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The role of somatic cell genetics in human gene mapping

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Introduction

Genetics based on parasexual events, like the transfer of genetic material of a different origin into the genome of a proliferating eukaryotic somatic cell and the segregation of donor genetic material from the fusion product, is called somatic cell genetics. Examples of this process are the fusion of somatic cells into hybrids, which can be considered as the transfer of the complete genome from one somatic donor cell to a recipient somatic cell, and also the transfer of isolated nuclei, metaphase chromosomes, genes or arbitrary DNA fragments of eukaryotic or prokaryotic origin into recipient cells. Although this definition does not explicitly exclude gene transfer in vivo somatic cell genetics in the animal kingdom has until recently[123] been almost exclusively a domain of cell culture in vitro.

Cell fusion and gene transfer methodology have developed during the last 20 years into versatile tools which can be used in many fields of biological and medical research. Numerous detailed reviews[31, 39, 41, 44, 96, 110] have followed up this development and can serve to trace both the history and the methodological specificities.

Not only have somatic cell genetics methods and their fields of application proliferated at an exponential rate, but Human Genetics, the specific subject of this issue, has also encountered an explosion of information during recent years since molecular geneticists have learned that the anatomy of the human genome and the functioning of its genes in normal or mutated cells can best be studied in man.

Somatic cell genetics has contributed significantly to our understanding of mutagenesis[9, 117], dissection of human genetic diseases by genetic complementation[5, 31], dosage compensation[124], analysis of malignancy[109, 122], gene expression, and gene regulation[15, 20, 92]. It has even opened up a new era in immunology by providing the methodology for production of hybridomas capable of secreting monoclonal antibodies of predefined antigenic specificity[63]. For the field of human genetics its major success, however, has been in gene mapping.

This review will cover the current application of somatic cell genetics to the analysis of the anatomy of the human genome considering complementary approaches like family studies, in situ hybridization and chromosome sorting. One major task will be to demonstrate how the somatic cell genetics approach can bridge the gap which previously separated by orders of magnitude the mapping resolution of genetics and molecular biology.

Sources of information on human gene maps

Many authors have reviewed the information on human gene mapping in general[39, 49, 56, 74, 99, 106, 108] or on specific fields of application, for instance medical genetics[28, 53, 75, 76, 107], mapping and analysis of oncogenes[17], characterization of gene families[18] or comparative genetics[87]. These reviews offer a digest of the state of the art. A complete collection of available information is compiled by regular Human Gene Mapping Conferences[46–52]. The committee reports of these meetings cover all available published gene mapping data. The material is evaluated using an arbitrary scale of credibility ranging from 'confirmed' to 'in limbo'. In addition to compiling an updated

catalogue of all mapped genes special care is given to standardizing nomenclature and to reporting mapping data important for neighboring fields of research like chromosome rearrangements in neoplasia, fragile sites, and comparative gene mapping.
Search for complete information on particular genes starting from these conference reports can, however, be a tedious task. For such a problem the Yale Human Gene Mapping Library, a data base maintained at the Dept of Biology of Yale University under the direction of Dr F.H. Ruddle probably provides the most complete source of references at present. A commercial database (GIRS, an adjunct of the journal Cytogenetics and Cell Genetics) collecting and distributing Human Gene Mapping information on line will be operating in the near future.

