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2. Schweizerische Gesellschaft für Vererbungsforschung
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Genetic Engineering

H. Gloor (Université de Genève): Introduction

Le terme "Genetic Engineering" (technologie génétique) s'applique en particulier aux méthodes qui permettent d'isoler de petits fragments sélectionnés de la substance génétique de n'importe quel organisme, et de les introduire dans des souches bactériennes où, selon les conditions, de tels fragments peuvent se maintenir ou même se multiplier rapidement. Tous les organismes étant parfaitement adaptés aux conditions de leur environnement, en tant que espèces sinon en tant que individus, les changements possibles de leur patrimoine héréditaire sont étroitement limités. Cependant, l'introduction artificielle de propriétés génétiques de provenance étrangère pourrait radicalement changer les activités d'un organisme, pourvu que de tels gènes étrangers s'harmonisent d'une façon ou autre avec les cellules de l'hôte et fonctionnent en parallèle avec les gènes de l'hôte. C'est pourquoi plusieurs Commissions gouvernementales, notamment en Angleterre et aux Etats-Unis, se sont déjà penchées sur la question des dangers éventuels du "Genetic Engineering" et des mesures à prendre pour les limiter.

Des méthodes, comparables à celles qui ont été mises au point pour certaines bactéries, pourront sans doute être appliquées aux organismes multicellulaires, plantes et animaux. Théoriquement, de telles méthodes pourraient frayer un chemin à de nombreuses applications, soit salutaires et profitables, soit dangereuses ou néfastes.

A part les moyens traditionnels et strictement génétiques, à savoir: les mutations, l'hybridation, la sélection; il faut relever qu'il existe une "technologie" auxiliaire déjà considérable à l'égard des organismes multicellulaires. Ainsi, on peut faire régénérer une plante à partir d'une seule cellule végétative, ou à partir d'un grain de pollen; on peut faire pénétrer une algue bleue dans une cellule de plante verte, conserver au froid des spermatozoïdes et même des embryons de mammifères; on peut fusionner deux embryons lesquels ensemble donneront un seul individu parfaitement viable. On peut fusionner des cellules plus ou moins différenciées provenant de différents individus ou encore de différentes espèces (même animales et végétales, paraît-il), transplanter des noyaux ou du cytoplasme, et ainsi de suite.

Dans le cadre de ce symposium, le docteur Bernard Mach du département de pathologie de l'Université de Genève discute les méthodes et les problèmes moléculaires du "Genetic Engineering", tandis que le docteur Nils R. Ringertz de l'Institutet för Medicinsk Cellforskning och Genetik, Medicinska Nobelinstitutet, Karolinska Institutet, Stockholm, traite des aspects de la cellule animale.

1. B. Mach (Department of Pathology, University of Geneva): Genetic Engineering at the Molecular Level

The technology of genetic engineering, or DNA recombination in vitro, is derived from the possibility of transfering in vitro segments of DNA, and therefore of genes, from one DNA molecule to another. The two DNA molecules can be of the same or of different species, with no limit for this DNA transfert among unrelated and distant species. One of the two partners in such a "gene transplantation" reaction must be a self-replicating DNA molecule, such as a virus or a bacterial plasmid, and capable therefore of functioning as a vector. After an in vitro transfer of a foreign gene into such a self-replicating structure (vector) the new hybrid DNA is used to infect the normal host of the vector. Clones of new recombinant vectors (virus or plasmid) containing foreign DNA genes are propagated, each containing a unique segment of foreign DNA which can now be amplified when the recombinant vector is replicated.

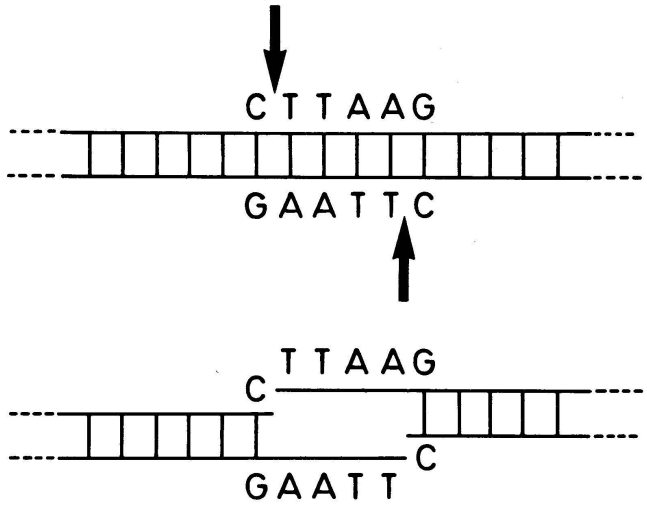
These segments result in the reaction, in vitro, of new genomes carrying and perpetuating genes belonging to another species. These developments have important implications for biological research as a whole, and in particular for the study of gene structure, function and regulation in the case of higher organisms. They may have practical implications as well for such problems as the large scale production of specific proteins.

Depending on the nature of the vector structure used (and into which foreign DNA is inserted) one can distinguish two broad categories of experiments. The first consists in DNA transfer into prokaryotes, using therefore a *prokaryotic vector,* such as a bacteriophage or a plasmid. The foreign DNA inserted and subsequently cloned and amplified, can either be of prokaryotic origin, or it may come from an eukaryote. Such experiments could involve the cloning of a specific mammalian gene sequence into a bacterial vector. The second type of transfer utilises a viral vector for the transfer of DNA (of whatever source) *into eukaryotic cell* hosts. Experiments of that second type are still rather preliminary.

