The Status of the Gene Map of the Human Chromosomes

At least one gene on each chromosome of man is known; some chromosomes are extensively mapped.

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Since the work of Sutton and Boveri in the first years of this century (*1*), it has been known that the chromosomes carry the "factors" that Mendel inferred in the 1860's and that Johannsen in 1909 called genes. Since the work of Thomas Hunt Morgan and his colleagues, particularly his student Sturtevant, in 1911 and the immediately following years, it has been known that the genes are arranged linearly along the chromosomes. Furthermore, it then became clear that the relative distance separating two gene loci on a chromosome could be inferred from the frequency with which recombination of traits determined by genes at these loci occurred among the offspring of particular parental pairs. Also in 1911, a specific gene, that for color blindness, was first assigned to a specific human chromosome, the X, by Morgan's associate at Columbia University, E. B. Wilson (*2*). In man the correct number of chromosomes, 22 pairs of nonsex chromosomes (the autosomes) and the sex chromosomes, either two X's (in females) or an X and a Y (in males), has been known since 1956 (*3*). In 1959, it was first inferred, from a study of humans with abnormal sexual development, that the Y chromosome (definitely established to be present in man by Painter in the early 1920's) carries a testis-determining factor (or factors). In the last 7 years new methods for staining the chromosomes ("banding techniques") have revealed distinctive regional landmarks permitting unique identification of each chromosome (*4*).

Thus, in exploration of the genetic planet that is the cell nucleus of man, the broad outlines of the continents and some of the gross details of their topography have been known for some time. It is only since 1968—and mainly in the last 5 years—that cartographic details of the chromosomes, the location of specific genes to specific chromosomes and regions thereof, have been determined (*5–10*).

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How Many Genes Does Man Have?

In man the number of structural genes—those that determine the amino acid sequence of polypeptide chains of proteins and occur in single copies so that mutations behave in a Mendelian manner—may be of the order of 50,000 (*11, 12*). The amount of DNA of man is sufficient for 50 to 100 times as many genes of average length. However, a large amount of the DNA is in a repetitive form that may play various regulatory, structural (centromere, for example), and functional roles in the chromosomes (*13, 14*). Some DNA codes for ribosomal RNA and transfer RNA (tRNA).

Specific Genes in Man

Genetics has been defined as the science of variation. Until the era of molecular genetics, without variation there could be no genetics. The presence of a gene was inferred from the existence of alternative forms of a given trait. In man more than 1200 gene loci have been confidently identified by this method (*10*). Examples include the biochemical and immunologic marker traits useful in chromosomal mapping by either family or somatic cell methods. About 900 gene loci are known because of the occurrence of disorders, most of them rare, resulting from mutation at these sites. A list of diseases determined by genes on the X chromosome is, for example, like a photographic negative from which a positive picture of the normal genetic constitution of the X chromosome can be deduced.

Molecular genetics brought us other ways to infer the existence of specific genes. The trinitarian dogma of DNA-RNA-protein and the related principle of one gene–one polypeptide chain means that, once the sequence of amino acid residues in a polypeptide chain is fully known, the existence of a gene that determines its structure can reasonably be inferred even though no variant form of the polypeptide has been discovered. Indeed, the principle of the colinearity of nucleotide bases in DNA and of amino acids in protein permits, within broad limits dictated by redundancy of the code, deductions about the chemical structure of the gene. The "Lepore method" of mapping (discussed later) is an example of how genetic inference can be drawn from protein structure.

Somatic cell genetics (*15*) provided us with yet further ways to identify specific genes. As is indicated later, in studies by cell hybridization, variation between species is substituted for variation within species. Thus, every protein can potentially be treated genetically. Rather extensive studies of inorganic pyrophosphatase (*16*) and of inosine triphosphatase (*17*) were conducted in search of electrophoretic polymorphism that might be subjected to family study, but no allelic variation was found. In contrast, the structural genes for those two enzymes have been assigned to chromosomes 10 (*18*) and 20 (*19*), respectively—by study of somatic cell hybrids. β_2-Microglobulin is another protein, the structural gene for which has been demonstrated by non-Mendelian means. The sequence of amino acids in its single polypeptide chain has been determined (*20*) and, by study of cell hybrids, the gene that determines this sequence has been assigned to chromosome 15 (*21*). Furthermore, by cell hybridization other phenotypes identifiable at the cellular level, for example, drug and virus susceptibility, can be studied genetically even though they have not been characterized biochemically.

The 50,000 structural genes postulated for man include those determining the basic amino acid structure of (i) all the enzymes of intermediary metabolism; (ii) all the structural proteins, such as the collagens; and (iii) all the proteins of special function, such as the hemoglobins and the immunoglobulins. They also determine the amino acid structure of many enzymes involved in processing of proteins, such as procollagen peptidase, glycosyltransferases, lysyl oxidase, and proline and lysine hydroxylases, necessary for formation of collagen fibers. Even the for-

mation of messenger RNA (the process of transcription) is a multistep process involving several enzyme-catalyzed reactions. Since a protein gene-product is produced, regulator genes such as lac repressor of *Escherichia coli* are structural genes as defined here, but have not been identified in mammalian cells—with the possible exception of the tyrosine aminotranferase regulator gene, *rTAT* (*22*). If this sort of Mendelian gene exists in man, it might account for a considerable portion of the postulated 50,000 genes.

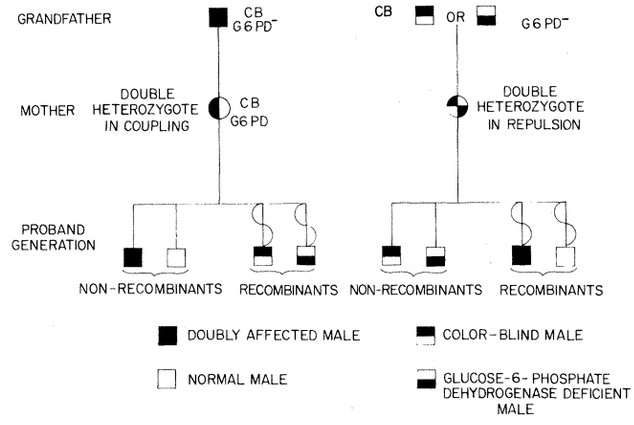


Fig. 1. "The grandfather method" in estimation of X linkage (*138*). Ideally, the recombination fraction can be derived simply by counting recombinant and nonrecombinant sons of doubly heterozygous women of known linkage phase.

Autosomal Versus X-Chromosomal Localization

A first step in the mapping of genes on chromosomes is the determination of which genes are on the X (or Y) chromosome and which are on one of the 22 pairs of other chromosomes, the autosomes. The characteristic pedigree pattern displayed by alternative forms of a character or by a particular familial disorder often allows us to identify genes carried on the X chromosome. Furthermore, pedigree patterns, supported by biochemical or statistical methods, may show that a given trait is determined by a gene on an unspecified autosome.

The autosomal or X-chromosomal localization of human gene loci identified by methods of family study, together with some demonstrated by the method of cell hybridization (see below), is given in *Mendelian Inheritance in Man: Catalogs of Autosomal Dominant, Autosomal Recessive and X-linked Phenotypes* (*10*). Although the entries are based on phenotypes, the listings purport to be catalogs of gene loci; no more than one entry per locus has wittingly been made. More than 100 loci on the X chromosome and more than 1100 on autosomes have been confidently identified. Obviously, only a small proportion of the genes of man has been identified—no more than a fiftieth, perhaps. The deficiency of known autosomal loci is proportionately greater than that of X-linked loci. Since the X chromosome is about 6 percent as long as the total haploid autosomal set, if a proportionate number of autosomal loci were known (and if the autosomes and X chromosome are genetically populated with equal density), autosomal loci would number over 1600. By the methods available until recently, variation at X-linked loci has been more "visible" than variation at autosomal loci. By parasexual methods described later, the probability of detecting a locus should not be dependent on whether the locus is autosomal or X-linked.

As was mentioned earlier, from information accumulated since 1959, the Y chromosome is essential to the development of the testes. As a rule, testes are present with the XY, XYY, XXY, XXYY, XXXY, and XXXXY karyotypes; they are absent when the Y chromosome is not present, regardless of how many X chromosomes are also present (*23*).

Histocompatibility antigens determined by the Y chromosome (H-Y) are known in mouse, rat, guinea pig, and man (*24*). Studies of Y chromosome aberrations suggest that the testis-determining locus and that for H-Y antigen [which may be the same (*25*)] are probably on the short arm of the human Y in the vicinity of the centromere. Good evidence for other genes on the Y chromosome is lacking (*26*).