Gene mapping in man

The genetic information contained in human cells is spread over 24 nuclear chromosomes and the mitochondrial DNA. Converging evidence from various sources has made it possible to estimate that the human genome contains 5×10^4 structural genes, which may be grouped in some 3000–15000 clusters[107]. Mapping the human genome involves partitioning this number of genes into maps of 24 individual linkage groups and determining their linear order on each of these chromosomes. The most advanced 'gene map' at present is represented by the 37 genes in the DNA of the mitochondrial genome[2].
The dissection of the nuclear genome for mapping can be achieved by meiotic segregation in families, mitotic segregation in somatic cell hybrids, natural tagging of particular chromosomes in individuals by chromosome aberrations, by sorting of individual metaphase chromosomes, by analysis of subchromosomal fragments using somatic cell genetics and/or genomic or gene sequencing procedures established by molecular biology.
Markers which have been used for mapping range from fragments of the DNA itself to differences in chromatin composition as reflected in chromosome morphology, from the products of coding genes, RNA and proteins, to genetic traits which characterize an individual in a population. Human gene mapping, as covered by the Gene Mapping Conferences, has mainly been restricted so far to a level of resolution which can identify 'genes' or at least DNA fragments. The results of purely molecular studies have not been included. Obviously the organization format of a conference, even with access to computer facilities, is inadequate to cover the burst of knowledge from restriction mapping and DNA sequencing. The ultimate goal for achieving precise molecular knowledge of the human gene map is to determine the nucleotide sequence of each chromosome and to delineate the position of specific genes within this sequence. The commingling of various methodological streams in gene mapping: classical Mendelian genetics, chromosome studies, somatic cell genetics, mapping with new technologies like chromosome sorting and of recombinant DNA methods has now opened up the field to achieve this goal.
The reason for human gene mapping and its potential both for understanding human biology and for medical application have frequently been outlined[28, 56, 76, 98] and will therefore not be stressed here again.

Assignment of genes to chromosomes

Two major genetic strategies are followed in dissecting the human genome; linkage studies in families based on meiotic segregation and analysis of somatic cell hybrid panels generated by parasexual events like cell fusion or gene transfer followed by mitotic segregation. Somatic cell genetics has so far been the most successful approach. About 60% of the gene assignments achieved until 1983 were performed by this method.
Studies correlating microscopic analysis of naturally occurring genetic imbalances which result from chromosomal rearrangements or faults in mitotic or meiotic division quantitatively or qualitatively with the presence of phenotypes has contributed considerably to the solution of particular regional mapping problems[8, 24, 56, 93]. Here, however, the use of cells with such anomalies will be discussed only as material for somatic cell genetic studies or two other important techniques developed more recently: in situ hybridization and chromosome sorting, which no longer require chromosome segregation by mitotic or meiotic means.

Somatic cell hybrid panels

Gene mapping by somatic cell hybridization is based on a series of parasexual events including fusion of somatic cells from different species, fusion of their nuclei, and mitotic segregation of part of the genetic material of one of the parents during the proliferation of the fusion products. In clones of cell hybrids retaining the complete genome of one parent (recipient) and one or several chromosomes of the segregating genome (donor) the concurrent segregation of biochemical phenotypes (markers) indicates that these genes are located on the same chromosome (syntenic). The corresponding retention of a donor chromosome or chromosome fragment and a donor gene marker allows the assignment of its gene. Formation and isolation of somatic cell hybrids by selection systems and by cell cloning as well as techniques for biochemical and cytogenetic analysis of the fusion products have been reviewed previously[14, 39, 58, 98, 102] and are standard methodology now.
It has been suggested that two types of collections of cell hybrid clones – frequently named panels – can be designed which should allow unambiguous assignments of genes to particular chromosomes: a 'single chromosome mapping panel', in which the cells of 24 unique hybrid clones contain one unique donor chromosome each, or a 'multiple chromosome mapping panel' containing clones with multiple donor chromosomes overlapping in such a way that a unique segregation pattern characterizes each chromosome[14, 58]. For human gene mapping a minimum number of five clones ($2^5 > 24$) of suitable configuration should be sufficient to perform this analysis[14].

Single chromosome mapping panel

Hybrid clones containing single human chromosomes can be generated by chance immediately after cell fusion or during the permanent process of chromosome segregation from established cell hybrids.
The desired chromosome should be retained in all cells of the clone whereas all other human chromosomes should

be lost. Many of the available hybrid clones with single human chromosomes have been obtained by frequent subcloning of clones retaining by chance a few human chromosomes. However, in this case there is no possibility of securing the retention of the single human chromosome, and the cloning procedure can be quite tedious. Therefore it is favorable if systems are available to select for the presence of a given chromosome and against the presence of others in one particular clone. In principle a number of selective systems could be utilized for this purpose based on drug resistance or sensitivity[10] or the requirements for particular nutrients or the presence of cell surface markers in combination with immunological or cell-sorting procedures[58].

The heterogeneity of these techniques, however, has probably limited their general use for the establishment of one panel. A way to circumvent this problem might be the use as parental cells in hybrid production of human cells in which the same selectable marker is translocated from its original site to other chromosomes. As an example, there exist numerous X-autosome translocations in which the selectable HPRT-locus is linked to autosomes[7]. Although several laboratories have used such translocations in fusion experiments nobody has made the effort to establish a complete single chromosome mapping panel from this material.

