patients with inborn errors of metabolism whose cells express their genetic defect in culture. Genetic variants of established heteroploid cell lines such as mouse fibroblasts (L), human HeLa, or Chinese hamster ovary (CHO) cells can be obtained by mutagenesis and selection. (For detailed review, see references 60 and 61.)

Littlefield's HAT selection system remains in wide use. Cells that lack the salvage pathway enzymes hypoxanthine guanosine phosphoribosyl transferase (HGPRT), an X-linked marker, or thymidine kinase (TK), on chromosome 17, cannot survive treatment with aminopterin, which blocks endogenous purine and pyrimidine biosynthesis. Such a deficiency can be the result of an inborn error as in the Lesch-Nyhan syndrome (HGPRT), or can be introduced by mutagenesis and selection with nucleoside analogues such as azaguanine (HGPRT) or bromodeoxyuridine (TK). These particular traits have the advantage that they are stable in the absence of selection pressure and that back selection can be readily performed.

Caution is necessary, however, to avoid uncritical use of selective markers in somatic cells since their genetic status as mutants and their biochemical nature often remain undefined. The expression of a recessive mutation in a diploid or heteroploid cell may depend on loss or inactivation of the homologous locus. Causes other than structural changes in an enzyme can contribute to drug resistance, including defective cell penetration and indirect effects on enzyme regulation. On the other hand, cross-reacting mutants (CRM) have been demonstrated in some $HGPRT^-$ cells (62, 63), and mapping of a trait to a particular chromosome certainly justifies its use as a genetic marker.

In practice, the efficiency of selection can be subverted in several ways. Apart from genetic heterogeneity, culture conditions such as cell density may counteract selection if metabolic cooperation between cells in contact permits the direct exchange of nucleic acid precursors and drugs via gap junctions (64, 65). The culture medium may contain serum enzymes (66) or other nucleosides that interfere with selection (67). Unexplained reversion to wild-type phenotype may occur in the course of a hybridization experiment (68–70). It is therefore important to characterize a particular marker before and after hybridization and to define its possible variation during changes in cell culture.

Another widely used selection system was devised by Puck and his co-workers who developed a family of nutritional auxotrophic variants of CHO cells (71). After treatment with a mutagen, cells that cannot grow in nutritionally deficient medium survive selectively, whereas growing cells are allowed to incorporate BUdR and can be killed by exposure to near-visible light. Mutants requiring glycine and adenine have been studied in most detail and illustrate the wide range of biochemical genetic studies possible with such a family of mutants (72, 73). (See Section III B.) Cell hybridization between pairs of CHO mutants results in stable retention of chromosomes. Studies with such homologous hybrids show that glycine- and adenine-requiring mutants are recessive and comprise four and six complementation groups, respectively. Heterologous hybridization with human cells and selection ir deficient medium result in the rapid loss of unnecessary human chromosomes, so that the different human chromosomes which relieve the auxotrophy can be identified. Nutritional and biochemical analyses can then be combined with genetic studies to determine the point of metabolic block in each cell. These mutants also lend themselves to the study of control genes that may be linked to the nutritional marker (74).

Drug-resistant variants and suitable selection procedures have been developed for other nucleoside salvage pathway enzymes such as adenosine phosphoribosyl transferase (APRT) on chromosome 16 (75–77), and uridine kinase (66). Drug markers for a variety of other cellular functions are also available (78), including agents which act on cell surface components. Ouabain-resistant mutants have been obtained from CHO and mouse L cells by one-step selection and remain stable in the absence of the drug (79). The variants are resistant to ouabain inhibition of ^{42}K uptake by intact cells and of Na^+/K^+ ATPase activity in isolated plasma membranes, but a specifically altered membrane component has not yet been identified. Human cells are 10^4-fold more sensitive to ouabain than rodent cells. Ouabain resistance is expressed as a codominant phenotype and can be used to select hybrids. In contrast, resistance to other drugs such as colchicine results from membrane impermeability and may be pleiotropic if several drugs share a common transport system (80).

Further development of selection techniques should permit the controlled production of hybrids carrying individual human chromosomes. Surface markers such as the species antigen, assigned to chromosome 11 (81), and β_2-microglobulin, assigned to chromosome 15 (82), can be exploited for this purpose. X-autosomal translocations make it possible to select against autosomal chromosomes by using an X-linked marker such as HGPRT (83). In turn, hybridization provides a genetic tool with which to analyze the biochemical nature of any group of closely related mutant cell strains. Cell mutants also provide suitable markers for attempted gene and chromosome transfer to somatic cells.

C. Enucleation

The recent development of an efficient method for enucleating mammalian cells by exposure to cytochalasin B and high-speed centrifugation (84–86) has made it possible to use Sendai virus-induced fusion as a tool to recombine subcellular fractions of different origins. The enucleation procedure yields a karyoplast fraction that contains the nucleus, 10–20% of the cell's cytoplasm, a portion of the plasma membrane, and a cytoplast fraction that contains the remaining cytoplasm and plasma membrane (87). The karyoplasts contain a variety of cytoplasmic organelles, but lack centrioles or microtubules and therefore lose their cell form and motility (88). These cell fractions cannot regenerate lost organelles and will die unless they can be "rescued" by fusion with intact cells (89) or other enucleated cell fractions (28, 29, 90). Chick erythrocyte nuclei have been reactivated after fusion with cytoplasts, and some of the nuclei have synthesized RNA after transfer, but continued viability of these reconstituted cells remains in question.

Micronucleation can be induced in mammalian cells by treatment with colchicine (91). Enucleation of such micronucleated cells make it possible to introduce a smaller portion of a cell genome into other cells by fusion.

The development of these techniques holds promise for future cell reconstitution experiments and for further study of nucleocytoplasmic relationships in somatic cells. Cytoplast fusion can also be used to introduce cytoplasmic markers into hybrids (Section IX) (92).