DNA transfer in vitro

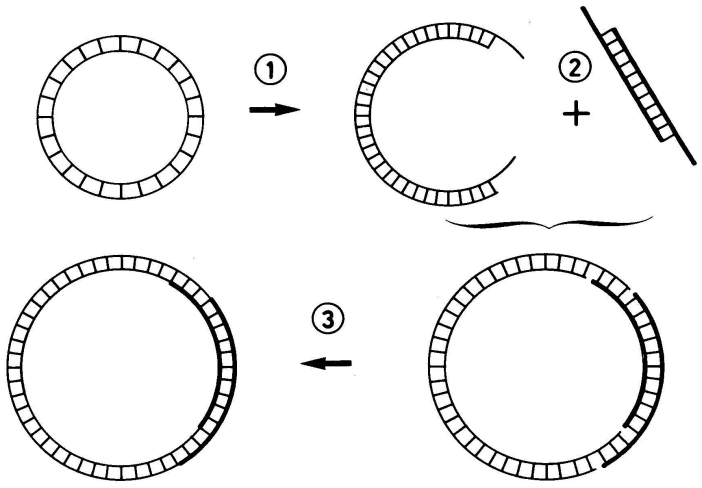
The first step in any of these experiments is to introduce a segment of DNA within a vector DNA molecule. This is achieved in vitro with a combination of biochemical reactions and it can be applied to any DNA molecules, whatever their source. The different molecules which are to be joined are first prepared with single-stranded tails of such structure that the single-strand tail of one DNA molecule is complementary to the single-stranded tail to another DNA molecule. End-to-end joining of DNA segments occurs therefore by the pairing of short complementary single-stranded stretches of the two DNA fragments.

Two biochemical mechanisms can produce DNA molecules capable of such end-to-end joining: a) fragments of DNA can be elongated enzymatically with single-stranded tails (on the 3' end of each of the two strands) with a short homopolymeric sequence. Two preparations of DNA, each



prepared with homopolymeric tails complementary to the other (for instance poly A and poly T) can be mixed and hybrid circles will be produced. Sealing and ligation can then be achieved enzymatically. b) Certain restriction endonucleases are capable of cleaving DNA molecules at very precise locations (specified by the nucleotide sequence along the DNA) and by cutting the two strands of DNA at a distance of about 3 to 5 nucleotide pairs. (see Figure 1). The enzymes therefore generate DNA fragments with short complementary tails and which are automatically capable of end-to-end joining. The crucial feature of this enzymatic cleavage is that DNA fragments produced by a given restriction endonuclease will be able to join to DNA fragments *from any source,* as long as these have also been produced by the same enzyme. Joining of such DNA fragments, followed by enzymatic ligation, will produce circular DNA molecules containing DNA of two different origins.

If one of the two partners in such an in vitro DNA joining experiment is a bacterial plasmid, or a bacteriophage, the resulting hybrid molecule, which



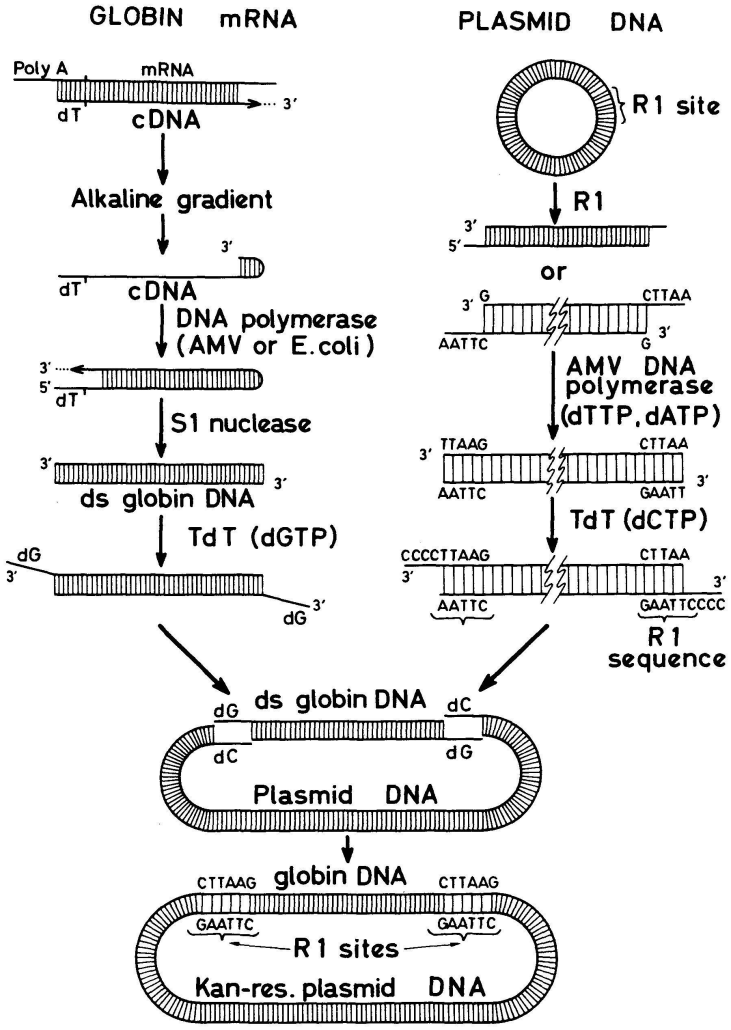
contains DNA, can function as normal phage or plasmid DNA and infect the host bacteria in which they will propagate. Great progress has been made in the past 2 or 3 years in the development of efficient vectors strains. These are derived from E. coli bacteriophage lambda and from plasmids of E. coli. One advantage of plasmids for these DNA transfer experiments is that, under certain circumstances, they can be amplified in their bacterial host and that large quantities of specific DNA, including specific foreign genes, can be prepared.