Recently, gene technology has allowed the engineering of viral vectors carrying prokaryotic or eukaryotic genes which can serve as selectable markers in tissue culture cells[30, 55, 81, 85]. These viruses integrate, probably at random, into chromosomes of various cell types, thus providing an attachment-point retaining their host chromosome or the genomic sequences neighboring the insertion site by selective media in the host cell.

This procedure offers the potential for introducing the same selectable marker into all 24 human chromosomes, each one in a separate cell. Outside the single insertion site these cells should remain unaltered. A series of cell hybrids originating from these 24 different human cells could retain the 24 different human chromosomes in selective medium. The unwanted supernumerary chromosomes in each clone, however, would still have to be eliminated.

An alternative fusion procedure which should produce hybrid cells with few or single donor chromosomes, microcell fusion, could solve the problem of unwanted chromosomes: Metaphase nuclei are split into microcells or minisegregent cells which contain few or single chromosomes packed into a cell-membrane-derived envelope[21, 26, 115]. After fusion with a recipient the production of hybrids with single donor chromosomes is obtained faster than with cell to cell fusion.

Unfortunately, it has turned out that at some time during this procedure donor chromosomes tend to be partially deleted or rearranged[58]. Thus the retention of intact chromosomes has to be carefully controlled. After all, if hybrids with single donor chromosomes are obtained and maintained under selective pressure to retain this chromosome, fragmentation of the donor chromosome can occur[82]. Hybrids with smaller donor fragments might have a higher division rate and overgrow the original clone.

The consequence of these pitfalls is that so far no laboratory has succeeded in producing a complete single chromosome mapping panel.

Multiple chromosome mapping panel

The problems encountered when producing a multiple chromosome mapping panel are not very different from the ones listed above. Theoretically the techniques for manipulating the donor chromosome contents in individual clones are available. In practice it has proved too much of an effort to set up the various selective procedures in a single laboratory[10, 58].

Therefore, generally, a pragmatic approach was adopted: A number of independent hybrid clones was generated and, after a period of growth, analyzed for the presence of donor choromosomes by cytogenetic and biochemical techniques. From this collection a series of clones was chosen as a mapping panel based on various criteria like their donor chromosome content, their relative stability, numerical relationship of donor and recipient genomes and growth characteristics. Clones which continued to segregate donor chromosomes at a high rate were avoided.

Usually the donor chromosome content in a series of hybrid clones is not favorable enough to give 5 clones overlapping in such a way that they could define each chromosome by a unique segregation pattern. Specific combinations of human chromosomes tend to cosegregate in human rodent hybrids, whereas other combinations are particularly rare[4, 78, 91]. In addition, the use of a panel containing only the minimum number of 5 different clones is prohibited by the high risk of encountering chromosome rearrangements in the clones. Both donor and recipient chromosomes do rearrange. The products, translocations or deletions within or between the species, are often not identified with the available cytogenetic techniques[37, 38, 79]. This pitfall is avoided with a certain amount of redundancy in the selected clone collection. In addition to selecting more clones than absolutely necessary for analysis a number of rules must be followed to minimize the risk of serious misinterpretations.

1) Clones in a mapping panel must originate from independent fusion events to avoid multiplication of errors due to rearrangements in a primary hybrid. It may even be helpful to use different donors to compensate for the individual variability of donor genomes.

2) All experiments must be performed with cells of the same batch, harvested and characterized at the same time. A new batch of the same hybrid clone has to be dealt with separately and to be characterized again since the clones are metastable; the donor chromosome content can remain the same during a long period of growth, but nevertheless, chromosome rearrangements, segregation and selection of particular chromosome combinations can occur unexpectedly within a short period of time, e.g. owing to selective pressure exerted by selective media, freezing/thawing, change of culture media or culture condition like starvation or accidental alteration of incubator temperature.