III. GENERAL PROPERTIES OF FUSED CELLS

The event of fusion initiates many dramatic changes in cell structure and function. In this

section, we review some of the general principles that determine the properties and evolution of heterokaryons and hybrids and consider methods for analysis of each class of fused cell. Several of these topics are dealt with in detail in later sections.

A. Heterokaryons

Usually, each parent contributes a nucleus, plasma membrane, and a variety of cytoplasmic organelles to the newly formed heterokaryons, although a nucleus or cytoplasmic constituents may be lacking in mammalian erythrocytes or enucleated cell fractions. The newly fused cell subsequently undergoes a striking reorganization of its cellular architecture. The plasma membrane becomes a mosaic structure as a result of redistribution of membrane components (93) (Section IV). Cytoplasmic organelles are originally distributed at random within the heterokaryon, but cellular organization is restored within a few hours by means of a colchicine-sensitive process. In macrophage homokaryons the nuclei assume a characteristic clockface distribution, and the giant cell develops a single centrosphere region with lipid droplets and mitochondria distributed peripherally (94). In huge syncytia formed by fusion of baby hamster kidney cells, the nuclei are redistributed in long parallel rows following their extensive migration along microtubules (95).

Nuclear changes are equally striking. When a condensed chick erythrocyte nucleus is introduced into the cytoplasm of an HeLa cell, for instance, the nucleus swells considerably and undergoes reactivation (Section V) (96). RNA and DNA synthesis are reinitiated when inactive cells are fused with cells engaged in nucleic acid synthesis (22) (Section VI). If the fused cell enters mitosis, the nuclear envelopes disappear, and the chromosomes become condensed. Very little is known about spindle formation and the mechanism of chromosome distribution to hybrid daughter cells. Many fused cells abort at this stage or within the next few divisions.

METHOD OF ANALYSIS: The study of heterokaryons depends mainly on methods of single-cell analysis. Identification of heterokaryons and their composition is easy initially, if the nuclei have a distinctive morphology, but may become difficult later. Nuclei can also be identified by prelabeling one nucleus with an isotopic marker or by exploiting the fluorescent staining properties of sex chromatin (97). Cytoplasmic markers are less reliable because of possible transfer to other cells, while plasma membrane markers may vary in expression after fusion.

Heterokaryon systems offer several advantages. It is possible to generate an unselected, large population of independent fusion events and to compare dosage effects in the absence of gene loss. Complementation studies are therefore feasible and have been used to discover heterogeneity in genetic diseases such as xeroderma pigmentosum (Section VII), galactosemia (98), maple syrup urine disease (99). and lysosomal storage diseases (Section X). Disadvantages of heterokaryon systems include heterogeneity of the fused cell population, low yield, and the difficulty in distinguishing preformed products from those formed after fusion. Unless turnover is extremely rapid, as with tyrosine amino transferase (100), the short lifespan of heterokaryons before entry into mitosis is a particular handicap. The use of γ-irradiation to prevent cytokinesis (101) is limited to particular cell types and may change the nuclear and cytoplasmic properties of the heterokaryon.

B. Hybrids

For the hybrid to evolve into an established cell line, at least one parent has to contribute a capacity to proliferate. The genotype of the hybrid depends in a striking manner on the species-relatedness of the parents. Intraspecific hybrids retain more than 90% of the sum of the parental chromosomes and remain remarkably stable over long periods (102). Interspecific human-mouse (3) and human-Chinese hamster hybrids are usually unstable and lose human chromosomes selectively while retaining an apparently complete set of rodent chromosomes. The rate of chromosome loss in interspecific hybrids can vary considerably. Hybrids formed with the CHO cell line originally used by Puck and co-workers lose human chromosomes particularly rapidly, within 2 wks, whereas in man-mouse hybrids the rate of chromosome loss is slower (103). The rodent chromosome complement is often duplicated in man-rodent hybrids, and these inter-species hybrids tend to lose human chromosomes more slowly. Human-rodent hybrids prepared by fusion of freshly isolated mouse, rat, or hamster embryonic tissues with a human heteroploid line, VA_2, can be more stable or even show a reverse pattern of rodent chromosome loss (104, 105).

The mechanisms that determine chromosome loss in hybrids are not known. Most of the human

chromosomes are probably lost in early divisions and, although chromosome loss may proceed with continued cultivation, these hybrids become sufficiently stable for genetic analysis. Human-mouse hybrids derived from independent fusions clearly retain different sets of human chromosomes, but further analysis of a wide spectrum of hybrids is needed to establish whether chromosome loss is truly random.

Hybrids formed between closely related rodent species show a more variable karyotype and may lose chromosomes of either parent. In the case of hybrids of mouse macrophage and Chinese hamster cells, the chromosomes of the nondividing macrophage parent are lost selectively and can be used for linkage analysis (106). Hybridization of a mammalian cell with avian erythrocytes can result in retention of a single avian chromosome (107) or, possibly, a fragment (108) (Section V).

The resulting hybrid displays a mosaic phenotype determined by the interactions between structural and regulatory genes retained from each parent. Genes involved in cellular functions that are common to many cell types, such as "household" metabolic functions, are readily expressed in hybrids whereas tissue-specific "luxury" functions are usually regulated in a more complex fashion and are often extinguished (Section XI).

METHOD OF ANALYSIS: The strategy of chromosome assignment and linkage analysis in hybrids which segregate chromosomes of one species has been reviewed by Ruddle (109). Multiple independent hybrid clones are isolated and expanded to provide sufficient material for biochemical and chromosome analysis. Subcloning and back selection can then be applied to recover a set of closely related hybrids which differ in the presence of particular chromosomes. Extensive species differences in primary structure of proteins provide a large library of suitable markers for analysis (110). Charge differences make it easy to distinguish between many human and rodent enzymes by simple electrophoretic procedures. In the case of polymeric enzymes, unique hybrid bands prove biosynthesis within a common cytoplasm. Antigenic species differences can also be exploited. Genetic analysis in intraspecific hybrids, on the other hand, has been limited by the paucity of strain-specific chromosomal markers, but follows the same general principles (111).