Figure 2 represents schematically the very simple sequence of events involved in an *in vitro* DNA recombination. Step 1 is the preparation of the vector (phage or plasmid), step 2 is the mixing, and pairing, of vector DNA and foreign DNA, both with complementary single strand tails, and step 3 is the final ligation of the hybrid circular DNA which is a viable phage or plasmid containing one or several additional genes of foreign origin.

Gene specificity

The construction of new recombinants carrying *one given gene* presents great technical difficulties, especially in the case of genes from higher organisms, where a single gene represents only one part in several millions in the entire genome. This question of gene specificity has not yet been solved in an obvious way but there are clearly three possible strategies:

1. Random insertion of unfractionated DNA fragments from the entire genome (with the creation of a large number of phage or plasmid recombinants) followed by screening or selection for recombinants carrying a specific gene. This so-called *"shot-gun"* approach requires the scaling up of screening procedures (usually based on in situ hybridization with a specific labeled RNA or DNA probe). It has the disadvantage of involving the construction of large numbers of recombinants containing unknown genes.
2. The second approach is based on the purification, or enrichment, of a given gene by various biochemical techniques followed by its insertion into a bacterial vector. The methodology of gene purification is still very complexe and progress along this second more sophisticated approach has been rather slow.
3. Because of the difficulties in cloning a specific gene from complexe organisms such as mammals, an alternative procedure has been developped. It consists in the in vitro synthesis of a gene (or portions of a gene) using a specific messenger RNA as a template. This has made possible the construction of E. coli plasmids carrying in vitro synthesized gene sequences from rabbit or mouse. The first success in such an experiment was in the case of rabbit and mouse globin; and the schematic representation of the different steps involved in such an experiment is presented in Fig. 3. When the messenger RNA coding for a given protein can be obtained either in pure form or as a majority component, this approach involves the creation of recombinants carrying only known gene sequences.



Possible uses and implications of in vitro DNA recombination

The possibility of "cloning" individual genes and to amplify them in simple bacterial vectors does represent a major technological breakthrough for biological research. It will make possible new approaches in the study of gene expression and gene control as well as in the study of the fine structure of genes and of controlling sequences. Crucial biological questions such as cellular differenciation, control of normal and malignant growth, hormone induction, genetic control of antibody variability will no doubt benefit in a decisive way from these new techniques.

In vitro DNA recombination will be utilized for the construction of bacterial strains capable of producing specific "useful" proteins. The commercial production of various hormones, such as insuline, by large scale cultures of bacterial recombinants carrying the specific genes, is forseeable in the near future. Other proteins of interest include viral proteins to be used as vaccine.

Great efforts will also be made to introduce the genes for nitrogen fixation into plants, an achievment which could have important consequences for agriculture and for the food problem as a whole.

And finally one must consider that the possibility of transfering foreign genes into animal cells might be used once for the treatment of genetic defects in human cells. Such a prospect involves technical steps which are clearly not possible now, and which may never be solved. It also carries at the same time a remarkable potential for medical progress and the worrysome possibilities of abuses inherent to such manipulations on human cells or even on individuals.

Although genetic engineering may never reach the state where it could be applied to humans; the mere possibility of such interventions has rightly generated grave concern among scientists and non scientists alike. Furthermore, the possibility that DNA recombination in vitro involving simple bacterial vectors might involved some health hazards, unknown in nature and extend, has also resulted in a serious and critical reflexion on the justification for such new research. The consensus is now that a strict control should be exerted on what kind of experiment should be allowed and not allowed and also on the very stringent safety conditions under which such type of research can be allowed to proceed. As a result of such concern, very strict rules are now applied to this field of research, which imply not only the physical conditions but also the use of bacterial or viral strains unable to survive outside a very complexe set of laboratory conditions. An open debate on the interest and possible benefits of certain types of these experiments, and on the rules imposed to prevent abuses in this field is most important. Also important is that such a debate be conducted in an objective serene climate.

2. N.R. Ringertz (Institute for Medical Cell Research and Genetics, Medical Nobel Institute, Karolinska Institutet, Stockholm, Sweden): Possibilities of Cell Biology for Genetic Engineering

The term genetic engineering is now used to describe experiments designed to modify the genetic material of bacteria, viruses and other prokaryote microorganisms. This technology owes its origin to recent progress in nucleic acid biochemistry and molecular biology. Thus, the discovery of restriction enzymes has made it possible to prepare DNA fragments in a form which makes it easy to recombine and join fragments from two different organisms into recombinant DNA molecules. Using these methods it is possible to construct specific gene sequences which can then be injected into bacteria where they will be replicated and thus amplified as the bacteria multiply. The consequences this may have for the biological properties of bacteria are presently the subject of intense studies and debate. In view of the rapid progress in "genetic engineering" with microorganisms it is now of interest to consider to which extent such manipulations can be performed with animal cells. It is also necessary to discuss the potential benefits and risks that such experiments may entail.