Distance Between Genes on the X Chromosome

A beginning was made first on mapping the X chromosome. In addition to the historical justification for discussing it first, the significance of linkage and the means by which it is detected and quantitated by family (recombinational) studies are most easily demonstrated by examples from X linkage. Usually the question in such studies is how far apart are two X-linked loci because, in most instances, the location of the genes on the X chromosome has been established or at least suspected by a characteristic pedigree pattern. Two exceptions—the gene for tyrosine aminotransferase regulator, *rTAT* (*22*), and that for X-linked surface antigen, *SAX* (*27*)—have been assigned to the human X chromosome only by the cell hybridization technique. Pedigree pattern alone does not provide conclusive proof of X-linked inheritance in a condition like testicular feminization (*Tfm*), in which affected males do not reproduce. Theoretically, family linkage studies can provide such proof. In the mouse, X linkage of *Tfm* is proved by linkage to X chromosome markers, but this has not been possible in man because of a relative paucity of marker loci; *Tfm* is not linked to the markers tested. Conclusive proof of X linkage of *Tfm* in man was provided by demonstration of Lyonization (random inactivation of one X chromosome) at the cellular level: in heterozygous females two populations of cells exist as to receptor for dihydrotestosterone—those with the same deficiency as in affected males and those with normal receptor (*28*).

Information on linkage of X-linked loci is provided by the sons of doubly heterozygous females (symbolized AaBb). When the phase of linkage in the female is known, that is, whether the two recessive genes are on the same chromosome ("in coupling," symbolized AB/ab) or on opposite chromosomes ("in repulsion," Ab/aB), the phenotypes of her sons give definitive information on linkage (Fig. 1). When new mutation is rare, the phase of linkage can be ascertained from the phenotype of the maternal grandfather. Porter and colleagues (*29*) used this approach in a study of linkage between the loci for color blindness and glucose-6-phosphate dehydrogenase (G6PD): by studying a population in which both loci are polymorphic (a Baltimore black population), 134 males with color blindness were first ascertained by screening more than 3648 schoolboys. The sibships of the color-blind males were tested for G6PD deficiency, with identification of ten families in which both G6PD deficiency and color blindness were segregating. Then the linkage phase of the presumptively doubly heterozygous mother of each sibship was ascertained by study of her father. It turned out that about 1 of 20 sons of doubly heterozygous females showed recombination. This means that the two loci, color blindness and G6PD deficiency, are very closely linked, having a

recombination fraction of about 5 percent, which at such a short interval is equivalent to a map distance of about 5 centimorgans (cM) (*30*). When the maternal grandfather was not available for testing, some information could still be derived from the pedigree (by methods such as that of lods described below), although the evidence is not as compelling as in the case of families with the grandfather available. Furthermore, the grandfather method may not be applicable to X-linked disorders that are genetic lethals, such as Duchenne muscular dystrophy, or have severe, although not complete, effects on reproduction in hemizygous-affected males. The linkage phase in a mother heterozygous for both an X-linked polymorphic trait and a rare X-linked genetic lethal cannot be determined if tests for heterozygosity are not available. Theoretically (*31*), about half the women heterozygous for an X-linked lethal inherited the gene from the mother who was also heterozygous; in the other half it came to them by new mutation in the sperm or ovum. The two possibilities cannot be distinguished if heterozygosity cannot be determined.

Immediately after the X-linked blood group, Xg^a, was discovered in 1962 (*32*), students of the X chromosome embarked on extensive exploitation of the new tool for mapping the X chromosome. Although a few loci were found to be genetically linked to the *Xg* locus (Fig. 2), many others, although clearly on the X chromosome and therefore syntenic (on the same chromosome) with the *Xg* locus, are not linked in a specific genetic sense (*33, 34*). This raised the question as to the genetic length of the X chromosome. In the mouse a correlation between genetic map length as gauged by linkage studies and genetic map length as gauged by chiasma counts can be confirmed (*35*). Thus, it would appear justified to estimate the genetic length of the entire haploid genome in man and that part of it in the X chromosome in the following way. Chiasma counts made on the autosomal bivalents in testicular meiotic material in man have averaged about 52 (*36*). If each chiasma represents a recombination fraction of 50 percent (*37*) and a genetic length of 50 cM, then the total genetic length of the human autosomes is about 2600 cM (*30, 38*). Since the X chromosome is about 6 percent as long as the haploid set of autosomes laid end to end, the genetic length of the X chromosome can be estimated to be at least 160 cM. Thus, there is ample opportunity for free recombination between many pairs of loci on the X chromosome. The above estimate of the length of the X chromosome is made on the basis of chiasma counts in autosomes in the male, whereas recombinations in the X chromosome occurs only in females. For many linkages, recombination is about 50 percent more frequent on the average, in females than in males (see later). Thus, the genetic length of the X chromosome may in fact be greater than 200 cM (Fig. 2).

What Genes Are on the Same Autosome?

Family study was, until as recently as 1967, essentially the only method for answering this question. By this method, it was first shown in the early 1950's that the loci for Lutheran blood group and secretor trait are on the same (still unspecified) autosome (*39*). Soon thereafter, the Rh and elliptocytosis gene loci (*40*), as well as the ABO gene locus and that for the nail-patella syndrome (*41*), were similarly found to be linked. (In the last few years the Rh and ABO linkage groups have been shown to be on chromosomes 1 and 9, respectively, and in specific regions of those chromosomes.)

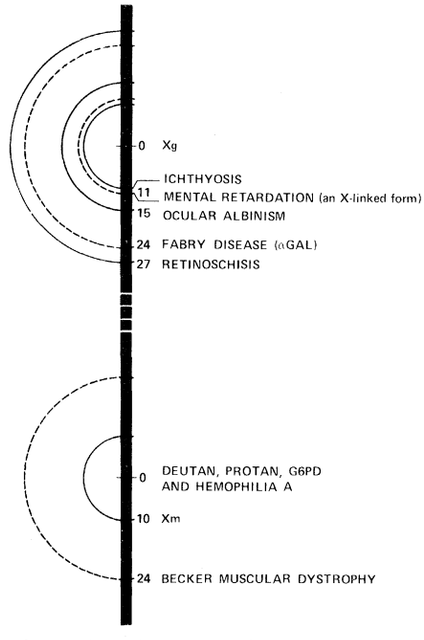


Fig. 2. Genetic map of the X chromosome based on data from family studies. [After Race and Sanger (*46*), who also gave information on linkages that have been excluded and some that are, in light of available data, probable but not firmly established.] The semicircles are used because the relative position of the loci in each cluster is unknown. The solid lines indicate established linkage, the interrupted lines very probable linkage. From studies of G6PD in hybrid cells, the color blindness cluster is known to be the distal portion of the long arm. The *Xk* locus, which determines Kell blood group "precusor substance," is closely linked to the *Xg* locus (*194*). What part of the X chromosome carries the *Xg* cluster is unknown.

The detection of autosomal linkage by the family method has distinct parallels to the X-linked case, but for several reasons the difficulties are greater. There must be allelic variability at the loci whose linkage is to be tested, just as families in which both color blindness and G6PD deficiency were "segregating" were needed for study of that linkage. As in X linkage, most information is provided if one parent is doubly heterozygous, especially if the other parent is doubly recessive—the so-called double backcross mating (*42*). Furthermore, precise deductions on recombination versus nonrecombination can be made if the linkage phase of the doubly heterozygous parent can be determined from the genotypes of his parents. The pedigree diagramed in Fig. 3 represents an ideal situation for study. Individuals in three generations have the nail-patella syndrome (indicated by filled symbols), and the family is segregating for ABO blood groups also. The linkage phase of the doubly heterozygous father in the second generation can be directly deduced. Clearly, in the sibship of the third generation there is one recombinant out of eight (individual III 5). Indeed, in extensive family data the ABO blood group locus and the nail-patella locus are found to be closely linked, with a recombination fraction of about 10 percent (*43*).