The identification of chromosomes in hybrids has been greatly improved by the development of empiric banding techniques, such as quinacrine fluorescence (112) and a variety of Giemsa's and other staining procedures (113–115). Mouse chromosomes can be distinguished from human chromosomes by their characteristic centrometric heterochromatin. *In situ* hybridization can also be used to identify regions of considerable redundancy (116). These procedures make it possible to identify individual chromosomes as well as regions of translocation.

The identification and correlation of gene products and chromosomes in man-rodent hybrids has made it possible to define syntenic relationships among products which segregate together and to assign their chromosomal location. Almost every human chromosome has been assigned at least one marker, and, in some cases, up to ten markers (14). A panel of hybrids containing a particular range of chromosomes should make it possible to map a great number of human genes in this way. Translocations (117), γ-irradiation (118), and agents such as adenovirus 12 (119, 120) which induce chromosome breakage at specific sites can be exploited for regional localization.

In addition to mapping structural genes, linkage analysis in hybrids makes it possible to assign control genes if reappearance of a trait can be correlated with the loss of a particular chromosome (74, 121). The participation of multiple genes in more complex cellular functions, such as antiviral activity, can also be identified (122, 123).

The chromosomal instability of proliferating hybrids, therefore, serves to generate systematic genetic variability upon which selective pressures can be brought to bear. Improved methods are still required to produce specific or more extensive chromosome loss in hybrids (124, 125).

In the remainder of this Review, we consider some of the subcellular properties of somatic cells that have been studied by the technique of cell fusion.

IV. PLASMA MEMBRANE

A. Heterokaryons

MEMBRANE FLUIDITY: Cell fusion makes it possible to combine two different types of cell membrane and observe subsequent membrane reorganization. Early studies with heterokaryons suggested that the species antigens of human and mouse tumor cells became intermixed after fusion (126) and that the plasma membrane ATPase of mouse macrophage became diffusely

redistributed over the surface of heterokaryons formed with melanoma cells (94).

Frye and Edidin (93) provided further evidence for an intramembrane-mixing process in newly formed heterokaryons. They used a double-labeling fluorescent antibody technique to detect cells which became mosaic for mouse *H-2* and human species antigens. Total intermixing of fluorochromes had occurred 40 min after adding Sendai virus and was also observed in some human-mouse hybrids. Inhibitors of protein synthesis did not affect the formation of mosaic cells, even after pretreatment for 6 h, nor did the use of dinitrophenol and sodium fluoride, which depleted cell ATPase content by 80%. At lower temperature, mosaic cell formation was prevented; and a plot of percent mosaic cells vs. temperature produced a sigmoidal curve. The chemistry, synthesis, turnover, or precursor pools of these antigens are not well defined, but it is unlikely that an independent drug-insensitive compartment for antigen synthesis exists in heterokaryons. The authors interpreted their findings as evidence for a temperature-dependent diffusion process within the membrane. Further studies suggest that the rate of intermixing may differ and that some restriction on antigen diffusion operates in other heterokaryons (127). The nature of an apparent phase transition observed between 15°C and 20°C has not yet been established.

These studies provided important evidence in support of the concept of membrane fluidity and the mosaic model of membrane structure (58). Similar conclusions have been drawn from subsequent studies on cap formation in lymphocytes as a result of antibody-induced redistribution of surface components within the plane of the plasma membrane (128). Further studies on heterokaryons with defined markers at a higher resolution may be useful, although it must be recognized that analysis of surface structures could be considerably complicated by capping, endocytosis, shedding, and masking. It would be of interest to know whether plasma membrane reorganization in heterokaryons also proceeds by a colchicine-sensitive process (94, 129).

RECEPTOR EXPRESSION: Apart from changes due to membrane intermixing, other factors may affect the expression of membrane components in fused cells. Surface markers, such as species antigens (126) common to many cell types, continue to be expressed in heterokaryons. In the case of erythrocyte heterokaryons, the antigens that are introduced at fusion decay, and there may be a significant lag period of several days before chick-specific surface antigens are resynthesized (Section V) (101). Other markers such as macrophage-specific Fc receptors are drastically altered as a result of fusion with cells such as mouse melanoma cells whch do not express them (94, 130). The ability of the macrophage to bind and ingest antibody-coated sheep erythrocytes disappears progressively after fusion, in proportion to the melanoma cell:macrophage ratio. The apparent extinction of the macrophage receptors is due to surface masking by melanoma-determined proteins, and the receptors can be unmasked by mild treatment with proteolytic enzymes such as trypsin. It is not known whether the protease relieves steric block of the receptor or whether it promotes clustering of membrane receptors dispersed after heterokaryon formation. The continued biosynthesis of cell-specific membrane receptors, such as the Fc receptor, has not yet been demonstrated in heterokaryons.

B. Hybrids

Surface antigens were used as cell markers in some of the earliest hybridization experiments (131), but their genetic analysis has lagged for several reasons. They are rather complex gene products, their chemical structure is poorly understood, and their detection in cultivated cells is often handicapped by variable expression and unreliable assays. Nevertheless, recent progress in this area suggests that cell hybridization will also prove useful in analysis of membrane structure and function.

Early studies showed that species antigens were readily detected in hybrids and that their structural genes were apparently widely distributed over the human genome, since they could still be detected in human-mouse hybrids that retained as few as four human chromosomes (3). Other studies suggested that antigen and chromosome loss could, however, provide a basis for eventual gene assignment (132). Puck and co-workers subsequently described a human species antigen linked to lactate dehydrogenase A (LDH A), in human-hamster hybrids (133) and a similar antigen in human-mouse hybrids has been assigned to chromosome 11 (81).