3) Selective pressure should be limited to the minimum time required to select the hybrid clones retaining the desired chromosome. Continuous growth in selective me-

dia induces or at least favors cells with rearranged chromosomes[82].
In addition it is very useful if the same batch of hybrids is used in a great number of experiments, since individual pecularities of a given clone can be more easily recognized if more markers of known location are tested with these cells[45].
The logistic problems with the production and continuous control of suitable hybrid panels have so far prevented the distribution of this material on a large scale by cell repositories or commercial enterprises and, at the same time, have stimulated the search for other more simple and straightforward approaches to gene mapping like chromosome sorting.

Regional mapping

If human cells with translocations of specific chromosomes are used for cell fusion with rodent cells, hybrid panels can be generated for regional assignment[38]. In these the segregation of a gene marker and fragments of a chromosome are analyzed. The combination of segregation data from these hybrids with data obtained by other mapping approaches using microscopically detectable chromosomal abnormalities[24, 56, 93] determines a region on a chromosome in which a given gene is localized, named 'smallest region of overlap (SRO)'[49]. The method potentially allows very fine mapping, even the establishment of a gene order within a microscopically detectable band. It is limited, however, by the number of available cells with defined defects.

Hybrid panels versus linkage studies in families

The use of somatic cell hybrid panels for human gene mapping offers a number of advantages over linkage studies in families, in particular the practically unlimited number of markers and the high progeny number of cells in culture, which have allowed many more human autosomal genes to be localized with somatic cell genetics than with family studies[74-76]. The impact of Mendelian genetics, however, has recently increased quite dramatically.

Markers

In somatic cell genetics, as opposed to family studies, allelic variance is no longer necessary for mapping. The differences in DNA sequence between the species allow the segregation analysis of human phenotypes (markers) on a hamster or mouse background[77, 89]. These differences are often reflected on the gene product level by the amino acid sequence of proteins.
Before the advent of recombinant DNA technology a great variety of techniques were applied to identify particular proteins and to allocate them to their species of origin; electrophoretic separation of enzymes by various separating gel types followed by specific staining was employed first[77, 89] and has been most successful. Immunological approaches identified particularly cell surface proteins[58]. 2-D-electrophoresis techniques split the cellular proteins into a great number of polypeptide spots, some of which could be assigned to one parental species[13]. This polypeptide mapping, however, has only rarely led to the identification of the protein generating a spot. This might be a reason why this powerful technique has not been exploited to its full capacity for mapping purposes. Another reason might be that the prospects of gene technology caused many of the groups which were capable of applying refined protein separating techniques to turn to the use of RNA or DNA itself as a marker. DNA or RNA probes used as markers can be sequences obtained by DNA synthesis, cDNA copied from isolated mRNA, or sequences cloned from gene libraries. These libraries in plasmid, cosmid, or viral vectors can compose the complete genome of a cell, or only the transcribed sequences[25, 112]. For the construction of chromosome specific libraries[73] the genome can be dissected by the formation of hybrids retaining single donor chromosomes[40, 68] or by mechanical isolation in a flow sorter[16, 36, 65]. All the techniques of somatic cell genetics listed below which allow the dissection of a particular chromosome into identified fragments provide material for the production of libraries of these pieces. The function of the sequences used as probes may be known, or they may be 'arbitrary fragments' of DNA[112].
The availability of DNA probes as markers dramatically increased the potentials both of somatic cell genetics and pedigree analysis. However, the same advantage which was present for proteins still holds for this new category of markers: No polymorphisms or variants within the species are required to map a region complementary to a given DNA probe to a particular chromosome or chromosome region via somatic cell genetics. Since the DNA is present in all nucleated cells the approach is no longer limited to genes of 'housekeeping' proteins expressed in cell hybrids.
Mapping of a gene by family studies requires it to be marked via a mutation to an allele, and needs information from families to follow its segregation. Due to the low number of suitable polymorphisms detectable on the protein level in the human population the establishment of autosomal linkage groups and their assignment to particular chromosomes was not very rewarding for many years.
Molecular genetics now helps to overcome this limitation to some extent, since a great number of inherited differences in DNA sequence can be detected with a reasonable amount of experimental effort, if they affect the size of fragments generated by the action of restriction enzymes[111]. These restriction fragment length polymorphisms (RFLP) can be detected by agarose gel electrophoresis, Southern blotting and hybridization with a labeled DNA or RNA probe[25, 111].
Studies at the beta-globin locus[54, 88] and at the human albumin locus[86] have demonstrated that about 1 nucleotide site in 85 to 100 is potentially polymorphic and about 1 nucleotide in 500 will actually differ between any two randomly chosen chromosomes because of linkage disequilibrium observed between markers within very short distances (100 kb or 0.1 centimorgan or less)[86].
The frequencies observed in these loci would suggest that if there are 10^6 bp of DNA on the average in 1 centimorgan, these would contain 10,000 potentially polymorphic markers (1/100 of 10^6). 2000 of these (1/500 of 10^6) would be informative for distinguishing between any