Some of the difficulties of autosomal linkage detection and quantification by the family study method are evident. To be mapped by the family method, a locus must not only show allelic variation; but, for efficiency, the marker locus (*ABO* in the above example) should have a reasonable degree of polymorphism so that, in testing for linkage with a rare autosomal dominant trait (such as a hereditary disorder of medical interest), one is likely to find that the affected individual is also heterozygous for the marker (*44*). It is no accident that ABO and Rh were the first two markers shown to be linked to specific rare dominants. At each of these loci most alleles are codominant, that is, per se identifiable when present; and at each there is a high order of heterozygosity. For similar reasons, HLA (the major histocompatibility complex) is a superb marker and several linkages with it have been established by family studies. Fortunately, the number of known marker loci has increased in the last 15 years as the extent of the genetic diversity of man has been demonstrated by biochemical and immunologic means (*45–47*). More than 50 useful polymorphic marker traits are now available for linkage studies in families. Some genetic centers (*48*) keep banks of deep-frozen red blood cells and serum from members of families, so that,

as new marker traits are discovered, their linkage relationships can be determined.

For most efficient analysis of autosomal linkage the traits must be dominant or, even better, codominant, so that heterozygotes can be identified as such from phenotype. However, if the biochemical defect is known in a recessive disorder (such as an inborn error of metabolism) and if the heterozygotes can be detected by, for example, specific qualitative or quantitative enzyme analysis, then the clinically recessive phenotype can in effect be converted into a dominant for purposes of linkage studies. Much less efficient linkage analysis can be done with unmitigated recessives (*49*). Quantitative, multifactorial traits are considered unsuitable for linkage study by Renwick (*34*), but not completely so by others (*50*).

One often has only two-generation data (Fig. 3, generations II and III) for linkage analysis. Even if three generations are available for study, the linkage phase of a doubly heterozygous parent may be ambiguous. For such data one can make use of the method of lods (acronym for "log odds"), which is now the cornerstone of linkage analysis of both multigenerational and bigenerational family data (*51*), this method having superseded earlier methods (*52*). The lods method tests the likelihood (*53*) of the particular constellation (distribution and combinations) of traits in the family. It does not give a yes-no answer, but rather gives the likelihood that such a constellation would have occurred if the two loci examined were linked with a given recombination fraction. The "odds" in the lod is a likelihood ratio, namely, the ratio of (i) the likelihood of the particular family constellation given a recombination fraction (theta, θ) of, let us say, 10 percent, to (ii) the likelihood if θ is 50 percent (which signifies no genetic linkage). The logarithm (to the base 10, by convention) of this likelihood ratio is used so that the values from different families can be summed. It is an advantage of the method that evidence from valuable "phase known" three-generation families can be combined with that from the individually less informative, but more available, two-generation families. Lods for various recombination fractions are determined by a formula for the given family (see legend for Fig. 3). For sibships, tables have been computed (*54*). The recombination fraction giving highest lod value is taken as the maximum likelihood estimate. Lod values of +3.0 or more (which means that the likelihood of linkage is 1000 times greater than the likelihood of nonlinkage) have, on the recommendations of Morton (*55*) and others, been taken as "very strong" evidence for linkage; a lod value of −2.0 or less is taken as "strong" evidence against linkage. In the method of lods, linkage is detected and estimated in one and the same procedure.

In the analysis of complicated multigeneration families in which many marker traits have been scored, and even with two-generation data, computer programs can be helpful (*48, 56, 57*). If, in a large kindred, a rare dominant disorder is studied for linkage with 50 marker loci, the number of pairs of marker loci is (50 × 49)/2, or 1225. Linkage of some pairs of loci have, of course, been either excluded or established, and therefore does not require further study. In any case, the task of analysis is formidable, and therefore well suited to computerization. Analysis of full kindreds by computer programs extracts additional information and avoids errors that may be created by splitting the kindred into multiple sibships for individual analysis.

As the chromosomal gene map "fills up," linkage of a given locus with multiple syntenic loci (that is, a linkage group) can be tested to advantage. Certainly, even though the data on no one of the pairs may be sufficient to prove linkage, the aggregated data may suffice. Combining lods on several pairs of syntenic loci (multipoint linkage analysis) presents special problems (*58*) but has advantages as well (see Fig. 4B).

The prior probability that two loci "selected at random" are on the same autosome was given by Renwick (*59*) as 1 in 18.5, or .054 (*60*). In further consideration of the prior probability Elston and Lange (*61*) concluded that, when one trait locus and 30 marker loci are studied, there is a priori more than a three-quarter probability that the trait locus is syntenic with at least one of the markers, and there is about a one-half probability that a linkage is mappable from recombination fraction. Increasing the number of marker loci has a benefit in addition to the increased chance of finding linkage; availability of more markers increases the chance of detecting nonpaternity that otherwise could obscure linkage and lead to false conclusions (*62*).

Heterogeneity is a problem for linkage analysis from at least two points of view. There might be occult rearrangement; for example, inversion, such that in one family or ethnic group two loci are linked whereas in another they are not. No example has been proved (*63*). Another possibility (which has been realized) is that what appears to be the same rare trait is in fact different in different families. The classic example is Morton's demonstration (*55*) that, whereas one form of elliptocytosis is closely linked with the Rh blood group locus, at least one phenotypically indistinguishable form is not linked to Rh.

For most pairs of loci, it can be shown that genetic recombination is greater in females than in males (*43, 57, 64*), so that the genetic distance separating two loci, as measured by the family study method, is greater in females; the physical distance is the same. This phenomenon is known in mice and is carried to the extreme in drosophila where no recombination occurs in the male. With a loose linkage, it may not be practicable to collect

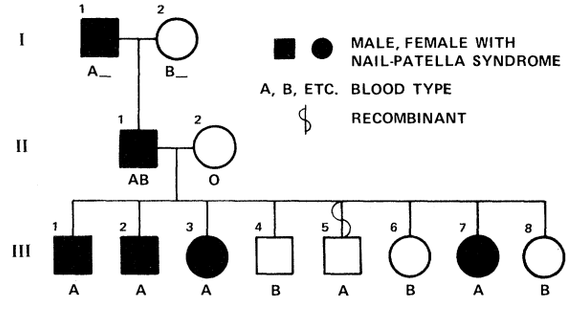


Fig. 3. Pedigree of the nail-patella syndrome (filled symbols) and ABO blood type. Obviously, in individual II 1, the nail-patella gene and the blood group A gene are "in coupling"; one of his eight children, III 5, is necessarily a recombinant. The recombination fraction is then ⅛. If only generations II and III were available or if the phenotypes of I 1 and I 2 did not give unambiguous information on their genotypes, so that linkage phase in II 1 was unknown, then the method of lods might be applied:

$$\text{Lod} = \log_{10} \times \frac{\text{likelihood of observed pedigree assuming } \theta = \theta}{\text{likelihood of observed pedigree assuming } \theta = .50}$$

For various values of θ (the recombination fraction) the likelihood of the pedigree is proportional to $\frac{1}{2}[\theta\,(1-\theta)^7] + \frac{1}{2}[\theta^7\,(1-\theta)]$ the first element relating to the possibility that *MPa* and *A* are in coupling and the second to the possibility that they are in repulsion, these alternatives being assumed equally probable (*51*). For a sibship of 7 and 1, the lod values are:

θ	.05	.10	.20	.30	.40
lod	.650	.787	.730	.503	.193

If three other identical sibships were available, total lods would exceed 3.0 for a recombination fraction of 1.10, thus providing strong evidence for linkage of the two loci with an interval of about 10 cM separating them.

family data in adequate volume to prove linkage when one looks at families with the mother as the doubly heterozygous parent or at all families together. Conversely, linkage may be detected when families in which the father is the doubly heterozygous parent are considered separately.

Differences in the frequency of recombination according to the age of the doubly heterozygous parent have also been sought (*65*). There may be a small negative correlation between recombination fraction and age, but this is probably of negligible magnitude.

Occasionally, especially in inbred groups, one expects to find two rare recessive disorders in the same family and even in an individual. Linkage (as opposed to pleiotropism) might be invoked to explain concurrence under the following circumstances (*66*). (i) The two disorders, each known to exist as a genetic entity in its own right, concur in a few families only; (ii) at least one person shows only one of the two disorders; (iii) each disorder shows full expressivity; and (iv) no biochemical or physiologic link between the two disorders is plausible. In each of four presumably unrelated Japanese families, Yamaguchi *et al.* (*67*) found two sibs with recessive congenital cataract and the very rare recessive blood type *ii*. All of seven non-*ii* sibs had no cataract. If this is not pleiotropism, linkage is, by the usual criterion, established (the sum of lods at $\theta = 0.00$ is + 3.4). Skre, Berg, and their colleagues in Oslo presented information on families suggesting linkage of albinism and cerebellar ataxia (*68*) and of Marinesco-Sjøgren syndrome and hypergonadotropic hypogonadism (*69*). The first of these two possible linkages reached a lod value of almost +3.0.