The expression of histocompatibility antigens in mouse hybrids has been analyzed by Klein and his colleagues (16, 134). *H-2* antigens are usually expressed as codominant products, but they can be suppressed in certain cells, either selectively or in

combination with other cell antigens (135). Ehrlich ascites tumor (EAT) cells lack detectable *H-2* antigens and are able to suppress in a nonspecific manner the *H-2*, C-virus-specific and L-cell antigens of mouse L cells after hybridization. This is probably due to surface-masking properties of the EAT cell acquired during propagation in histoincompatible animals. *H-2* can be re-expressed in EAT hybrid clones, presumably following chromosome loss, but the chromosomes responsible for suppression have not been identified. In contrast, the lack of *H-2* in another cell type, a subline of the TA_3 ascites carcinoma, behaves like a recessive trait and can be reversed by fusion with other mouse fibroblasts, as can the Moloney virus-determined surface antigen of YACIR lymphoma cells (136). Detailed analysis of malignant phenotype of hybrids showed that *H-2* and polyoma-specific tumor antigen expression are independent of tumorigenicity.

The major human histocompatibility (HL-A) antigens are linked to PGM_3 and have been assigned to chromosome 6, mainly on the basis of family studies (137, 138). β_2 microglobulin, which is physically associated with HL-A molecules at the cell surface, has been assigned to chromosome 15 (82). The gene for the heavy chain of immunoglobulin, with which β_2-microglobulin shares 20–30% sequence homology, has not yet been assigned.

It has been claimed that the Xg^a antigen, an X-linked marker, can also be detected in hybrid cells (139). If confirmed, this should prove useful in studying the position of the Xg locus on the X chromosome and in determining whether Xg^a is subject to X inactivation.

Surface receptors that permit cell penetration by poliovirus (140, 141) and diphtheria toxin (142, 143) have been assigned to chromosomes 19 and 5, respectively. Cell hybridization has also been used to study the expression of more complex membrane functions, such as electrical excitability and chemosensitivity in neuroblastoma cells (144, 145), and the role of membrane junctions in intercellular communication (146). Human diploid fibroblasts that show electrical coupling in culture and have gap junctions are able to correct the defect of noncoupling mouse fibroblasts, which lack gap junctions, by hybridization. As the hybrid cells lose human chromosomes, which have not yet been identified, clones are found among the segregants which have reverted to the noncoupling and junction-deficient trait of the mouse parent cell. Some of the restored hybrids were peculiar in that, while they were coupled electrically, they were not seen to transfer fluorescein, perhaps due to alteration of the passageways in the hybrid.

Tissue-specific macrophage Fc and complement receptors are selectively extinguished after hybridization with cells which lack these receptors, but the mechanism of suppression is not known (147). Lymphocyte-specific membrane receptors, however, have been detected recently in hybrids derived from human lymphoid cells and mouse fibroblasts (W. F. Bodmer, personal communication).

The assignment of the determinants for membrane antigens and receptors to individual chromosomes provides a range of potential selection systems for controlling the chromosome constitution of hybrids and for generating closely related cell variants that differ with respect to particular membrane functions, and it provides a basis for further study of the regulation of receptor expression.

V. NUCLEAR REACTIVATION IN HETEROKARYONS

During differentiation, the chick erythrocyte loses the ability to synthesize DNA, RNA, and most protein, its chromatin becomes condensed, and its nucleolus disappears. The dormant nucleus can be reactivated by cell fusion with HeLa cells, or other metabolically active cells, in a process involving complex nucleocytoplasmic interactions (for a review, see references 1 and 148).

The erythrocyte nucleus swells rapidly, its chromatin becomes more dispersed, and it undergoes drastic physico-chemical changes which manifest themselves in an increased binding of basic intercalating dyes, such as acridine orange and ethidium bromide, and an increased susceptibility to thermal denaturation (149). Nuclear dry mass increases mainly due to migration of human nuclear proteins into the chick nucleus. RNA synthesis is initiated, and newly labeled species of high molecular weight, polydispersed RNA appear in the chick nucleus (101). DNA replication occurs within 24–48 h. Human nucleolus-specific proteins migrate into the chick nucleus (150), and a morphologic nucleolus is reformed within 2–4 days, the exact time depending on the maturity of the chick erythrocyte. Chick ribosomal RNA is synthesized, and chick-specific markers such as surface antigens (101) and receptors, HGPRT (151, 152), and nucleolar antigens (150) are produced.

The chick nucleolus plays a key role in the transfer of chick messenger RNA from nucleus to cytoplasm (153). The new chick products appear parallel with the formation of a nucleolus, and their synthesis can be prevented, or reversed (154), by selective destruction of the nucleolus by UV-microbeam irradiation.

Ringertz and his co-workers have studied the intracellular migration of proteins and antigens during nuclear reactivation with the aid of specific antibodies, some of which were obtained from patients with systemic lupus erythematosus (150, 155). Human nucleolar and nucleoplasmic antigens could be detected in chick nuclei by immunofluorescence soon after fusion with HeLa cells, whereas chick-specific nucleolar antigens appeared in both chick and HeLa nuclei only after synthesis of chick-specific proteins. Since antigens characteristic of human cytoplasm did not enter the chick nuclei, reactivation was associated with selective concentration of human nucleospecific macromolecules.

In subsequent experiments, autoradiographic techniques were combined with re-isolation of chick nuclei from heterokaryons (156). It was shown that preformed or newly synthesized human or mouse proteins rapidly enter chick nuclei during the early stages of reactivation (157, 158). This transfer was independent of chick nucleic acid synthesis and was unaffected by UV-irradiation of the chick erythrocyte before fusion. Electrophoretic and chromatographic analysis of ^{3}H-labeled proteins accumulating in the chick nuclei showed preferential uptake of many subgroups of nonhistone proteins and an altered histone composition, with an increase of f1 histone, derived from the HeLa cell, and loss of chick-specific f2c histones (159). These studies provide evidence for a cytoplasmic pool of HeLa nuclear proteins. Improved characterization of these proteins may prove difficult, and the species origin of individual proteins, such as RNA polymerase, has not yet been established. The possible role of altered nuclear membrane permeability and exchange of ions between nucleus and cytoplasm during nuclear reactivation is unknown.