Recent advances in the field of somatic cell genetics have resulted in techniques which make it possible to generate animal and plant cells with altered genomes (Fig. 1) such as *mutant cells* with specific genetic defects, *virus transformed cells* in which viral genes have been added to the cellular genomes, *hybrid cells* containing genetic material from two different cell types, and *reconstructed cells* in which the nucleus may be derived from one cell type and the cytoplasm from another. The aim of this presentation is to discuss possibilities of preparing animal cells with altered genomes. For references and a more detailed discussion of this subject see ref. 1)..

Mutant cells

The term mutant is used to describe cells or cell lines which show a stable and heritable alteration in phenotype. Mutants can be isolated by culturing cells from patients suffering from inherited metabolic disorders or by treating normal ("wild-type") cells *in vitro* with mutagens. In the latter case, exposure to mutagens (X-irradiation or chemical mutagens) is followed by selection for mutant cells. Often one uses special culture media or drugs in order to kill normal cells and allow a special class of mutants to accumulate. *Drug resistant, enzyme deficient mutants* have been used in testing the relative importance of different pathways of purine and pyrimidine synthesis and in studies of metabolic cooperation and cross-feeding between cells. *In vitro* studies of cells sensitive or resistant to cytostatic drugs have provided information about the mechanism of action of these drugs. This type of information is important in cancer chemotherapy. *Auxotrophic mutants,* that is cells requiring special nutrients not required by normal cells, have been used for genetic analysis of nutritional requirements. *Temperature sensitive mutants,* are cells which grow at certain temperatures (permissive temperatures) but not at other temperatures (nonpermissive temperatures) at which normal cells multiply. Many of these mutants appear to be due to slight base changes in DNA which cause single amino acid substitutions in proteins thereby causing them to become nonfunctional when the temperature is changed from the permissive to the nonpermissive temperature. The protein affected may be one which is necessary for DNA, RNA or protein synthesis in general or one which has a specific enzymatic function in a metabolic pathway. Other temperature sensitive mutations affect complex cell functions, for instance progress through the cell cycle, mitosis, susceptibility to viral infection or ability to undergo transformation by tumor viruses. The study of mutant cells by biochemical techniques makes it possible to test the direct involvement of a specific gene product in the control of a given phenotype. Furthermore mutant cells may be used in cell fusion experiments to facilitate the isolation of hybrid cells. As will be discussed below this represents an important genetic technique which makes it possible to explore the organization and function of genes in human and animal cells.

1) Ringertz NR, Savage RE (1976): *Cell Hybrids.* Academic Press, New York.

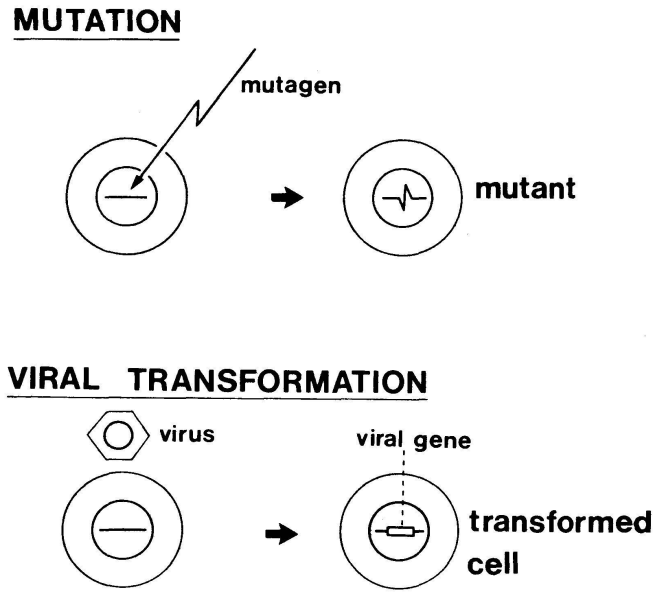


Fig. 1 Schematic illustration of mutation and viral transformation.

Virus transformed cells

Treatment of normal cells *in vitro* with DNA or RNA tumor viruses can cause a heritable alteration in their growth regulation known as transformation. This process is believed to be analogous to *in vivo* transformation of normal cells to tumor cells. Cells which have been transformed continue to multiply *in vitro* after normal cells have undergone senescence and stopped multiplying. Usually transformed cells do not contain detectable quantities of infectious virus. Using nucleic acid annealing it is, however, possible to detect a small number of viral genomes which are covalently linked to host cell DNA (Fig. 1). In some transformed cell lines the viral genomes are defective and correspond only to one or two genes. One of these genes is believed to specify the transformed phenotype and a special virus-induced nuclear antigen. Further studies of viral transformation genes will no doubt provide valuable information about virus-induced carcinogenesis.

Cell hybrids

The technique of cell hybridization involves a spontaneous or induced fusion of two different types of cells (A and B) into multinucleate *heterokaryons* containing A and B type nuclei within a common cytoplasm (Fig. 2). Most heterokaryons die soon after fusion but some of those which contain only one A nucleus and one B nucleus survive and complete a normal cell division. The binucleate heterokaryons then give rise to two mononucleate *hybrid cells (synkaryons)* each of which contains a complete set of A and B chromosomes. Many types of hybrids show great vitality and are capable of multiplication in tissue culture for many years.