What Genes Are on Specific Autosomes?

The family methods described above can be adapted to the assignment of loci to specific autosomes (*70*); a chromosomal variant present in heterozygous form is used as one "locus" in the linkage test. For example: a "normal" familial chromosomal variation [or, better termed, heteromorphism (*71*)], namely, a chromosome 1 that was unusually long because of an "uncoiled" region, was used in family studies to achieve the first specific assignment of a locus to an autosome. Roger Donahue, then a graduate student in human genetics at Johns Hopkins, studied his own family, in which he found this chromosome variant, now referred to as *lqh* (for heterochromatin on the long arm), and showed that the Duffy blood group locus is carried on chromosome 1 (*72*). The finding was confirmed by Ying and Ives (*73*) and others. Soon thereafter Robson and colleagues (*74*) used familial translocations to show that the locus determining the α-polypeptide of haptoglobin (*Hp*α) is on chromosome 16, and Magenis and colleagues (*75*) used an inherited morphologic peculiarity they called "fragile site" to map *Hp*α to a specific region of the long arm of 16, namely 16q22 (*76*). Lamm *et al.* (*77*) assigned the major histocompatibility complex (HLA) to chromosome 6 by study of a family with an inherited pericentric inversion involving that chromosome.

Table 1. Clone panel for assignment tests or regional mapping.

Hybrid clones	Human chromosomes							
	1	2	3	4	5	6	7	8
A	+	+	+	+	−	−	−	−
B	+	+	−	−	+	+	−	−
C	+	−	+	−	+	−	+	−

The family method has serious limitations. Genetic constraints, such as the necessity for allelic variation and the preference for dominant inheritance, have been mentioned. Human families, especially these days, are small in size and geographically dispersed. Study of more than two generations may be difficult because generation time is long. Because of the limitations, progress in chromosome mapping was slow when the methodology was limited to the family approach. Up to 1968, the watershed year when the Duffy blood group locus was assigned to chromosome 1 by the family method (*72*) and the first chromosome assignment (*78*) was achieved by the method of cell hybridization (the thymidine kinase locus to a group E chromosome), five pairs of linked loci, one triplet and two tight linkage groups had been identified, but no autosomal assignment had been achieved.

Mapping by Somatic Cell Hybridization

Somatic cell genetics has provided a highly productive approach to chromosome mapping (*15*). The first step in the method is fusion (*79*) of human somatic cells with those from another species, a process facilitated by inactivated viruses (*80*) such as Sendai (*81*) and by some chemical agents (*82*). The nuclei of the hybrid cells contain the chromosomes of both parent cells.

In subsequent cell divisions there is a progressive and preferential loss of human chromosomes from man-mouse or man-hamster hybrid cells. The resulting clones of hybrid cells have only certain ones of the chromosomes of the original human parent cell. The process simulates Mendelian segregation and assortment, which occur in meiosis. Somatic cell hybridization is, as Haldane (*83*) put it, an "alternative to sex." [Other parasexual (*84*) systems for genetic analysis were previously developed in drosophila (*85*), aspergillus (*86*), and bacteria (*87*).] Precise identification of chromosomes and parts of chromosomes, for precision in mapping, has been possible with various "banding techniques" (*4*) and other new methods (*88*). The new techniques also permit differentiation of mouse and human chromosomes in hybrid cells (*89*).

Whereas allelic intraspecies variation is necessary for mapping by family linkage studies, interspecies variation in cellular phenotypes—for example, in the electrophoretic mobility of specific enzymes—can be used to identify the presence of the human gene for those enzymes in particular hybrid clones.

In the *synteny test*, the location of two gene loci on the same chromosome of man is demonstrated by the consistently concordant presence or absence of two human phenotypes in many clones derived from an interspecies hybrid cell line. (Occasional exceptions to consistent concordance can be accounted for on the basis of chromosomal breakage and rearrangement.) In the *assignment test*, the location of a specific gene locus on a specific human chromosome is demonstrated by concordance between the presence or absence of the specific chromosome and the specific human phenotype in many hybrid clones. For *regional mapping*, that is, mapping of a human gene locus to a specific segment of a chromosome, a human cell with a chromosomal rearrangement is used as one parent in the hybrid; a translocation breakpoint divides a chromosome into two independently segregating units that can be individually studied in the assignment test.

With the best of methods to encourage cell fusion, hybrid cells constitute only a small proportion of the cells in mixed cultures. Consequently, Littlefield's adaptation (*90*) of Szybalski's HAT (hypoxanthine-aminopterin-thymidine) selection system (*91*) for the isolation of hybrid cells free of both parental types provided a major impetus to somatic cell genetics. In the HAT system, one parental cell is deficient in thymidine kinase (TK), an enzyme of the pyrimidine salvage pathway, and the other "parent" is deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme of the

purine salvage pathway. If the cultures are treated with the antimetabolite aminopterin, both the purine and thymidylate de novo synthetic pathways are blocked (*92*), and the cells become dependent on exogenous sources of preformed purine (as hypoxanthine) and pyrimidine (as thymidine). In the HAT medium the parental cells, which are not able to use one or the other of the exogenously supplied substances, die; only the complemented hybrid cells survive. Once they are obtained free of parental cells, the hybrid cells can be used for mapping of any loci. Further selection systems specific for each chromosome are useful in ensuring the retention of specific single chromosomes in cell lines derived from the original hybrid.

Creagan and Ruddle (*93*) have taken a systematic approach to gene mapping by means of a clone panel. In the usual approach, for seeking association (or concordance) between specific cellular phenotypes and chromosomes, many clones are studied because of redundancy in the patterns of chromosomal segregation. However, from a large repertory one can select a few clones that are optimally complementary, and therefore unnecessary and time-consuming redundancy can be eliminated. Table 1 illustrates how three properly selected clones can provide a unique binary signature for each of eight chromosomes; five clones suffice for 32 (2^5). With an optimal panel of five clones, one can compare the cellular phenotypes with the unique binary signature of each of the 24 chromosomes (22 autosomes plus the X and Y chromosomes), and have some redundancy, which is useful because it reduces error arising from incorrect scoring of the cellular phenotypes.

Given an ideal panel, it is necessary only to expand each clone and store samples in the frozen state. Periodic checks must be made on the human partial karyotype and the phenotype profile of each clone. Clone panels can be created to assist in assignment of loci to regions of chromosomes. The availability of reliable clone panels should make it possible for biochemical, virologic, and other laboratories not involved primarily in human gene mapping to contribute to advancement of the field.

The first successful synteny test was that performed in 1969 by Nabholtz *et al.* (*94*), who showed that the loci for HGPRT and G6PD are syntenic, a fact already known indirectly from family studies showing that each is X-linked. Somatic cell hybridization was used to demonstrate the first autosomal synteny (in 1970) by Santachiara *et al.* (*95*) and Ruddle *et al.* (*96*) for the loci LDH_B (lactate dehydrogenase B) and PEP_B (peptidase B). The first assignment of a marker that, unlike TK, is nonselectable, namely, LDH_A to chromosome 11, was made by Boone *et al.* in 1972 (*97*). The first regional assignment by use of a chromosomal translocation in somatic cell hybridization was that of *G6PD, PGK,* and *HGPRT* (*98*) to the long arm of the X chromosomes by Ricciuti and Ruddle in 1973 (*99*) using the KOP translocation (*100*). Subsequently, use of translocations with breakpoints at various places along the X chromosome permitted ordering of these loci and the centromere as follows: centromere : *PGK* : *HGPRT* : *G6PD* (*101*).

Regional mapping has been facilitated by organization of a "human mutant cell bank" for analysis, storage, and distribution of human cell lines with chromosomal translocations or deletions (*102*). Families of interest are usually detected through a child with abnormalities resulting from an unbalanced translocation. The balanced translocation, which may be found in one parent and other relatives of the proband, is especially valuable for regional gene mapping. The unbalanced translocation, which is monosomic or trisomic for parts of chromosomes, can be used for gene dosage or other studies.