Reactivation of the chick erythrocyte nucleus in mammalian cell cytoplasm is incomplete in one respect. Although a variety of chick-specific proteins can be produced, hemoglobin synthesis ceases after transient stimulation (1). Ringertz and his colleagues fused chick erythrocytes with rat myogenic cells, which differentiate in vitro, in an attempt to reactivate the erythroid phenotype or to reprogram the chick nucleus to a myogenic phenotype (160). Although intracellular antigen migration was observed (161), neither hemoglobin nor chick myosin was produced in virus-induced heterokaryons. (Rare mononucleated cells, the progeny of unidentified cells, did contain chick myosin.) Heterokaryons formed by spontaneous fusion between chick and rat myoblasts produced both chick and rat myosins, indicating that simultaneous translation of chick and rat RNA's for myosin was possible in a common cytoplasm. Similarly, no chick albumin was detected after reactivation of chick erythrocyte nuclei in rat hepatoma heterokaryons (162). The basis for the differential expression of cell-specific and nonspecific products is not known.

As a result of the reactivation process, DNA synthesis in the erythroid nucleus may lag and be asynchronous with that of its partner nucleus. If the chick chromosomes undergo premature chromosome condensation before complete replication (Section VI), they are readily lost at mitosis, although chick genes for HGPRT (108) and TK (163) can be incorporated in hybrid cell lines that contain no detectable chick chromosomes. If DNA synthesis in the heterokaryon is prevented until the chick erythrocyte nucleus is fully reactivated, as in heterokaryons derived from adenine requiring CHO mutants and chick erythrocytes that have been starved for adenine, both nuclei enter the S phase and mitosis in synchrony, and intact chick chromosomes may appear and segregate in the resultant hybrids (107).

VI. CELL CYCLE REGULATION IN HETEROKARYONS

Cell fusion offers a unique tool with which to study the regulation of growth in eukaryotic cells, by making it possible to combine cells at different stages of their cycle. In such heterophasic fusions, nuclear synchrony is usually imposed at the time of DNA synthesis (S) and during the initiation of mitosis (M) (for review, see references 164–166).

A. DNA Synthesis

DNA synthesis is rapidly induced in the nuclei of G_1 or G_0 cells after fusion with S-phase cells, whereas G_2 nuclei can neither reinitiate DNA synthesis nor inhibit the S phase (164). Mouse peritoneal macrophages, for example, become arrested in G_0 during the course of their differentia-

tion. Upon fusion with actively proliferating mouse melanoma cells, dormant macrophage nuclei show induction of a wave of DNA synthesis (94). The signals for initiation of S are contributed by the melanoma cell since DNA synthesis in the macrophage nucleus is under the control of the melanoma cell cycle and depends on melanoma RNA synthesis (167) and protein synthesis (168). During the early phase of swelling, melanoma-derived proteins that initiate DNA synthesis enter the macrophage nucleus, which plays a passive role. The kinetics of S-phase induction in different nuclei are correlated with their heterochromatin content, presumably reflecting the ease of access of the replicating system to the template.

The cytoplasmic inducers of DNA synthesis have no effect on the duration of S or on the programmed sequence of chromosome replication (169). The factors lack species or cell specificity, and nothing is known about their nature or relationship to cytoplasmic factors which stimulate DNA synthesis in cell-free systems (170, 171).

B. The Duration of G_2

The presence of G_1 or S nuclei inhibits progression of G_2 nuclei into M until DNA replication has been completed (164). This effect has been ascribed to heterophasic condensation and decondensation of chromatin within the same cell. On the other hand, normal G_2 components can partially overcome G_2 delay induced by X-irradiation or by inhibition of protein synthesis, thus allowing transition into mitosis.

C. Mitosis

When a cell in interphase (I) is fused with a mitotic cell, the interphase nucleus may lose its nuclear membrane and nucleolus and undergo premature chromosome condensation (PCC) in a process that resembles normal prophase in many respects (165, 172, 173). The morphology of the prematurely condensed chromosomes varies according to the position at the time of fusion of the interphase cell in its cycle. The prematurely condensed chromosomes in G_1 contain a single chromatid which is very long and coiled, and in G_2 they have two chromatids that are more condensed though still more extended than metaphase chromosomes. PCC in the S phase results in uneven chromosome condensation and a fragmented, pulverized appearance which varies in relation to its stage of DNA replication. It is possible to display constitutive heterochromatin and other banding patterns in such condensed chromosomes (174). These studies suggest that PCC in G_2 and probably also G_1 represent condensation of interphase chromatin into chromosomal elements similar to those normally formed at metaphase. Electron microscope studies of PCC have also been reported (175, 176).

Unlike the analogous condensation of chromosomes observed in endosperm of *Haemanthus* (177) and after nuclear transplantation into maturing *Xenopus* oocytes (178), the induction of PCC in virus-fused cells is not accompanied by formation of a spindle (165). Prematurely condensed chromosomes adopt a position adjacent to the metaphase chromosomes and pass into the progeny of metaphase-interphase fused cells in a varied and complex, apparently random, pattern of segregation. Prematurely condensed chromosomes that are incorporated into daughter nuclei can replicate and reenter mitosis in synchrony with the rest of the genome and thus be functionally retained by the progeny (179). The fusion products of mitotic cells and S-phase cells with PCC are less likely to survive and may lose more chromosomes than hybrids formed by homophasic fusion. The integration of chromatin fragments, generated by PCC, into the genome of another cell could account for the acquisition of chick HGPRT by mouse fibroblasts fused with chick erythrocytes (108). The retention of the chick trait can be unstable, and the nature of the association between chick DNA and mouse DNA in this system, however, is unknown (180).