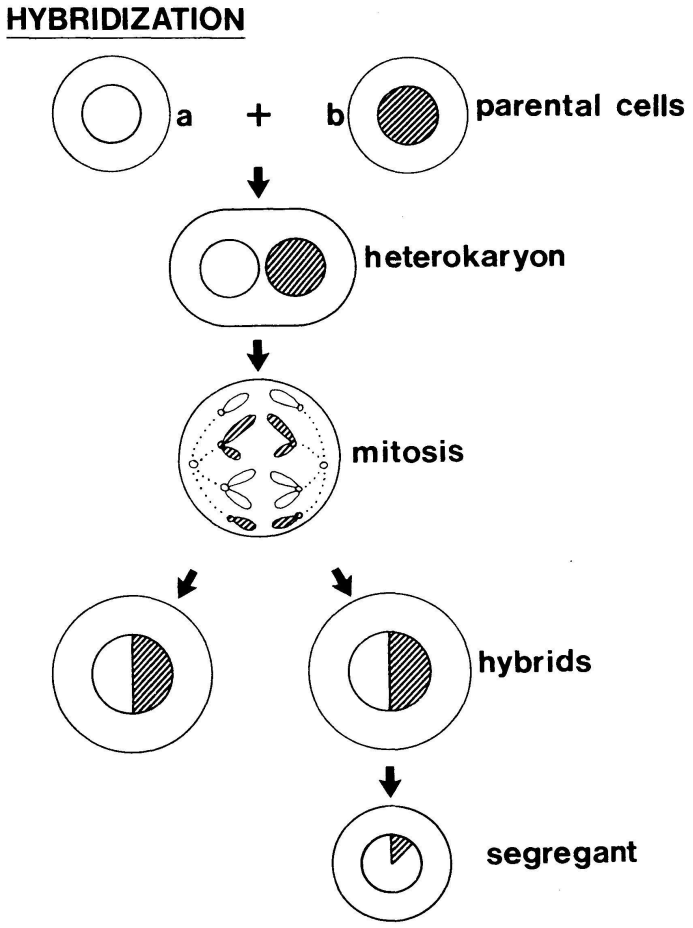


Fig. 2 Schematic summary of somatic cell hybridization. Fusion of two different cells, A and B results in a binucleate heterokaryon which in some cases divides to form two mononucleate hybrids (synkaryons) each of which contains a complete set of A and B chromosomes. If such hybrids are cultured over longer periods of time there may be elimination of chromosomes from one of the parental cells (chromosome segregation). A segregant may lose practically all chromosomes from the B cell while at the same time retaining a complete set of A chromosomes.

A wide variety of animal, human and even plant cell types, have been used as parental cells in these fusions. When cells of different organisms are fused *interspecific hybrids* are produced. In these cases, the parental cells differ at least with respect to genotype if not phenotype as well. *Intraspecific hybrids* are obtained by fusing two different cell types from the one species. In these cases the parental cells share a common genotype but differ in morphological, immunological, or functional properties, that is in phenotype.

The most common method of inducing cell fusion is to add *inactivated Sendai virus* to a suspension or monolayer of cells. Several chemical methods

of inducing cell fusion have been suggested. These involve the use of lysolecithin, polyethyleneglycol and artificially produced liposomes.

Heterokaryons, the immediate products when two different cells fuse, have been useful in the analysis of nucleocytoplasmic interactions and the role of the cytoplasm in the control of nuclear activity. Fusion of cells differing in their activity with respect to DNA and RNA synthesis has shown that the active partner almost always stimulates the nucleus of the inactive partner to synthesize more RNA and/or DNA. There is suggestive evidence that the signals which trigger the increased activity are proteins which migrate from the cytoplasm into the inactive nucleus. The signals appear not to be species specific since active cells of mouse origin can stimulate inactive human, rabbit or chick as well as mouse nuclei.

If one of the parental cells in a fusion happens to be in mitosis, while another is in interphase, the nucleus of the latter cell is induced to undergo premature chromosome condensation (PCC). The morphological appearance of the PCC filaments varies depending on whether the interphase cell happens to be in G_1, S, or G_2 phase. Therefore, the PCC phenomenon can be used to analyze the chromosome condensation cycle during interphase and factors which trigger mitosis. Furthermore, it represents a method of visualizing chromosomes in nondividing cells (e.g. sperm), and of scoring the immediate effects of chromosome breaking agents.

Heterokaryons have been useful in the analysis of factors which regulate phenotypic expression and in gene complementation analysis of human genetic disease. Restoration of a missing function in a heterokaryon formed by the fusion of two mutant cells is an indication that the two cell types suffer from different genetic defects. Using this approach it has for instance been possible to show that the Xeroderma pigmentosum syndrome in Man can be divided into 4 - 6 complementation groups. Each of these groups probably corresponds to a specific genetic lesion, each representing an enzyme or factor required for the repair of UV-induced DNA damages.

In order to study mononucleate hybrid cells (synkaryons) they usually have to be separated from the parental cells and from polyploid cells arising from the fusion of like cells. This can be achieved by single cell cloning or by using culture conditions which favour the growth of hybrid cells while inhibiting the parental cells. Selection for hybrid cells is commonly based on the use of mutant parental cells. The "HAT" selection system can be used as an example of this. Mutant cells deficient in hypoxanthine-guanine phosphoribosyl transferase ($HGPRT^-$) are obtained by selection with thioguanine or from patients suffering from the Lesch Nyhan syndrome. These cells are then fused with cells deficient in thymidine kinase (TK^-). The latter type of mutant is obtained by selection with bromodeoxyuridine. The genetic defects of the mutant cells are of little importance during growth on normal tissue culture media, since the relevant enzymes are only involved in salvage (reserve) pathways for nucleotide synthesis. When the main biosynthetic pathways for purine and pyrimidine nucleotides are blocked by the folic acid analogue aminopterin, normal cells ($HGPRT^+$ TK^+) can survive if supplied with exogenous hypoxanthine and thymidine whereas the mutant cells die because of their inability to synthesize nucleotides from hypoxanthine ($HGPRT^-$ cells) or from thymidine (TK^- cells). Hybrid cells formed from fusion of $HGPRT^-$ with TK^- cells can be isolated by selection in a

