Somatic Cell Hybrids: Impact on Mammalian Genetics

The development of techniques for in vitro cultivation and fusion of somatic cells has provided a powerful impetus to the study of mammalian genetics. These techniques are now being employed for the mapping of mammalian —including human—chromosomes, for the study of differentiation in highly complex mammalian cells, and for research on the etiology of cancer.

Until 1960, most scientists believed that only the germ cells—the eggs and sperm—of a species were capable of fusing to form single cells that could reproduce themselves; however, in that year, G. Barski and his colleagues at the Institut Gustave Roussy in Paris found that somatic cells could also unite to form hybrids that contained a single nucleus and were able to divide. More recently, the demonstration that hybrids between cells of different species are possible as well as intraspecific hybrids, the use of inactivated Sendai virus to increase the rate of cell fusion, and the development of mutant cell lines and of media selective for hybrids have sparked research efforts.

At present, some of the most rapid progress is in the area of human chromosome mapping. Such mapping requires both the establishment of gene linkage—if two or more phenotypes or gene expressions are always associated with one another, their genes are probably linked or located on the same chromosome—and also the identification of the chromosome that carries the linked genes. In addition, a complete map includes the distances between genes and their order on the chromosome.

Although somatic cell approaches to mapping chromosomes vary from laboratory to laboratory, most investigators are exploiting certain characteristics of hybrids between cells of two different species. Mary Weiss and Boris Ephrussi, now at the Centre de Génétique Moléculaire, Gif-sur-Yvette, France, first demonstrated the feasibility of producing such interspecific hybrids. In clones of these hybrids, the chromosomes of one species are preferentially lost during several generations of cell culture. For example, in human-mouse hybrids, the human chromosomes are lost.

The ability to control which chromosomes are lost and thus which remain would greatly facilitate genetic analysis. One method of achieving this goal depends upon a procedure originally developed by John Littlefield of Harvard Medical School, Boston, Massachusetts, for the isolation of certain hybrids from mixtures of hybrid and parent cells. The technique requires the use of parent cells that are mutants, each lacking the capacity to synthesize a different enzyme. Littlefield's selective culture medium does not permit the reproduction of the defective parent cells; however, any hybrids that synthesize both enzymes can multiply in the medium.

Human Gene Mapped

Weiss, with Howard Green, who is now at the Massachusetts Institute of Technology (M.I.T.), Cambridge, used these methods to identify a particular human chromosome as the carrier of the gene for the enzyme thymidine kinase (TK). For their experiments they employed a mouse parent cell that lacked TK and was thus unable to reproduce in Littlefield's selective medium; the human parent cell was not a mutant but it divided very slowly compared to the other cell types in the mixture. Consequently, mouse-human hybrids that possessed the gene for TK —which was derived from the human cell—multiplied rapidly in the selective medium to form discrete colonies that could be isolated for further study. Any hybrid that lost the human chromosome carrying the TK gene would no longer be viable. According to Green, hybrid clones selected in this manner contained both the enzyme TK and a human chromosome of the E series—probably chromosome 17. The addition of a chemical that is lethal for cells containing TK killed most of the hybrids; only those that lost the TK gene could survive in this medium. Green found neither the enzyme nor the E group chromosome in the few surviving cells. This observation provided additional evidence for the association between the gene for TK and chromosome 17.

Hybrids between hamster and human cells lose their human chromosomes even faster than the human-mouse hybrids. Theodore T. Puck and F. T. Kao, at the University of Colorado Medical Center in Denver, have developed several mutant strains of Chinese hamster ovary cells that are dependent on added nutrients such as glycine for growth and reproduction. The nutritional requirements of the cells may then be used as the basis of hybrid selection; for example, a glycine-free medium can be used to select for hamster-human hybrids containing the human genes for glycine synthesis. The availability of these genetic markers has also enabled Puck to investigate gene linkages. He has determined linkages between several genes, including those responsible for the activity of the enzymes lactate dehydrogenase B and serine hydroxymethylase, an enzyme required for glycine synthesis.

One of the disadvantages of the use of somatic cell hybrids for genetic analysis is the difficulty of proving that the cell population under investigation is indeed hybrid. Identification of marker chromosomes—chromosomes that are distinguishable as characteristic of a given species—from both parent cell lines in the hybrid is one method of achieving this goal. Such karyotyping is an extremely tedious process; however, the recent introduction of specific chromosome staining techniques enables the identification of all chromosomes.

The existence of hybrids may also be confirmed by studying the products of gene expression—namely, the enzymes and proteins of the hybrids. The enzymes of mice and men are sufficiently different to be distinguished with such physicochemical techniques as electrophoresis. Frank Ruddle and his associates at Yale University, New Haven, Connecticut, have used both of these approaches to determine linkages and chromosome assignments for a variety of human genes. According to Ruddle (*1*), investigators in his laboratory and in others have employed somatic cell genetics to assign 30 genetic markers to human chromosomes (13 autosomes and the X sex chromosome) or to linkage groups.