Instead of inducing PCC in the interphase nucleus, the mitotic chromosomes may themselves clump together, become enclosed in a nuclear envelope (NE), and resemble "telophase-like nuclei" (181–183). The frequency of these two possible outcomes, PCC formation vs. NE formation, depends upon environmental pH and the metaphase/interphase ratio. Nuclear envelope formation in some systems predominates at pH 8.5 and a metaphase/interphase ratio of 0.33.

Neither PCC- nor NE-inducing factors have been isolated, but they are not cell specific or species specific. Cations such as spermine, putrescine, and Mg^{++} promote PCC, whereas spermidine, negatively charged compounds, and 17 β-Estradiol inhibit PCC induction (184). Indirect evidence that proteins play a role in PCC induction comes from experiments by Matsui and co-workers (185) who found that inhibition of protein

synthesis during the last hour of G_2 reduced subsequent PCC induction. Rao and Johnson found that when mitotic cells had been prelabeled with [^{3}H] amino acids during G_2 there was substantial migration of labeled material to the prematurely condensed chromosomes (165). The nature or specificity of these labeled molecules has not been demonstrated.

The induction of PCC provides a unique tool with which to study the mechanism of chromosome condensation, to enable visualization of chromosomes in differentiated interphase cells, and to analyze the effect of X- and UV-irradiation in G_1 cells (186).

VII. DNA REPAIR IN FUSED CELLS

By analogy with bacterial systems, it is often assumed that mammalian cells also effect UV-induced DNA repair by incision, excision, repair replication, and strand rejoining, but the enzymes involved have not been characterized. Cell fusion studies with fibroblasts obtained from patients with an inborn error, xeroderma pigmentosum (XP), have brought new insights into the DNA repair process in somatic cells. Cultured cells from these patients show diminished unscheduled DNA synthesis (UDS) after UV-irradiation and probably lack an early endonucleolytic activity (187). The XP syndrome is due to a rare autosomal recessive gene defect, and patients showing the syndrome include those with the "classical" form of cutaneous photosensitivity and malignancy as well as others with additional involvement of the central nervous system (the De Sanctis Cacchioni syndrome). Patients with classical XP show further heterogeneity in the level of UDS demonstrable in culture (188).

Heterokaryons formed between XP fibroblasts and normal fibroblasts show rapid restoration of UDS (189), and at least three complementation groups have been defined by fusion of XP cells among themselves (97, 190). These conclusions are based on experiments with autoradiography, as well as density labeling, and isopycnic centrifugation of DNA (191). Further evidence that complementation restores an early incision event was obtained by following the disappearance of dimer-containing sites from DNA of UV-irradiated XP heterokaryons with the aid of a UV-specific endonuclease purified from *Micrococcus luteus* (192).

The factor that is responsible for UDS in normal cells is apparently present in excess and in a preformed form, since it can restore UDS after fusion with multiple XP cells and in the absence of further protein synthesis (189, 193). It probably enters the XP nucleus very rapidly after fusion. A dosimetric analysis has been performed to try to identify heterozygous carriers, but this approach is complicated by uncertainty with regard to the relative importance of nuclear transfer, synthesis, and turnover rates in controlling the reaction.

The human factor that restores UDS also enters chick erythrocyte nuclei during reactivation in HeLa cytoplasm, but not in XP fibroblast cells (194). The chick nucleus itself does not complement the deficiency in XP nuclei even after reactivation. Hamster cells can complement the human XP cell defect in segregating hybrids, confirming the absence of species specificity for the factor (195).

Cell fusion has thus been useful in demonstrating genetic heterogeneity, but further progress awaits identification of the enzymes involved. The use of UV-irradiation in the selection of hybrids also has potential interest (195).

VIII. RIBOSOMES AND PROTEIN SYNTHESIS

The formation and function of the nucleolus during chick nuclear reactivation and in the expression of chick gene products in heterokaryons have already been discussed (Section V). In this section, we review studies on ribosomal RNA and related proteins in hybrid cells.

A. RNA

The study of RNA in interspecific fused cells depends on the availability of suitable species markers. The 28S ribosomal RNA (rRNA) from the larger ribosomal subunit of a number of species can be distinguished by polyacrylamide gel electrophoresis (196) or by specific ribosome dimerization (197). Eliceiri and Green first observed that only mouse 28S RNA could be detected in human-mouse hybrids that contained up to 35 human chromosomes per cell (198). The 32S human RNA precursor was also not found. These results were confirmed in subsequent studies (199, 200).

Both mouse and hamster ribosomes were found in mouse-hamster hybrids, however, and the hamster-specific ribosome dimers contained dimers of hamster 28S RNA (201). Both types of rRNA were synthesized in hybrids containing only about one-half the haploid number of either mouse or

hamster chromosomes, although a disproportionately higher percentage of RNA of the species contributing the majority of the hybrid cell chromosomes was present. Mouse-rat and human-hamster hybrids resemble mouse-hamster and human-mouse hybrids, respectively, with regard to 28S RNA content (202).

The mechanism that accounts for the absence of human 28S RNA in human-rodent hybrids is not known. The genes for rRNA have been assigned to the satellite regions of human chromosomes 13, 14, 15, 21, and 22 by *in situ* hybridization (203). Detailed karyotypic studies with newer techniques have not been reported for the human-mouse hybrids although several human acrocentric chromosomes have been identified in some hybrids (202). It is unlikely that all of the human chromosomes that bear RNA genes are absent in every case, but individual chromosomes, such as those bearing the 5S RNA gene assigned to chromosome 1 by *in situ* hybridization (204), could also be obligatory for 28S RNA expression. Also of interest is the recent report by Marshall et al. (202) that human-rodent heterokaryons, which show no chromosome loss, continue to produce both species of 28S rRNA for as long as 10 days after fusion, in contrast with their corresponding hybrids, although the proportion of human RNA did diminish with time.