The family and somatic cell methods are complementary and mutually potentiating. The family method can give information on the distance between, and ordering of, loci known by somatic cell methods to be syntenic. Cell hybridization studies may permit assignment to a specific chromosome of a linkage group determined by family studies. For example, Ruddle *et al.* (*103*) assigned the Rh linkage group (defined by a combination of family and cell hybrid studies) to chromosome 1 by somatic cell assignment of one of its members (*PepC*); Westerveld *et al.* (*104*) assigned the HLA linkage group to chromosome 6 by somatic cell assignment of PGM_3; Westerveld *et al.* (*105*) assigned the ABO linkage group to chromosome 9 by somatic cell assignment of AK_1. Consistent findings by the two methods strengthens conclusions on mapping. For example, at the workshop on human gene mapping held at Yale University in 1973, cell hybridizers (*106*) reported assignment of enolase to chromosome 1; and students of families (*107*), working in ignorance of the cell hybrid findings, reported linkage between enolase and Rh, which had previously been assigned to chromosome 1. Figure 4A is a map of chromosome 1 based on information derived mainly from cell hybrid studies, and Fig. 4B is a map of part of the same chromosome based on recombinational data.

Although cell hybridization has greatly accelerated human chromosome mapping since 1968, the family method has also been productive. More than 30 autosomal linkages have been established by the family method during this period.

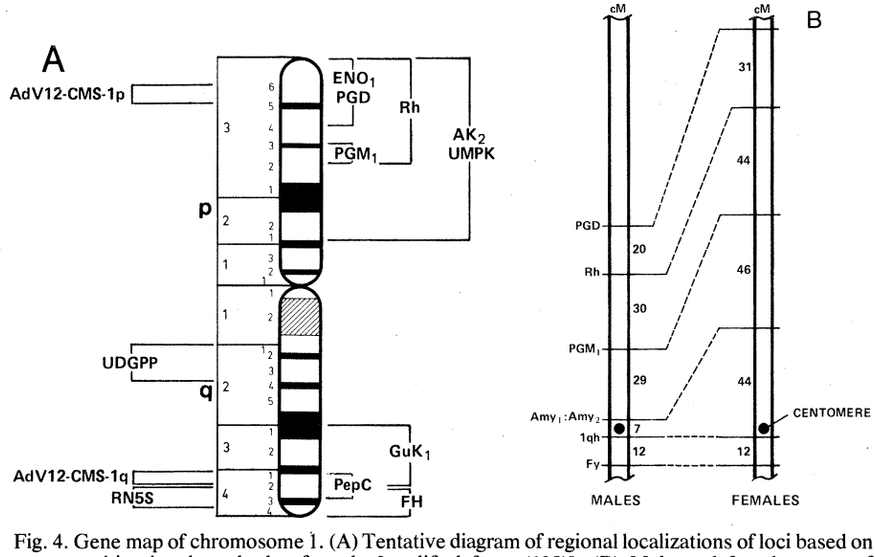


Fig. 4. Gene map of chromosome 1. (A) Tentative diagram of regional localizations of loci based on nonrecombinational methods of study [modified from (*195*)]. (B) Male and female maps of chromosome 1 (mainly its short arm) based on multipoint linkage analysis of family data (*196*). The map units are centimorgans and were derived from the recombination fraction using Carter-Falconer mapping function (*38*). [Modified, with permission, from diagram by Merritt and Karn (*177*).] The short arm of chromosome 1 represents about 4.3 percent of the total autosomal length and, by extrapolation from chiasma counts, should have a genetic length in the male of at least 110 cM. Study of a chromosomal rearrangement (*197*) suggests that *Fy* is located in the 1q2 region, a conclusion consistent with the multipoint mapping. In addition to the linkages represented in (B), the locus for one form of elliptocytosis and that for α-fucosidase are closely linked to the *Rh* locus.

A Different Form of Hybridization

A third approach to gene mapping is in situ hybridization of RNA (or DNA) of specific type to chromosomes. If the RNA involved in the hybridization by reannealing is radioactively labeled, one can, by autoradiography, identify the chromosomal location of the specific genes from which that RNA is normally transcribed. By this method, genes for 18*S* and 28*S* ribosomal RNA have been localized to the short arms of chromosomes 13, 14, 15, 21, and 22 (*108*) and those for a special type of ribosomal RNA (5*S* RNA) to the long arm of chromosome 1 (*109*). Also this method has resulted in the proposal that the loci for the α and β chains of hemoglobin are on the long arm of chromosome 2 and the long arm of a B group chromosome (that is, either 4 or 5) (*110*). Because, on theoretical grounds, the method would seem to be insufficiently sensitive to demonstrate localization of the globin genes, the report was initially met with skepticism (*111*). A critical test may be the same experiment in the mouse, where the chromosomal localization of the α and β hemoglobin genes is known through standard linkage studies. When Atwood *et al.* (*112*) did this type of experiment in the mouse, labeling was observed in chromosome 11, which is known to carry the α gene in the mouse. The chromosomal location of human α and β globin genes is being studied by an independent method based on C_0t analysis (*113, 114*). Yet another approach to mapping human globin genes (*115*) depends on induction of hemoglobin gene expression in human somatic cells by hybridization with the mouse erythroleukemia cells, first isolated by Friend and co-workers (*116*), and treatment with dimethyl sulfoxide (*117*).

Work in other species suggests that the genes for histones and genes for immunoglobulins, being present in multiple copies, can be mapped by available techniques of nucleic acid hybridization. Theoretically, mapping can be done with any RNA gene product that can be obtained in sufficient amount, purity, and radioactivity. Progress toward mapping the genes for the collagens, which represent at least 10 percent of protein synthesized by fibroblasts at some stages, has recently been reported (*118*).

The Lepore Method

This might be used as the name of a fourth approach for deducing close linkage of gene loci. In its amino acid sequence, one polypeptide chain (the non-α chain) of an anomalous hemoglobin called Hb Lepore is a curious molecular hybrid (*119*). One part (at the NH_2-terminal end) has the sequence of the normal δ-polypeptide chain that occurs in hemoglobin A_2; the rest has the sequence of the normal β-polypeptide chain that occurs in hemoglobin A. (Actually one must speak of the hemoglobins Lepore because several, differing by the site of the δ-β fusion, are known.) Close linkage of the β and δ loci has been demonstrated by family studies; in a total of 61 opportunities for recombination, none was observed (*120*). Close linkage was also indicated by marked linkage disequilibrium of sickle cell hemoglobin and hemoglobin B_2, mutants at the β and δ loci, respectively (*121*). Probably the gene for the anomalous Lepore chain originated by nonhomologous pairing and unequal crossing-over (Fig. 5). This interpretation has been strengthened by finding patients in Japan and in Africa with an "anti-Lepore" hemoglobin, that is, one with a non-*a* polypeptide that is the reciprocal product of the accident postulated to cause the Lepore gene (*122*).

Hemoglobin Kenya (*123*) is a similar fusion hemoglobin chain that tells us that the γ and β loci are also closely linked (*124*). The conclusion is that the genes for the non-α chains of hemoglobins F, A, and A_2 are closely clustered, having originated through the process of gene duplication. Among the immunoglobulins, Lepore-type variant proteins have been found that tell us that the genes for different types of immunoglobulin G (IgG) are likewise closely linked (*125*).

Gene Dosage Methods

Deletion mapping is a means for demonstrating synteny and for assigning genes to specific regions of autosomes (*126*). The method was used to confirm the synteny of the *Rh* and *6PGD* loci, previously shown by standard family studies to be linked. In 1972 Fialkow and associates (*127*) described a patient with chronic myeloid leukemia who from red cell typing was inferred to be hemizygous at both of these loci (that is, expressed only one form of each gene), although the findings in the parents indicated that he should have been heterozygous and that the patient was indeed heterozygous at many other loci. Thus, a single deletion was presumed to have included both syntenic loci. Similarly, assignment of the *Rh* locus to the short arm of chromosome 1 by somatic cell genetics (*103*) was confirmed by deletion mapping. In a patient with a presumably preleukemic state, Marsh *et al.* (*128*) found both Rh-negative and Rh-positive red cells; they also found a cell line with a partially deleted chromosome 1, permitting them to conclude that the *Rh* locus is on the distal portion of the short arm of that chromosome. Salmon *et al.* (*129*) observed a 76-year-old woman with two populations or erythrocytes, one heterozygous (phenotype 2-1) at the AK_1 locus and the red cell type A_1, the other homozygous (phenotype 1) at the AK_1 locus and "A faible, de type A_X" [a weak type A] at the ABO locus. Thus, the linkage of AK_1 and ABO, first shown by the family method in 1967, appeared to be confirmed by concurrent deletion of the loci in a malignant clone. These three examples illustrate the study of hematologic malignancies for mapping by the deletion principle.