medium containing Hypoxanthine, Aminopterin and Thymidine (HAT-medium). Hybrids contain one chromosome set which is $HGPRT^-$ but TK^+ and one that is $HGPRT^+$ and TK^-. Therefore, they are able to produce HGPRT and TK enzyme and to utilize exogenous hypoxanthine and thymidine for nucleotide synthesis. Thus when combined in one cell the two parental genomes complement each other and make it possible for the hybrid cell to survive on HAT-medium. The unfused parental cells, however, are killed by this medium.

In addition to the HAT medium there are a number of other selective systems in which the parental cells are temperature sensitive or auxotrophic mutants. When the two parental cells differ with respect to their gene mutations, the hybrids can be isolated because of the gene complementation phenomenon. This type of analysis also illustrates an important application of the cell hybridization method, namely the genetic analysis of metabolic pathways and cell function.

Intraspecific hybrids (e.g. mouse + mouse) usually show chromosome numbers which approximate or are a little below the expected sum if one assumes that one cell of one type and one cell of the other type have fused (1 + 1 hybrids). As the cells are maintained in culture over long periods of time the chromosome number undergoes a slight decrease. Interspecific hybrids (e.g. mouse + man) on the other hand usually show extensive chromosome elimination. Presumably *chromosome segregation* in the hybrid population is due to abnormal mitoses and overgrowth of variants with a reduced chromosome complement. In interspecific hybrids chromosome segregation usually involves the preferential elimination of chromosomes of one species while the chromosomes of the other species are selectively retained. Thus human chromosomes are selectively eliminated in man + rodent hybrids. Since many homologous isozymes of man and mouse can be distinguished by differences in their electrophoretic mobility it is possible to establish if there is coexpression of both genomes or if a human enzyme activity has been lost because of loss of a specific human chromosome. By analyzing the expression of a variety of human markers in many different clones it is possible to establish that some markers always occur together and that they are always lost as a group *(linkage analysis).* In many cases the retention or loss of a marker can be correlated with a specific chromosome *(synteny testing).* Hybrid cells can also be used to establish the linear order in which genes occur on individual chromosomes, measuring distances between genes and relating these data to the structure of the chromosomes *(chromosome mapping).* One method of mapping genes is to induce massive chromosome fragmentation by treating one of the parental cells or the hybrids with chemical or physical mutagens, causing chromosome breaks. By using appropriate mouse mutants as one of the parental cells, and selective media on which the cells have to retain a specific human gene in order to survive, it is possible to obtain hybrid clones that retain a broken chromosome carrying the complementing human gene. Different clones differ with respect to the size and break points of the chromosome fragment. Detailed cytological examination of the chromosome fragments and assaying for linked genes by isozyme analysis make it possible to map the exact localization of genes down to the molecular level.

One of the main applications of the cell hybridization technique in cell biology has been in the analysis of gene expression and cell differentiation. Hybrid cells with complete or reduced chromosome sets have been examined for general characteristics such as morphology, growth rate, contact inhibition and senescence, complex physiological and immunological properties, specific gene products e.g. enzymes, hormones, immunoglobulins, and for sensitivity to specific drugs. Any of these characteristics can be used as a marker if the two parental cells differ with respect to it. Obviously a distinction has to be made between properties common to all cells of a given organism *(constitutive markers)* and properties which are expressed only by certain differentiated cells *(facultative markers).* A number of different gene expression patterns have been observed; *coexpression* of constitutive and facultative markers, *dominance* or *recessiveneness* of drug resistance markers, *extinction* of facultative markers and in some cases *activation* of new properties not expressed by the parental cells. Although these observations are important for our understanding of how gene expression and cell differentiation are controlled in eukaryotic cells they do not yet provide a clear picture of how gene activity is regulated in animal cells.

The technique of cell hybridization has also gained wide applications in the analysis of malignancy and transformation. It is an important tool in tumor virology where it has been used to map virus integration sites, virus rescue and virus detection, analysis of factors determining the susceptibility of different cell types to viral infections, and analysis of cellular mechanisms inhibiting viral replication or modifying viral gene expression.

Reconstruction of cells by fusion of cell fragments

A new technique which promises to be of great interest in the study of gene regulation and nucleocytoplasmic interactions in animal cells involves fusion of cell fragments. The technique used for generating cell fragments is based on the use of the drug cytochalasin B. If animal cells are exposed to this drug marked changes occur in the shape of the cells and frequently the nuclei are extruded into the tip of long protrusions which are connected to the main cytoplasm via narrow stalks. These stalks frequently break spontaneously, thus causing a loss of nuclei from some cells. The efficiency of *enucleation* can be increased to close to 100% by centrifugation. Usually glass discs with monolayers of cells are placed cell-side down in centrifuge tubes containing a cytochalasin solution. During centrifugation the nuclei are pulled out of the cytoplasms which remain attached to the discs. The cytoplasms (anucleate cells) have an abnormal shape immediately after centrifugation but if returned to normal culture medium they resume the form of the intact cell and continue to synthesize protein. The cytoplasms remain metabolically intact for 12 - 36 h before they round up and die.