Further studies are needed to establish the detailed karyotype and the possible presence of RNA precursors before ascribing the loss of human 28S RNA in the interspecific hybrids to selective inactivation of nucleolar activity, similar to that described in other systems (205, 206). It would also be interesting to examine the RNA's of rodent-human hybrids, which show more stability or reverse segregation (104), as well as early stages of hybrid cell evolution before prolonged selection in vitro (207).

B. Proteins

The processing of RNA may involve the function of specific proteins. Toniolo and Basilico isolated a temperature-sensitive hamster cell mutant which failed to produce 28S rRNA at nonpermissive temperatures, possibly due to a defect in processing, since hamster 28S RNA could be restored by hybridization with mouse cells (207).

The genetic analysis of ribosomal proteins has lagged because of the paucity of electrophoretic markers (208), ascribed to strong conservation of these proteins, and is further complicated by variation associated with physiologic activity (209). Drug-resistant mutants and immunologic markers have not yet been developed.

Nonribosomal proteins involved in protein synthesis, however, can be studied readily by somatic cell hybridization. The aminoacyl-tRNA synthetases, which occur in a soluble form or as a multienzyme complex, comprise a family of such proteins.[2] Tryptophan-tRNA synthetase from mouse and man can be readily distinguished by electrophoresis and has recently been assigned to chromosome 14. Cell mutants with temperature-sensitive leucyl-tRNA synthetase have also been isolated (210) and could be useful for further hybridization studies.

IX. MITOCHONDRIA

Cell hybrids provide a novel tool with which to study the interactions between nucleus, cytoplasm, and mitochondria in mammalian cells. Segregating interspecific hybrids are most useful for this purpose since some species of nuclear and mitochondrial DNA, rRNA, and proteins can be distinguished by physico-chemical techniques. The fate of mitochondria contributed by each parent cell at the time of fusion has not been studied.

A. DNA

In the earliest studies, human-mouse hybrids that were segregating human chromosomes were examined by density gradient centrifugation and were found to contain only mouse mtDNA (211, 212). Recent investigation by Coon and co-workers showed that both human and rodent mtDNA's could be demonstrated in a new set of human-rodent hybrids which either were more stable or showed "reverse" segregation (Section III B) (104). These hybrids segregated either human or rodent chromosomes, and each species of mtDNA could be distinguished by specific in vitro hybridization with RNA prepared from highly purified DNA templates. Both types of mtDNA were found in clonal populations, excluding cell admixture, and either species could be lost with further cultivation. A positive correlation was found between the loss of nuclear DNA and the loss of mtDNA of each species, though not strictly so. The segregation of mtDNA proceeded further and more rapidly than that of nuclear DNA. In

[2] Denney, R., and I. Craig. Localization of a gene for tryptophanyl-tRNA synthetase (EC6.1.1.2) to human chromosome 14. Manuscript submitted for publication.

addition, there appeared to be a strong tendency to lose human mtDNA. The chromosomes that could play a role in maintaining long-term replication of mtDNA in these hybrids have not been identified.

Horak and co-workers also put forward evidence that these hybrids contain a high proportion of recombinant mtDNA molecules (213). Rodent and human mtDNA's were identified after CsCl gradient centrifugation by specific hybridization with their complementary RNA. In several hybrid strains, the mtDNA's derived from the two species did not separate in the gradients, and this result was interpreted as evidence for linkage between sequences from the two parental mtDNA's. Sheared samples of DNA gave similar results, excluding the formation of catenates, but the exact nature of the linkage and the structure of the molecules containing both types of sequences have not been demonstrated. Recombination has been demonstrated in yeast mtDNA, but genetic markers on the mitochondrial genome are required to evaluate the frequency and possible significance of mtDNA recombination in animal cells (214).

B. RNA

Eliceiri has observed both mouse and hamster 18S and 16S mtRNA in some interspecific hybrids, but no detailed correlation has been made with chromosomes or mtDNA content (215).

C. Proteins

Although mitochondria synthesize proteins, many proteins located in mitochondria are presumed to be nuclear coded and are transported from the cytoplasm which produces both mitochondrial (m) and cytoplasmic (s) forms in some cases. The distribution of three mitochondrial enzymes, citrate synthase (CS), NAD-malate dehydrogenase (MDH_2), and aspartate aminotransferase (AAT) has been studied in human-mouse hybrids segregating human chromosomes and lacking human mtDNA (216, 217). The human and mouse forms of CS can be distinguished by electrophoresis, and human-mouse heteropolymers of CS were found in hybrids that retained mostly mouse enzymes (218). Segregation analysis showed that human CS is linked to lactate dehydrogenase B (LDH B) and peptidase B, and its gene has been assigned to chromosome 12. The mitochondrial forms of MDH_2 and AAT can be distinguished from the cytoplasmic enzymes by electrophoresis, and specific mouse antibodies to the human mitochondrial enzymes were used to identify the human enzymes in human-mouse hybrids and to assign MDH_2 to chromosome 7 (219). Recently, fumarate hydratase (FH), another citric acid cycle enzyme, has been assigned to chromosome 1 (220), and superoxide dismutase (SOD) to chromosome 6 (221).

These studies confirm that these mitochondrial products are nuclear coded, that cytoplasmic and mitochondrial forms of human MDH_2 and AAT are unlinked, and that consecutive citric acid cycle enzymes, CS, MDH_2, and FH, as well as AAT, are all unlinked. Although further studies on isolated mitochondria are needed, these studies imply that human MDH_2 is able to integrate into organelles controlled by mouse mtDNA.

Mitochondria contain a distinctive thymidine kinase (mTK) as well as a cytoplasmic variety (222). The mTK differs from this and another cytoplasmic form, with respect to electrophoresis and phosphate donor specificity. Species differences among mTK have also been described by Kit and co-workers (163). Mouse-primate hybrids with a reduced complement of primate chromosomes contain only mouse mTK, while retaining the primate cytosol TK which formed the basis for selection of the hybrids. These findings suggest that the cytosol and mTK genes are coded on different chromosomes.