The fact that most of this has been achieved within the past year is indicative of the impact of cell fusion techniques on genetic analysis. Ruddle expects that gene markers will be assigned

to all of the human chromosomes before the end of 1973. When one marker gene is known for each chromosome, further assignments can be made by demonstrating that an unmapped gene is linked to one of the markers. This is not as simple as it may appear, because linkage analysis requires the examination of a large number of clones to show that the two phenotypes are invariably present together.

The determination of gene order and distances on the chromosomes is even more difficult. Ruddle, however, states that the use of chromosome translocations—the shifting of a fragment of one chromosome onto another—may permit the solution of this problem. Translocations can arise spontaneously in the hybrids; alternatively, cell fusion may be achieved with a human cell line that already bears a known translocation, for example, the translocation of chromosome 21 onto chromosome 15, a condition that is occasionally found in the human and may result in the production of progeny with familial Down's syndrome. In either case, normal linkage relationships will be disrupted and new ones created. The use of overlapping translocations should allow the localization of the gene under investigation to a discrete region of the chromosome.

The National Institute of General Medical Sciences, Bethesda, Maryland, has recently initiated a program of collecting and distributing mutant human cells for use in medical research. According to Ruddle, one of the objectives of the program is the organization of a bank of human cells with chromosome translocation—up to 500 different types—for use in this kind of chromosome mapping.

Classical techniques for genetic analysis are difficult to apply to the human because of the long generation times, the low number of progeny produced, and the inability to carry out matings that would be most enlightening from a genetic point of view. Nevertheless, most investigators do not believe that somatic cell techniques will supplant classical human genetic analysis but rather that the two approaches are complementary. The classical geneticist can use information gleaned by the somatic cell geneticist—that two genes are closely linked on the same chromosome—and then apply population and kindred analysis to determine their order and distance apart. Thus, the combination of these two approaches is a potent one.

Current progress toward the elucidation of a human chromosome map represents only a beginning; as more powerful techniques emerge they will ultimately permit the development of a human chromosome map comparable in detail to the one for the bacterium *Escherichia coli*. Such human (and other mammalian) chromosome maps will have important implications for medical genetics; for example, they will permit a better understanding of the genetic defects that occur as a result of chromosomal aberrations and may suggest therapies for specific conditions. By the use of these maps, genetic abnomalities that cannot now be detected before birth may be recognized by examining the chromosomes of fetal cells taken from samples of amniotic fluid. They may also contribute to the understanding of cell differentiation.

Although each somatic cell of an organism contains the total genetic information for that organism, only parts of this total are expressed in differentiated cells. Understanding differentiation thus requires an understanding of the factors that regulate gene expression. Attempts to apply concepts of gene control derived from investigations of the relatively simple bacterial systems to mammalian cells have met with only limited success. Fusion of two mammalian cells that express different phenotypes and observation of the fate of the phenotypes in the hybrids may help in the determination of the factors that control expression of genes. Three results of hybridization are possible: extinction, in which a phenotype present in one parent cell but not the other is no longer expressed; continued expression of the phenotype in the hybrid; and activation of a phenotype that was expressed in neither parent. All of these possibilities have been observed in one or more laboratories.

Richard Davidson, who is now at Harvard Medical School, Boston, Massachusetts, and Ephussi found that hybrids resulting from the fusion of pigmented hamster melanoma cells with unpigmented mouse fibroblasts were not pigmented (*2*). According to these investigators, the chromosome loss from each of more than 100 unpigmented hybrid clones was quite small—less than 15 percent of the total chromosome complement. Thus they thought it unlikely that all of the clones had lost the genes required for making melanin and they interpreted the results as indicative of negative control—that is, the fibroblasts produced some factor that prevented the synthesis of an enzyme essential for melanin production. (In positive control, a genetically controlled activator would turn on the expression of some gene.)

More recently, Davidson found that a gene dosage effect is operative in the regulation of pigment synthesis. If melanoma cells, with double the usual chromosome number, are fused with fibroblasts, approximately half of the hybrids do synthesize melanin. Davidson believes that these results are still consistent with the idea of negative control but concedes that other interpretations are possible. At present, he cannot explain why half of the hybrids are unpigmented. Because chromosomes carrying essential genes may have been lost, a complete genetic map would be valuable in situations such as this. It would enable the investigator to determine, by chromosomal analysis, whether or not the necessary genes are present.

Expression of the differentiated parent cell phenotype continues in other hybrid cells. John Minna, working in the laboratory of Marshall Nirenberg at the National Heart and Lung Institute, Bethesda, Maryland, prepared numerous clones of hybrids between mouse fibroblasts and neuroblastoma cells. The latter cells have many of the properties of differentiated neurons, including the capacity to respond to acetylcholine, to synthesize enzymes required for the formation and breakdown of neurotransmitters, to form projections called neurites, and to generate action potentials. Some of the hybrids retained all of the properties of the neuroblastoma parents; others were more like the fibroblasts; and others expressed some, but not all, of the neuroblastoma functions.