The nuclei, on the other hand, can be collected from the bottom of the centrifuge tubes. These nuclei differ from nuclei prepared by other techniques in that they are surrounded by a thin rim of cytoplasm and an intact plasma membrane. These cell fragments, which are referred to as *minicells* or *karyoplasts,* in many cases contain less than 10% of the cytoplasm of the intact cell and are capable of RNA and DNA synthesis for several hours

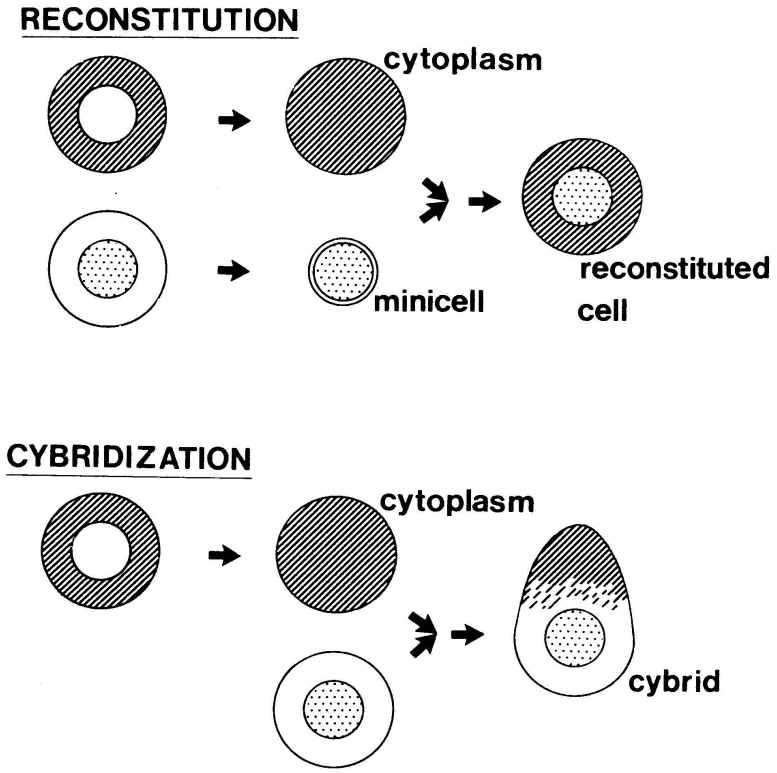


Fig. 3 Enucleation of cells by cytochalasin treatment and centrifugation of animal cells gives two types of cell fragments: nuclei (minicells) and cytoplasms. Both types of fragments are surrounded by an intact plasma membrane and may be fused to give a reconstituted cell. Viable cells may be reconstituted by combining nuclei and cytoplasms from different types of cells. If an enucleated cell (cytoplasm) is fused with an intact cell, the result is a cytoplasmic hybrid (cybrid).

after enucleation. Under the conditions of preparation and culture which we have used minicells from several different cell types have been unable to regenerate a cytoplasm and to form cell colonies. Instead the minicells have undergone lysis and have died within 24 - 48 h of enucleation.

Since nuclei and cytoplasms prepared by the cytochalasin enucleation technique are surrounded by an intact plasma membrane carrying receptors for Sendai virus they can be fused together with inactivated virus to give *reconstituted cells* (Fig. 3). It is also possible to add cytoplasm from one cell to an intact cell. In this case the result is a cytoplasmic hybrid *(cybrid),* that is a cell containing a cytoplasm which is a mixture of that of two different cells. One of the chief problems in such experiments lies in recognizing and distinguishing the intact parental cells, the reconstituted cells and cybrids. Four types of markers have been used to identify the origin of individual cells and small colonies of cells arising from reconstructed cells: morphological markers (nuclear size and shape), artificially produced markers (ingestion of polystyrene beads of different size classes and/or labelled with different fluorochromes), DNA content and functional markers. The functional markers (mutant cells defective in specific enzyme functions or resistant to specific drugs) are useful not only for identification purposes