All the above hybrids were isolated after selection for nuclear coded functions, but selection for mitochondrial functions may also be feasible. Chloramphenicol (CAP) resistance can be expressed at the level of the mitochondrion and may be coded for by mtDNA. Bunn and co-workers used CAP-resistant mutant $HGPRT^-$ mouse fibroblasts, isolated after ethidium bromide treatment, to prepare enucleated cytoplasts ("93% pure"), which were then fused with CAP-sensitive mouse TK^- cells (92). Fusion products were obtained at high frequency, were resistant to CAP, and contained nuclear markers and the chromosome complement of the CAP-sensitive parent. Control experiments were performed to show that the hybrids could have resulted from fusion with cytoplasts rather than residual intact cells. It has not yet been proved that CAP resistance in these hybrids is due to function of a mitochondrial gene. This type of approach could also be extended to interspecific hybrids where it should be possible to select for the retention of human mitochondrial functions.

X. LYSOSOMES

Structural, control, and architectural genes all play a part in the production of lysosomal acid hydrolases such as β-glucuronidase (223). Genetically and structurally interrelated isozymes have been identified for several acid hydrolases, but the molecular modifications that accompany biosynthesis and intracellular transport are still obscure. Ganschow was the first to study a possible lysosomal marker in cell hybrids (224). Using a heat-labile strain variant, he showed that the pattern of glucuronidase expression in intraspecific mouse hybrids resembled that in heterozygous animals and that both parental genes were therefore expressed. Since then, a variety of soluble acid hydrolases has been studied in segregating interspecific hybrids with the aid of electrophoretic and antigenic markers. Chromosome assignment reveals that acid hydrolases and their isozymes are not linked to one another and has provided preliminary evidence that control and modification of these enzymes can also be studied by somatic cell hybridization.

A. β-D-N-Acetylhexosaminidase

The human lysosomal enzyme occurs in at least two major molecular forms, hexosaminidase A (hex A) and hexosaminidase B (hex B). Linkage analysis of interspecific hybrids assigned hex A and hex B to chromosome 15 and 5, respectively (219, 225, 226). When a combination of immunologic and electrophoretic techniques was used to identify hex A and hex B with certainty, it was noted that hex A was never expressed in hybrids in the absence of hex B (227).

Several autosomal recessive lipid storage diseases are associated with hexosaminidase deficiency. Among these are Tay-Sachs disease, in which hex A is lacking and Sandhoff's disease in which both hex A and hex B are lacking (228, 229). Intergenic complementation has been demonstrated in heterokaryons formed by fusing Tay-Sachs fibroblasts with Sandhoff cells. Hex A, absent in both parental cell types, appears 2–6 days after fusion and resembles the normal hex A with respect to electrophoresis and heat lability. The possible structural relationship between A and B and the molecular defects in the storage diseases is still uncertain.

B. Acid Phosphatase

The gene specifying lysosomal acid phosphatase ACP_2 has been assigned to chromosome 11 with the aid of human-rodent hybrids (230). A heteropolymeric enzyme was demonstrated in the hybrids and is compatible with a dimeric structure for the enzyme. The hybrid cell enzymes are β-glycerol phosphatases, have an acid pH optimum, show a lysosomal distribution after differential centrifugation of cell homogenates, and exhibit structure-linked latency. The gene for ACP_1, the nonlysosomal acid phosphatase present in red cells, has been assigned to chromosome 2 (231).

Using different rodent-man hybrids, Shows and Lalley also obtained evidence for a dimer structure and for segregation of human acid phosphatase isozymes (232). Some clones lacked the mouse isozymes, and the authors suggested that a human chromosome, which they did not identify, was responsible for its extinction since the mouse enzymes reappeared in some subclones, presumably after further segregation of human chromosomes. Extinction was specific for acid phosphatase and did not affect unrelated acid hydrolases. The "regulatory" gene was not linked to that coding for human acid phosphatase. On the basis of rather limited evidence, the authors postulated that the regulator was a sialyl transferase which modified the processing of the mouse enzyme.

Acid phosphatase activity, which is readily inducible in unfused mouse macrophages, also disappears by an unknown mechanism from macrophage heterokaryons after fusion with acid phosphatase-poor melanoma cells (94).

C. α-Galactosidase

The gene specifying this enzyme has been assigned to the X-chromosome by study of hamster-human hybrids (233).

Though characterization of the enzymes and their lysosomal association is incomplete, these studies show that the soluble lysosomal enzymes can be studied readily by hybridization. The study of less soluble, membrane-associated constituents of lysosomes awaits the development of suitable markers. Tissue-specific differences also offer opportunities for further research.

XI. CONCLUSION

This review has served to illustrate the diversity of experimental problems in cell biology that can be studied by cell fusion techniques. The spectacular growth of gene mapping by cell hybridization will undoubtedly extend to many other areas of cell

physiology and also provide a basis for further study of gene regulation in somatic cells.

Gene assignment and our understanding of the mechanisms that control the expression of tissue-specific traits in somatic cells, however, have lagged, by comparison (13, 234, 235). Initial experiments indicated that this class of phenotypic markers is subject to selective regulation and is often extinguished in hybrids. Recent studies show that such traits as melanin production (236, 237) and liver-specific enzymes (238) can also be expressed in hybrids, provided the gene dosage of the differentiated parent is doubled or segregation of chromosomes from the nondifferentiated parent occurs. Peterson and Weiss have shown that hybridization of a mouse fibroblast with a rat hepatoma cell could activate the fibroblast gene for albumin (239). Similar activation of the human leukocyte gene for albumin was achieved by hybridization with mouse hepatoma cells (240). Segregation analysis after reactivation should enable the mapping of tissue-specific traits in the future. These remarkable studies suggest that cell hybridization will also provide unique insight into the process of cell differentiation.

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