The first assignment by deletion mapping (not previously known by another method) was reported in 1973 by Ferguson-Smith and associates (*130*), who used a chromosomal aberration to assign acid phosphatase to the distal end of the short arm of chromosome 2. The assignment was subsequently corroborated by family studies of chromosomal rearrangements (*131*), by studies of somatic cell hybrids (*132*), and by trisomy mapping (*133*). In a case of partial deletion of chromosome 12, Mayeda *et al.* (*134*) used the principle of deletion mapping to show that the LDH_B locus, already known by somatic cell genetics (*135*) to be on that chromosome, is situated on the short arm. Some sporadic cases of retinoblastoma have been shown (*136*) to have an interstitial deletion of a short segment of the long arm of chromosome 13, best demonstrated by banding techniques. The mechanism of the tumorigenesis is unclear; the observation merely indicates the presence on chromosome 13 of a locus (or loci) having something to do, at least indirectly, with retinoblastoma.

Regional mapping of the X chromosome by the deletion method is foiled by the usual preferential inactivation of partially deleted X chromosomes (*137, 138*). Thus, if a karyotypically normal father and mother are Xg(a+) and Xg(a−), respectively, an offspring might be Xg(a−) with deletion of either the long arm or the short arm of one X chromosome; the same findings would be expected whether the *Xg* locus is on the long arm or the short arm. Indeed, such families have been observed (*139, 140*).

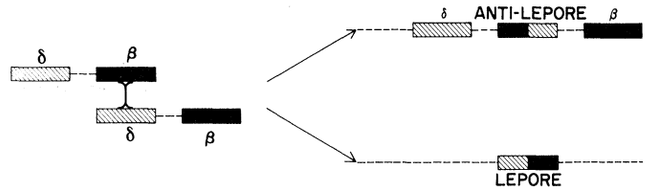
Negative information on assignment should be useful. Thus, in seeking assignments by study of families with chromosomal variants, various parts of the karyotype may be excluded as the location of given loci, even though no positive infor-

mation is turned up. Clearly, exclusion mapping is inefficient. Accumulation and collation of the data are difficult (*141*). The application of banding techniques improves greatly the quality of the data on deletions. An inconsistency between exclusion mapping data and a presumed new assignment can be a useful caution.

Trisomy or duplication mapping (that is, finding that a person trisomic for part or all of a chromosome has both alleles of one parent or has approximately 50 percent more of a particular gene product) is, like deletion mapping, a gene dosage method. When leukocyte alkaline phosphatase was found to be elevated in trisomy 21 [Down syndrome (*142*)] and lower than normal in patients with chronic myeloid leukemia and the Philadelphia chromosome (which was thought then to be a partially deleted chromosome 21), a locus controlling alkaline phosphatase was thought to be on chromosome 21. This view was shaken by the discovery that the activities of several other enzymes were also high in trisomy 21, including G6PD, which of course is X-linked. It was largely destroyed when the Philadelphia chromosome was found by banding techniques to be derived from chromosome 22, not from the "mongol chromosome" (chromosome 21), and when it was shown to be a translocation (usually from 22q to 9q) rather than a deletion (*143*). Although no primary assignment has been achieved by trisomy mapping (*144*), at least five assignments have been corroborated thereby: red cell acid phosphatase (ACP_1) on chromosome 2 (*133*), glutathione reductase to chromosome 8 (*145*), galactose-1-phosphate uridyltransferase to chromosome 3 (*146*), and "antiviral protein" (*147*) and soluble superoxide dismutase (*148*) to chromosome 21. Furthermore, regional mapping has been possible by the dosage method. Superoxide dismutase was assigned to 21q22 (*148*). Study of instances of partial trisomy or monosomy of the short arm of chromosome 12 permitted regional assignment of the *GAPD* and LDH_B loci (*149*), and similar regional mapping of the *NP* locus was achieved by study of four different partial trisomies of chromosome 14 (*150*). Recently, the *ABO-AK*$_1$*-NPa* cluster has been assigned to the distal end of the long arm of chromosome 9 (9q34) by the gene dosage method (*151*). Study of hemoglobin synthesis in patients with duplication of a segment of the long arm of chromosome 4 suggests that the α globin locus may be somewhere in the segment 4q28-4q34 (*152*). (See the earlier discussion of assignment of the hemoglobin loci by nucleic acid hybridization.)

In a subpopulation of trisomic persons (with the Down syndrome, for example) the frequencies of particular alleles at a polymorphic locus are altered from that of the parent population, if the locus is on the trisomic chromosome. This approach to mapping has been unproductive (*153*).

Fig. 5. The origins of Lepore and anti-Lepore hemoglobins through nonhomologous pairing and unequal crossing-over, involving closely linked δ and β loci.



Analysis of the phenotype of trisomies [for example, trisomy 21, Down syndrome (*143*)] and of deletion syndrome (for example, 5p-, cri du chat syndrome) has taught us little about the specific gene content of the involved chromosomes. Even the information that the main features of Down syndrome are due to trisomy of the distal pale band of the long arm of chromosome 21, 21q22 (*144*), tells us nothing about individual genes carried by that segment. The converse, that knowledge of what genes are on the triplicated segment tells us nothing about the pathogenesis of the phenotype, is not necessarily true.

Other Methods

As a method of human chromosome mapping, comparative gene mapping (mapping by homology) is useful mainly in relation to the X chromosome. Ohno's law of the evolutionary conservatism of the X chromosome is so compelling (no exceptions in mammals are known to us) that demonstration of X linkage of loci in any mammal is convincing evidence that the same holds in man (*154*).

The homologies in the autosomes of man and nonhuman primates and the course that evolution of the karyotype has taken have been revealed in improved clarity by the banding techniques (*71*). The full extent of genocartographic homology is unknown. The homology of the major histocompatibility regions of mice (*155*), rhesus (*156*), and man is well established. Four γ-immunoglobulin heavy chain loci are closely linked in man, mouse, and rabbit (*157*). The β-γ hemoglobin cluster seems to exist in the mouse as well as in man (*158*). In both rat and mouse, the albinism (*c*) locus (probably the structural gene for tyrosinase) is linked to the β-hemoglobin locus (*157*), but nothing is known of this in man.

Classic linkage analysis (called here the family method) remains the mainstay for determining the linear order of genes on a chromosome and the distance separating loci. As was mentioned earlier, the order of genes can also be determined by study of a set of different variants involving the same chromosome, for example, different X-autosome translocations, in hybrid cells. At least two further methods for ordering genes are now available. Goss and Harris (*159*) devised an ingenious method combining the use of interspecific cell hybrids and ionizing radiation, which causes fragmentation and rearrangement of chromosomes. In the experiments of Goss and Harris, the "rescue" of irradiated human cells by fusion with hamster cells yielded hybrid cell lines in which normally syntenic loci were separated from each other. This segregation would be expected to occur at a frequency that is a function of the distance separating the loci. They applied the method to the X chromosome and obtained results of the order of *PGK*, *αGAL*, *HGPRT*, and *G6PD* loci (*160*) consistent with the conclusions from the standard use of interspecific somatic cell hybridization (*99*). The X-linked cell surface antigen (*SAX*) has, furthermore, been mapped to the distal portion of the long arm of X by this method (*161*). Furthermore, in studies of chromosome 1, Goss and Harris (*161a*) demonstrated the applicability of the method to other chromosomes. The method combines features of family linkage studies and synteny testing in hybrid cells. The data generated can be subjected to the same sort of multipoint linkage analysis (*58*) that is used in combining lods on several pairs of syntenic loci (see earlier). From comparing maps of chromosomes 1 and X obtained by their method with cytogenetic maps of these chromosomes at metaphase, Goss and Harris (*161a*) concluded that the statistically derived distances between loci are related to the amount of Giemsa light-band material separating them.