two randomly chosen chromosomes[86]. The actual number may be much larger if the number of polymorphic sites in noncoding regions exceeds that of coding loci[12]. Even if only one in 10 polymorphisms might be recognized by restriction analysis there is a good chance that a single copy cloned probe will identify a polymorphism[108].

This suggests that an enormous number of markers exists for linkage studies by pedigree analysis. The human chromosomes could be covered with a network of well-spaced polymorphic markers[6]. Close linkage would determine the chromosomal location of any gene or fragment, alleles of which are segregating in human families. Because of the RFLPs detected so far only a few are highly polymorphic[112, 119], the actual number of polymorphic loci required to cover all human chromosomes will be considerably higher than the 150 originally anticipated to be the lower limit[6].

While this network is set up, using mapped DNA polymorphisms will be more efficient in linkage tests. However, once the location of a reasonable number of genes is known, the mapping of new markers no longer requires the assignment by somatic cell hybrids or other techniques. Owing to this development the availability of marker systems no longer limits chromosome mapping by family studies.

Care has to been taken when physical distances on chromosomes are related to genetic distances, since recombination frequencies can vary by as much as 60-fold between sites on the same chromosome[101].

Progeny number

The second advantage of somatic cell genetics over family studies was the unlimited number of progeny of established cell hybrids in culture compared with the low progeny number in man. For linkage studies the difficulties of obtaining adequate numbers of informative pedigrees were serious. In addition to the problems of obtaining statistically significant data, the presentation of the results in the literature did not always allow a comparative evaluation.

These shortcomings are being solved now as a result of progress in organization and cooperation mainly promoted by the Human Genetic Mutant Cell Repository at the Institute for Medical Research, Camden, New Jersey[90] and the Human Gene Mapping Conferences. Collections of fibroblasts and lymphocytes established from members of selected families[119] are distributed via the Camden Repository. In a cooperative effort all segregation data of all markers tested in this material can easily be added in a uniform manner to the previously compiled information. A rapid assignment of any new polymorphic marker into a detailed genetic map is thus easily at hand. It should, however, be borne in mind that the high number of meioses which has to be analyzed for obtaining statistically significant segregation data will render mapping studies at a level of resolution below 1 centimorgan a tedious task.

Mapping by direct hybridization

Molecular biological techniques and sophisticated equipment have allowed the development of procedures by which DNA probes can be assigned directly to metaphase chromosomes which have been isolated and fixed on a surface: in situ hybridization and chromosome sorting.

In situ hybridization

For in situ hybridization, metaphase chromosome preparations on a microscope slide are hybridized with highly labeled DNA probes. The radioactive signal is detected by autoradiography. This approach was previously limited to the detection of repetitive gene families. Technical improvements which increase the signal generated by the hybridized probe now allow identification of low and single copy genes[29, 43, 72]. In situ hybridization combined with appropriate cytogenetic resolution extends the precision of gene mapping and complements other mapping methods. Zabel and coworkers[127] could assign a series of genes to specific regions of human chromosomes which showed up to 1000 Giemsa-bands per haploid set of chromosomes. At this level of resolution one band represents on the average 3×10^3 kbp(kilobasepairs)[56]. This is of the order of three centimorgans. Unless new cytogenetic techniques are developed which extend bands on metaphase chromosomes considerably more, this level of resolution is likely to be the highest which can be reached with this technique.

Translocations could separate loci which are less than one centimorgan apart. In the same way as these are useful in somatic cell hybrids they can allow in situ hybridization to one or the other side of the breakpoint[84] and thus increase the mapping accuracy.