The reasons why some properties of neuroblastoma cells are maintained in the hybrids while others are lost are still unclear. According to Minna, the explanation could involve chromosome loss by the hybrids or the addition of a negative regulator with the fibroblast genome. He points out, however, that clones that had very low amounts of the enzyme acetylcholinesterase did have fewer chromosomes than those with high acetylcholinesterase activity. Although the use of these hybrids enables the generation of a large number of variant phenotypes for genetic analysis, at present it is not possible to correlate phenotypes with specific chromosomes.

Minna found that certain phenotypes

appeared to be associated in the hybrid clones that he analyzed. He states that this could be due to either an inadequate sample size, to functional association of the phenotypes, or to coordinate control and expression of some genetic functions. Ruddle and F. Arthur McMorris, who is now at M.I.T., reported similar results for their neuroblastoma-fibroblast hybrids. They also found an example of gene activation. The enzyme choline acetyltransferase is inactive in both neuroblastomas of sympathetic origin and in fibroblasts, but Ruddle and McMorris found one clone of hybrids between these two cell types in which enzyme activity was very high. The mechanism for this gene activation is as yet unknown.

Ruddle and his colleagues are now investigating another apparent instance of gene activation in hybrids between mouse hepatoma cells and human leukocytes. The hepatoma cells, but not the leukocytes, synthesize serum albumin, a product characteristic of differentiated liver cells. In one hybrid clone, which had lost a large number of human chromosomes, human albumin was synthesized in addition to mouse albumin. Ruddle thinks that it should be possible to use this system to map the location of the human albumin gene; moreover, the hepatoma system may be a prototype for others that can be used to map genes coding for the specialized products of differentiated cells.

The extreme complexity of mammalian cells has undoubtedly been a major reason for the difficulty in unraveling the mechanisms responsible for differentiation of these cells. Because of this complexity, Ruddle believes that it is premature to attempt to describe the phenomena observed thus far in terms of positive or negative control—concepts that were derived from studies of bacterial systems.

The Nature of Malignancy

Malignancy may be considered to be either a positive quality—that is, one caused by the presence of information that transforms a normal cell to a tumor cell—or it may be considered to be a negative quality—that is, one caused by the absence of a factor or factors normally inhibiting cell division. In order to distinguish between these possibilities, Henry Harris at Oxford University, Oxford, England, and George Klein, at the Karolinska Institutet, Stockholm, fused a line of highly malignant cells with a line that was much less malignant. The fact that the resulting hybrids were far less malignant than the highly malignant parent suggested that the malignancy was due to a defect in that parent that was compensated by the less malignant strain. Cells taken from the tumors that did form when the hybrids were injected into mice had fewer chromosomes than the other hybrids; this implied that these cells had somehow lost the information that suppressed malignancy.

Again, interpretation of the results of such experiments is somewhat equivocal. J. F. Watkins (*3*), also at Oxford University, has pointed out that a "principle of indeterminancy" applies to experiments that attempt to determine whether malignancy is a positive or negative characteristic. The cell lines used thus far for the fusion experiments have a range of chromosome numbers rather than a definite single value; and hybrids do tend to lose some chromosomes. Therefore, it is currently impossible to prove that a particular hybrid has not lost chromosomes. The absence of malignancy in a clone that results from a given cross between a malignant and a nonmalignant parent could then be due to either a suppression of malignancy by the normal cell or to the loss of the gene or genes conferring malignant properties. However, according to Watkins, the theory that malignancy results from the loss of genetic information that would normally inhibit cell growth and division is consistent with observations in other laboratories concerning changes in the surface properties of tumor cells.

Cell fusion may not only be important for the study of oncogenesis, but it may also be involved in the etiology of cancer. Harris and Klein (*4*) found that tumors could be produced in mice by the injection of a line of tumor-inducing mouse cells. Although these cells lacked an enzyme required for growth in Littlefield's selective medium, the medium would support the growth of some cells derived from the tumors. The enzyme deficiency had apparently been remedied. The logical source of the enzyme would be the cells of the host mice; and, in fact, the cells that reproduced in the selective medium had antigens characteristic of the host mice. They also had a chromosome number approximately equal to the sum of the chromosomes in the cells of the test animals plus those in the tumor-inducing cells.

In another experiment, Harris and Klein used test mice with an identifiable chromosome marker; they found the marker in the tumor cells that grew in the selective medium. According to Harris, such in vivo cell fusion would be one way of increasing the genetic variability of cells and thus giving rise to some that are able to reproduce faster than normal. Further experimentation—in particular, a more thorough examination of the chromosomes of tumors—is needed to establish the validity of this hypothesis.

The utilization of cell fusion techniques has made possible rapid advances in the mapping of human chromosomes. Research on cellular differentiation and oncogenesis is not progressing as fast as had once been hoped—a fact undoubtedly due to the extreme complexity of the mammalian cell and its regulatory mechanisms. Nevertheless, the development of somatic cell hybrids has provided a valuable tool for the investigation of mammalian cells.—JEAN L. MARX

References

1. F. Ruddle, *Nature*, in press.
2. R. L. Davidson, *In Vitro* **6**, 411 (1971).
3. J. F. Watkins, *Int. Rev. Exp. Pathol.* **9**, 115 (1971).
4. F. Wiener, E. M. Fenyö, G. Klein, H. Harris, *Nature* **238**, 155 (1972).