Benign ovarian teratomas are diploid like other somatic cells, but originate by parthenogenesis after the first stage of meiosis (*162*). The evidence for the last statement is the homozygosity of chromosomal and biochemical markers in the tumor in the face of heterozygosity of the host female. The two chromatids of one chromosome give rise to the pair of

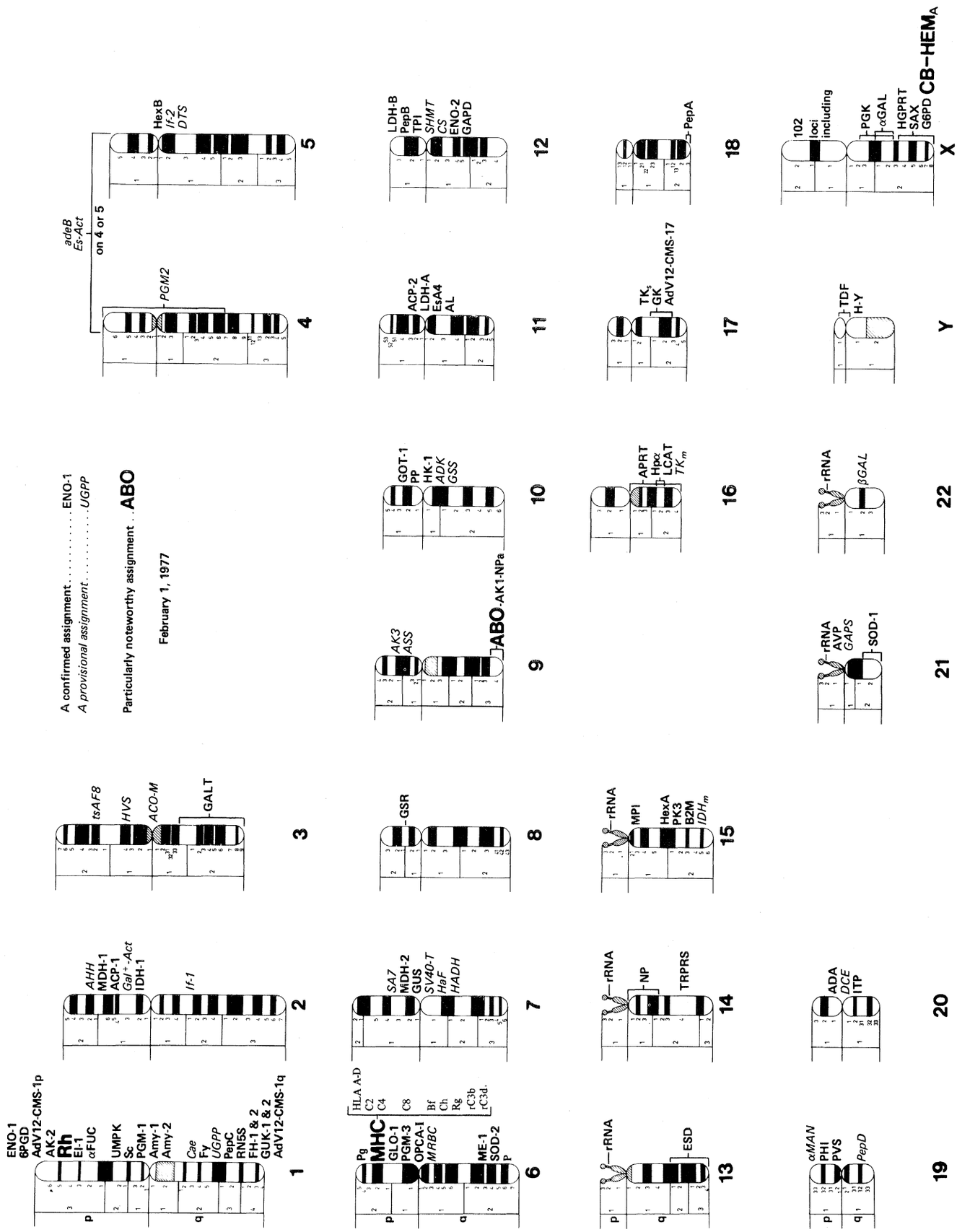


Fig. 6. A diagrammatic synopsis of the gene map of the human chromosomes. The banding patterns and numbering (*76*), are those given in the 1975 supplement to the report of the Paris Conference (*71*). An assignment is considered confirmed if found in two laboratories or several families; it is considered provisional if based on evidence from one laboratory or family. Additional assignments considered tentative (evidence not as strong as that for "provisional") or based on inconsistent results of different laboratories are not included.

Fig. 6. Human chromosome map. The symbols are explained in the following key.

ABO	ABO blood group (chr. 9)
ACO	Aconitase, mitochondrial (chr. 3)
ACO-S	Aconitase, soluble (chr. 9)
ACP-1	Acid phosphatase-1 (chr. 2)
ACP-2	Acid phosphatase-2 (chr. 11)
ADA	Adenosine deaminase (chr. 20)
adeB	FGAR amidotransferase (chr. 4 or 5)
ADK	Adenosine kinase (chr. 10)
AdV12-CMS-1p	Adenovirus-12 chromosome modification site-1p (chr. 1)
AdV12-CMS-1q	Adenovirus-12 chromosome modification site-1q (chr. 1)
AdV12-CMS-17	Adenovirus-12 chromosome modification site-17 (chr. 17)
AHH	Aryl hydrocarbon hydroxylase (chr. 2)
AK-1	Adenylate kinase-1 (chr. 9)
AK-2	Adenylate kinase-2 (chr. 1)
AK-3	Adenylate kinase-3 (chr. 9)
AL	Lethal antigen: 3 loci (a1, a2, a3) (chr. 11)
Amy-1	Amylase, salivary (chr. 1)
Amy-2	Amylase, pancreatic (chr. 1)
ASS	Argininosuccinate synthetase (chr. 9)
APRT	Adenine phosphoribosyltransferase (chr. 16)
AVP	Antiviral protein (chr. 21)
Bf	Properdin factor B (chr. 6)
β2M	β2-Microglobulin (chr. 15)
C2	Complement component-2 (chr. 6)
C4	Complement component-4 (chr. 6)
C8	Complement component-8 (chr. 6)
Cae	Cataract, zonular pulverulent (chr. 1)
CB	Color blindness (deutan and protan) (X chr.)
Ch	Chido blood group (chr. 6)
CS	Citrate synthase, mitochondrial (chr. 12)
DCE	Desmosterol-to-cholesterol enzyme (chr. 20)
DTS	Diphtheria toxin sensitivity (chr. 5)
El-1	Elliptocytosis-1 (chr. 1)
E11S	Echo 11 sensitivity (chr. 19)
ENO-1	Enolase-1 (chr. 1)
ENO-2	Enolase-2 (chr. 12)
Es-Act	Esterase activator (chr. 4 or 5)
EsA4	Esterase-A4 (chr. 11)
ESD	Esterase D (chr. 13)
FH-1 & 2	Fumarate hydratase-1 and 2 (S and M) (chr. 1)
αFUC	Alpha-L-fucosidase (chr. 1)
Fy	Duffy blood group (chr. 1)
Gal$^+$-Act	Galactose + activator (chr. 2)
αGAL	α-Galactosidase (Fabry disease) (X chr.)
βGAL	β-Galactosidase (chr. 22)
GALT	Galactose-1-phosphate uridyltransferase (chr. 3)
GAPD	Glyceraldehyde-3-phosphate dehydrogenase (chr. 12)
GAPS	Phosphoribosyl glycineamide synthetase (chr. 21)
Gc	Group-specific component (chr. 4)
GK	Galactokinase (chr. 17)
GLO-1	Glyoxylase I (chr. 6)
GOT-1	Glutamate oxaloacetic transaminase-1 (chr. 10)
G6PD	Glucose-6-phosphate dehydrogenase (X chr.)
GSR	Glutathione reductase (chr. 8)
GSS	Glutamate-γ-semialdehyde synthetase (chr. 10)
GUK-1 & 2	Guanylate kinase-1 & 2 (S & M) (chr. 1)
GUS	Beta-glucuronidase (chr. 7)
HADH	Hydroxyacyl-CoA dehydrogenase (chr. 7)
HaF	Hageman factor (chr. 7)
HEM_A	Classic hemophilia (X chr.)
Hex A	Hexosaminidase A (chr. 15)
Hex B	Hexosaminidase B (chr. 5)
HGPRT	Hypoxanthine-guanine phosphoribosyltransferase (X chr.)
HK-1	Hexokinase-1 (chr. 10)
HLA	Major histocompatibility complex (chr. 6)
Hpα	Haptoglobin, alpha (chr. 16)
HVS	Herpes virus sensitivity (chr. 3)
H-Y	Y histocompatibility antigen (Y chr.)
If-1	Interferon-1 (chr. 2)
If-2	Interferon-2 (chr. 5)
IDH-1	Isocitrate dehydrogenase-1 (chr. 2)
IDH_m	Isocitrate dehydrogenase, mitochondrial (chr. 15)
ITP	Inosine triphosphatase (chr. 20)
LCAT	Lecithin-cholesterol acyltransferase (chr. 16)
LDH-A	Lactate dehydrogenase A (chr. 11)
LDH-B	Lactate dehydrogenase B (chr. 12)
αMAN	Lysosomal α-D-mannosidase
MDH-1	Malate dehydrogenase-1 (chr. 2)
MDH-2	Malate dehydrogenase, mitochondrial (chr. 7)
ME-1	Malic enzyme-1 (chr. 6)
MHC	Major histocompatibility complex (chr. 6)
MPI	Mannosephosphate isomerase (chr. 15)
MRBC	B-cell receptor for monkey red cells (chr. 6)
NP	Nucleoside phosphorylase (chr. 14)
NPa	Nail-patella syndrome (chr. 9)
OPCA-I	Olivopontocerebellar atrophy I (chr. 6)
P	P blood group (chr. 6)
PepA	Peptidase A (chr. 18)
PepB	Peptidase B (chr. 12)
PepC	Peptidase C (chr. 1)
PepD	Peptidase D (chr. 19)
Pg	Pepsinogen (chr. 6)
PGK	Phosphoglycerate kinase (X chr.)
PGM-1	Phosphoglucomutase-1 (chr. 1)
PGM-2	Phosphoglucomutase-2 (chr. 4)
PGM-3	Phosphoglucomutase-3 (chr. 6)
6PGD	6-Phosphogluconate dehydrogenase (chr. 1)
PHI	Phosphohexose isomerase (chr. 19)
PK3	Pyruvate kinase-3 (chr. 15)
PP	Inorganic pyrophosphatase (chr. 10)
PVS	Polio sensitivity (chr. 19)
Rg	Rodgers blood group (chr. 6)
Rh	Rhesus blood group (chr. 1)
rRNA	Ribosomal RNA (chr. 13, 14, 15, 21, 22)
rC3b	Receptor for C3b (chr. 6)
rC3d	Receptor for C3d (chr. 6)
RN5S	5S RNA gene(s) (chr. 1)
SA7	Species antigen 7 (chr. 7)
SAX	X-linked species (or surface) antigen (X chr.)
Sc	Scianna blood group (chr. 1)
SHMT	Serine hydroxymethyltransferase (chr. 12)
SOD-1	Superoxide dismutase-1 (chr. 21)
SOD-2	Superoxide dismutase-2 (chr. 6)
SV40-T	SV40-T antigen (chr. 7)
TDF	Testis determining factor (Y chr.)
TK_m	Thymidine kinase, mitochondrial (chr. 16)
TK_s	Thymidine kinase, soluble (chr. 17)
TPI	Triosephosphate isomerase (chr. 12)
TRPRS	Tryptophanyl-tRNA synthetase (chr. 14)
tsAF8	Temperature-sensitive (AF8) complementing (chr. 3)
UGPP	Uridyl diphosphate glucose pyrophosphorylase (chr. 1)
UMPK	Uridine monophosphate kinase (chr. 1)