In situ hybridization requires considerable cytogenetic experience. The method is limited to DNA sequences with a single or at least infrequent location in the genome. Hybridization of a specific DNA probe to Southern blots of genomic DNA shows, in many instances, a series of restriction fragments hybridizing with different intensities at given stringency conditions. These bands can sometimes be assigned to different chromosomes. Since the hybridization characteristics of a given probe with genomic DNA should be known before in situ hybridization experiments, electrophoresis of genomic DNA and analysis of hybridizing fragments on gel blots with the labeled probe are still necessary. Therefore the use of the direct technique is not faster or less laborious than the use of a suitable cell hybrid panel. Consequently, to achieve maximal resolution, in situ hybridization has mainly been used in combination with the other approaches for regional mapping of genes on particular chromosomes, although occasionally it has been used to localize genes directly on previously unidentified chromosomes[95].

Spot blots

The development of sophisticated dual laser sorters offers another opportunity for direct assignment of genes to chromosomes. Isolated fixed metaphase chromosomes are stained and sorted according to two parameters onto nitrocellulose filters[3]. Lebo and coworkers recently improved the resolution, to identify 22 human chromosome fractions with 21 unique spots[69]. 3×10^4 chromosomes of each type were sorted directly onto nitrocellulose filters to avoid DNA extraction or restriction enzyme digestion. A panel of filters (spot-blot) was sorted in 2 days and

each panel could be used five times to map genes. In addition to confirming known localizations of human genes, the authors localized the previously unassigned skeletal muscle glycogen phosphorylase gene on chromosome 11[69].

As for somatic cell hybrids and in situ hybridization, translocations separating fragments of chromosomes are useful for regional assignment[69].

The resolving power of this direct method is limited to individual chromosomes or, at best, chromosome fragments. This is the capacity of a panel of somatic cell hybrids. In addition, multiple locations of homologous sequences on one chromosome are not easily detected. If somatic cell hybrids were easier to provide on a large scale there would be no immediate need for dot blot mapping, particularly since the equipment required for generating these dot blots is expensive and requires experienced handling. However, if dot blots should become available commercially or on a non-profit basis, they would be extremely useful gene mapping tools for many scientists working with human DNA samples without direct access to genetic expertise.

Fine mapping of chromosomes

A human chromosome, on average, spans 10^5 kilobasepairs (kbp) of DNA. The somatic cell genetics methods for regional gene assignments described above at present dissect the chromosome into regions of about 10^4 kbp each. For favored regions for which a high number of cells with cytogenetic peculiarities is available, e.g. the short arm of chromosome 6[93] a better resolution down to a level of a single chromosomal band or even lower (10^3 kbp) will be obtained by the combined efforts of somatic cell genetics, Mendelian genetics and chromosome studies. For the greater part of the genome, however, there is a gap in resolution between the power of molecular biology and the mapping procedures described above which spans between 10^2 and 10^4 kbp.

The tendency of donor chromosomes to fragment during fusion is on the one hand a major problem hampering the production of cell hybrid panels, but on the other hand can be exploited to bridge this gap by introducing fragments of subchromosomal size into recipients. The fusion products can serve for the alignment of the genes around a selectable biochemical marker and can thus cover an intermediate level of resolution which is not easily reached with the search for SROs in hybrid panels.

On the basis of transfer into somatic cells several strategies have been devised for dissecting individual human chromosomes down to the size of individual genes, and for multiplying single pieces in recipient cells for isolation and analysis. The general methodology has been reviewed and summarized in a series of diagrams by Shows and Sakaguchi[106].

Induced breakage of donor chromosomes

Goss and Harris[32] induced donor chromosome breakage by irradiation and subsequently transferred these cells to recipients by hybrid formation. By counting the frequency of cotransfer of genes known to be syntenic the authors determined a gene order for the human X-chromosome[33] and estimated the distance between the linked human genes thymidine kinase (TK) and galactose kinase to be 1.2 centimorgans[34].

In hybrid clones containing a single human chromosome human genes are present in the haploid state. Therefore single recessive mutations become detectable. The use of mutagenized subclones containing specific deletions in the human chromosome makes possible the convenient mapping of other genes carried on the human chromosome[57].