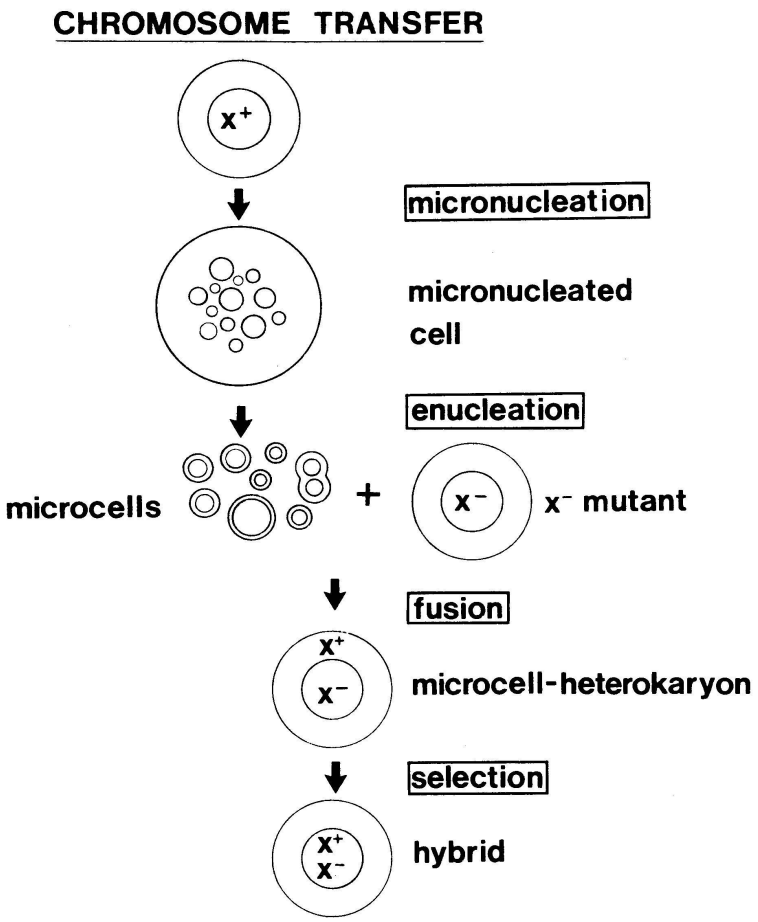


Fig. 4 Transfer of chromosome(s) from one cell to another by microcell fusion. Microcells are subdiploid miniature cells which are generated by first inducing micronucleation with microtubular poisons. Micronucleated cells are then enucleated to give microcells. The smallest microcells have a DNA content equivalent to single chromosomes. Fusion of microcells from normal cells with a mutant cell (x-) can be used to introduce a complementing chromosome (x+). By using appropriate selection methods one may be able to predetermine which microcell hybrids survive.

but also to obtain the progeny of the desired reconstructed cells by growth on selective media. Using this approach we have been able to reconstitute viable cells by combining nuclei and cytoplasms from different sublines of an established rat myoblast line. Also some combinations involving nuclei and cytoplasms from two different species have been successful. Thus viable cells capable of cell multiplication have been obtained by combining nuclei from rat myoblasts with cytoplasms from mouse fibroblasts. The fact that animal cells can be reconstructed by fusing two nonviable cell fragments from two different species and two different cell types indicates that it may be possible to generate a fairly wide spectrum of reconstituted cell types. Specific areas of research in which reconstitution techniques should prove useful include regulation of gene expression, the stability of the differen-

tiated state, interactions between the nucleus and cytoplasm, and the dependence of mitochondria and other cytoplasmic organelles on nuclear genes.

Using the cytochalasin enucleation technique it is also possible to prepare another type of cell fragment. *Microcells* are prepared by centrifuging micronucleated cells in the presence of cytochalasin (Fig. 4). Such cells can be obtained in large numbers by treating normal mononucleated cells with microtubular poisons (colchicine, colcemid, vinblastine etc.). After an extended metaphase nuclear membranes reassemble around individual or small groups of chromosomes which are scattered throughout the cytoplasm of the dividing cell because of abnormal anaphase movements. As a result the genome is divided into a large number of micronuclei in which the chromosomes are dispersed into interphase chromatin. Enucleation of micronucleated cells results in subdiploid microcells containing one or several micronuclei surrounded by a rim of cytoplasm and a plasma membrane. The smallest members of a microcell population have a DNA content equivalent to single chromosomes while the larger microcells clearly must contain more than one chromosome. As with minicells most of the subdiploid microcells are metabolically intact in the sense that they persist in culture for a short time and exclude trypan blue. Using inactivated Sendai virus, microcells may be fused with intact cells to give heterokaryons. There is tentative evidence that some microcell heterokaryons divide and give rise to mononucleate hybrid cells. Fusion with microcells, therefore, appears to offer a method by which a small part of the genome of one cell can be introduced into another cell. If the recipient cell is a mutant which is unable to grow on a selective medium it may be possible to isolate hybrids in which a complementing chromosome has been introduced by fusion with a microcell (Fig. 4). Thus, although it will be difficult to fractionate microcells and obtain those containing a specific chromosome, and although fusion is likely to be random, it is possible to control which hybrids one obtains. Microcell hybrids containing mouse chromosomes and only one specific human chromosome could be useful in chromosome mapping. Other applications could be in gene complementation analysis designed to distinguish structural and regulatory mutations, and in the analysis of integrating sites for tumor viruses.

Concluding remarks

Recent progress in somatic cell genetics has made it possible to generate many different types of genetically altered cells. The techniques are based on the use of mutagens, virus transformation, cell fusion, and methods of preparing and recombining cell fragments. With these techniques it is now possible to analyze a large number of important biological and medical problems. Undoubtedly it is also possible to repair genetic defects in mutant cells by introducing complementing chromosomes from normal cells. In spite of this, it is not likely that such techniques will be useful for clinical medicine within the foreseeable future. The main reasons for this belief are (a) that the problems in repopulating an individual suffering from a genetic disease with cells "repaired" in vitro appear to be exceedingly difficult.

There may also be a risk that the repaired cells give rise to tumors; (b) There are at least 1'500 distinguishable human diseases which are known to be genetically determined but most of these diseases are rare or very rare. Furthermore even if the patients show exactly the same symptoms they may differ with respect to their individual genetic lesions. The cost of mapping the nature of the genetic lesion in each individual and then designing a genetic therapy based on genetic engineering with cells or DNA would be prohibitive.

In view of the recent discussion about the dangers of recombinant DNA research it may be appropriate to ask if there are any dangers in genetic engineering with human or animal cells. So far there are no indications that there would be any serious risks in this type of research. On theoretical grounds it has been pointed out, however, that cell hybrids between animal and human cells may represent an intermediate host in which animal viruses can be adapted to grow on human cells by mutation or recombination with human cellular DNA. Clearly this possibility must be explored. It appears, however, to be a risk which may be controlled by using known methods for containing pathogenic microorganisms.