Microcell-mediated gene transfer

Cells arrested in metaphase in response to mitotic inhibitors[26] or treated with cold shock[22, 115] split up their nuclei into micronuclei of various sizes, which can be collected encoated by part of the cellular membrane by centrifugation in the presence of cytochalasin[22]. The resultant 'microcells'[22] or 'minisegregant cells'[105], the smallest of which contain single chromosomes, can be fractionated according to size and used in fusion experiments. The use of microcells, at least in a number of experiments[26, 27], has allowed the introduction of single chromosomes into the recipient genome. However, since frequently chromosomes introduced with this technique do not escape lysosomal degradation[27, 58], the approach can be listed among the procedures resulting in the transfer of chromosome fragments.

Chromosome-mediated gene transfer

Transfer of genetic material into mammalian cells using isolated metaphase chromosomes followed by selective retention of a marker gene results in a broad range of transferents with transgenomes[121] consisting of several donor chromosomes[125, 126], of cytologically detectable fragments of donor chromosome material[61, 62, 83] or, most frequently, of small cytologically undetectable fragments including the selectable gene[104, 121].

Since this approach has been carefully reviewed several times[106, 121], I shall mainly focus on recent developments which make the method extremely useful because it can bridge the gap in resolution between molecular biology approaches and gene mapping by cell hybrids or other techniques, i.e. the range between 10^2 and 10^4 kilobasepairs (kbp) which covers closely-linked genes at a distance of approximately 0,1–10 centimorgans.

The retention of the transgenomes in recipients depends on the presence of genes which can be selected for in cell culture, like hypoxanthine phosphoribosyltransferase (HPRT) or the TK genes. Because there are only few of these genes large parts of the genome could not easily be retained after transfer of metaphase chromosomes. In a number of cell lines from patients, the selectable genes are introduced by rare translocation events into other chromosomal sites. These cells can be used as donors. They are, however, too rare to cover the human genome completely[7].

Gene cloning procedures have recently led to the isolation of selectable genes of eukaryotic or bacterial origin inserted in viral vectors, which can be integrated to function in eurkaryotic genomes at many locations[30, 81, 85, 94]. Thus not only can each human chromosome be selectable by specific media, as described above, but even each chromosomal region.

As vehicles to transfer genes into the genomes of cells, retroviruses appear to be ideal[80]. Their viral RNA genome is efficiently transmitted into the recipient cell and integrated into the chromosomes as DNA. Integration is specific with respect to the viral genome. Its plasticity allows the packaging of DNA inserts up to at least 7 kbp. In contrast, the previously used papovaviruses (e.g. Simian virus 40, SV 40) can package foreign DNA only up to 2.5 kbp[81]. In addition they have only a limited host range, whereas retroviruses have a wide host range and can infect a variety of cell types. Their viral long terminal repeats provide efficient signals for initiation and termination of transcription[19, 116].

If these selectable markers can be introduced into a single chromosomal site of one cell the selectable region can be analyzed directly for cosegregation of test markers. If, however, the selectable sequences are inserted at different locations in the donor genome, a second selective system is required to identify one particular transgenome in the population of transferents.

Procedures have been worked out for a detailed genetic analysis of any specific region of a mammalian genome to which one or more genes have already been mapped[30,118]. In one series of experiments Weis and colleagues[118] infected a murine cell line multiply with a defective recombinant murine leukemia virus that contains a neomycin-resistance gene. This gene, which also confers resistance to a substance (G 418) toxic in eukaryotic cells, was thus introduced at multiple sites in the mouse genome. Genomic fragments containing both the G 418-resistance gene and the murine H-2 gene were obtained by screening resistant transferent cells obtained after chromosome-mediated gene transfer for expression of H-2 antigens. From the transfer method used the authors anticipated that the transferents should receive about 1% of the donor genome and that they would have to screen about 100 chromosome recipients to find the desired cell. Actually they found two suitable clones by screening 30. These received less DNA than is required to encode genes 5 centimorgans apart. However, transgenomes of larger sizes can be expected to be obtained with this approach, cloned into cosmid libraries and used for rapid chromosome walking experiments. Since the method appears to introduce 0.1–1% of the donor genome into the recipient cells one can expect that one should be able to co-transfer genes that are separated by as much as 20 centimorgans[118].

This dissection via chromosome mediated gene transfer, if extended to other chromosome regions, and combined with molecular cloning methods, can provide a series of about 100 to 200 cosmid minilibraries covering the whole genome. If the size and the sequential order of adjacent fragments are known the speed of chromosome walking[11, 114] could be increased considerably. This will be particularly useful for narrowing down the position of disease genes starting from linked RFLP genes[119] and eventually pave the way to identification, sequencing and functional characterization of the relevant genes.

Mechanical dissection of chromosomes

A molecular map of large portions of mammalian chromosomes can be approached without the use of gene transfer techniques. Since DNA-microcloning procedures successfully work with very small amounts of DNA[103], the mechanical sampling of parts of metaphase chromosomes under microscopic control provided sufficient material to establish a gene library of one particular chromosome region containing the T-complex of the mouse on chromosome 17[97]. This procedure is only applicable to chromosomes which can be unambiguously identified in a metaphase spread and provides material only for molecular analysis.

DNA-mediated gene transfer

The transfer of total genomic interphase DNA into mammalian cells using calcium phosphate precipitation[35, 106], polyethyleneglycol-mediated transformation[60], or microinjection[1] followed by selective isolation of transferent cells, results in the retention of fragments which are often not much larger than the selected gene.

Combined with molecular cloning techniques this approach has allowed the isolation of selectable genes of eukaryotic origin[70, 71, 81, 120]. Cotransfer of selectable prokaryotic or eukaryotic genes with other sequences for which no selection system was available permitted the retention of both, obviously due to fusion of the DNA fragments during the transformation process[106]. This method has contributed much to our current knowledge of the mechanism and regulation of gene expression[23, 81, 102, 113, 122]. It is, however, probably not very useful for the mapping of long distances because fragments of various origins can be combined and cotransferred[113]. Alien sequences can be eliminated by repeated transfer steps. As afterwards the maximum size of the transgenomes is not much more than 50 kbp[99] the DNA-mediated gene transfer offers little advantage over chromosome walking for the fine dissection of unidentified parts of the human genome. Its major potential for mapping purposes is as a functional indicator system for molecular cloning of eukaryotic genes, based on their expression in cultured cells[64, 100].

This can be exploited both for the analysis of individual mutations within a previously identified gene, as has been demonstrated for the beta-globin cluster in thalassemias[55], and for the identification and isolation of genes for which a functional assay is available.

In the most commonly used method, total genomic DNA provides donor sequences for gene transfer. Transferent cells are isolated by selection[64]. After a series of secondary transformations cells that are free of most nonessential transforming DNA sequences are isolated. The target gene can then be rescued by construction of a recombinant DNA library and identified with repeat DNA sequences[55, 64] or linked vector probes[42, 71].

To shorten the molecular cloning part of this procedure and to minimize the risk of cotransfer of unrelated genomic fragments a series of specific cosmid shuttle vectors has been developed[66] containing genes which are selectable both in eukaryotic and prokaryotic cells. These vectors can serve as cloning vehicles for preparing genomic recombinant DNA libraries and gene isolation, as expression vectors for both transient and stable transformation in tissue culture cells and as shuttle vectors between bacterial and mammalian cells[67]. Compared with the usual DNA transfection method the functional gene sequences

can be recovered faster and more efficiently. Primary transformants appear to retain the original input pattern whereas secondary transformants contain various rearrangements[67]. An obvious limitation of the shuttle vectors is imposed by the size of the insert in cosmid cloning. Genes larger than 45 kb would not be represented in their entirety within a recombinant cosmid and could not be cloned with this technique.

Prospects

Somatic cell genetics laid the foundation for successful human gene mapping about 15 years ago, and since then has contributed to the majority of the more than 800 autosomal assignments performed up to 1984. Now it can again play a crucial role since it has the potential to fill the gap between the maps of the geneticists and those of the molecular biologists.

Obviously, though, the number of localized genes which have been characterized to some extent at the molecular level is still very small, and a complete map at the level of genes or even less at the level of DNA-sequences is not within reach. In addition, the variance between analogous DNA-sequences of different individuals or even more between populations or races is too profound for 'the' DNA-sequence of one chromosomal region or one chromosome to be determined. However, if the methodology continues to develop at the same rate as it has during the past few years, one can foresee that within a short time the location and sequence of most genes responsible for serious monogenic inherited diseases will be known. This opens up new horizons for diagnosis and, eventually, even therapy at the DNA-level.

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