chromosomes in the diploid cells of the teratoma. If crossing-over has occurred between the centromere and the locus in question, the tumor in a heterozygous host will also be heterozygous. The frequency of heterozygosity in a "population" of ovarian teratomas from heterozygous females should be a function of the distance of the locus from the centromere: the proportion that is heterozygous is expected to vary from 0 (for loci very close to the centromere) to 0.67 (for loci far from the centromere). [Crossing-over occurs at the four-strand stage. In the heterozygote ($A_1A_2B_1B_2$), a given chromatid, let us say A_1, may undergo crossing-over with any one of three other chromatids. Two of the three possibilities will give rise to heterozygotes (*163*).] Preliminary data (*164*) suggested that the PGM_3 locus (*98*) may be about 17 units from the centromere (on chromosome 6). The polarity of a linkage group can potentially be determined by the teratoma method, and the interval between two syntenic loci can be estimated by this method even though there is no way to determine it more directly. Another method of centromere mapping consists of family studies of hereditary heteromorphisms revealed by C banding techniques (for centromeric heterochromatin). Although no linkages have been identified by this technique, the proximal segments of the several chromosome arms have been excluded as the site of genes for blood groups and other marker traits that have not been otherwise mapped (*165*).

Status of the Map

The gene map of the human chromosomes (Fig. 6) now shows assignment of at least one structural gene locus to all 22 autosomes plus the X and Y chromosomes (*166*). More than 20 loci have been assigned to chromosome 1. It is a matter of great intellectual satisfaction that the chromosomal localization of the relatively long-known ABO and Rh blood group loci is now established. Of more than 100 loci assigned to the X chromosome, some information on position (arm or region or position relative to other loci) is available for at least 16. Again, knowing the precise localization on the X chromosome (that is, on the distal portion of the long arm) of the color blindness loci and the locus for classic hemophilia A—some of the genes longest identified in man—is intellectually satisfying.

Not capable of full representation in Fig. 6 is a considerable body of data concerning regional mapping of individual chromosomes. Some of these data (for chromosomes 1 and 6) are presented in Figs. 4 and 7. Also not represented are 13 linkage groups which have been established by family studies but not yet assigned to specific autosomes. These include the secretor-Lutheran-myotonic dystrophy group, the first autosomal linkage demonstrated in man.

The traits for which genes have been mapped cover most of the range of biochemistry, virology, immunology, and medicine: enzymes of purine, carbohydrate, amino acid, and lipid metabolism; an aminoacyl-tRNA synthetase; disorders such as a specific form of hereditary cataract and the nail-patella syndrome; susceptibility of cultured cells to toxins such as diphtheria and to viruses such as polio; structural proteins such as collagen; blood clotting factors; proteins involved in resistance to infection; cell surface antigens; gene regulators; peptide hormones such as chorionic gonadotropin; and others.

Scientific Usefulness of the Map

Understanding of evolution, of chromosomal organization in relation to genetic control mechanisms, and of the pathogenesis of some neoplasia and some malformations is advanced by knowledge of the gene map.

Banding methods, which reveal many more details of chromosome morphology than did previous techniques, have been useful in describing the karyotypic differences and similarities (*71*) between, for example, man and the chimpanzee, which from data on amino acid sequence of proteins and other considerations appears to be man's closest primate relative (*14*). The chimpanzee has 23 pairs of autosomes. The 22 autosomal pairs of man may have been arrived at by centric fusion of two ancestral acrocentric chromosomes to form human chromosome 2. Most of the other karyotypic differences between man and chimpanzee are accounted for by a limited number of pericentric and paracentric inversions (*71, 167*). Evolutionary trees based on karyotypic similarities have been devised.

The gene content of structurally homologous chromosomes has also been compared. In the chimpanzee as in man (*98*), ENO_1, AK_2, PGM_1, and *PEP-C* are on chromosome 1; *MDH* (malate dehydrogenase) is on chromosome 2; LDH_A is on chromosome 11; LDH_B is on chromosome 12; SOD_S is on chromosome 21 (*168*). Trisomy 21 in the chimpanzee (*169*) has a clinical picture closely similar to the Down syndrome (*143*) in man. In both the chimpanzee and the African green monkey, the loci for the enzymes thymidine kinase and galactokinase (GalK) are on a chromosome morphologically similar to chromosome E17 of man (*170*). Adenovirus-12 induces a gap in the short arm of the chimpanzee chromosome homologous to human 17 and in the long arm of the human chromosome 17 (*171*). In the chimpanzee, TK and GalK are on the short arm of 17, not the long arm as in man, and banding studies suggest that a pericentric inversion is responsible for this difference between man and chimpanzee. The genes coding for the 5*S* RNA of ribosomes are on the long arm of the longest chromosome (No. 1) of man, two species of chimpanzee, the mountain gorilla, the orangutan, and the baboon (*172*). Discrepancies in map relationships have been found more often in species evolutionarily more remotely related to man than the chimpanzee.

Homology of mammalian X chromosomes as to gene content (Ohno's law) was mentioned earlier (*173*). No information is available concerning homology for gene order on the X chromosome (*174*). The locus for a male antigen (H-Y) has been conserved on the Y chromosome through several hundred million years of vertebrate evolution (*175*).

Tetraploidization, suggested as a step in the origin of the human karyotype (*176*), finds some support in similarities of banding patterns of pairs of human chromosome homologs (*177*). For example, the morphologic similarities of chromosomes 11 and 12 are consistent with tetraploidization, which is also supported by the fact that the LDH_H locus is on the short arm of chromosome 11 and the LDH_B locus is on the short arm of chromosome 12. Along the same line, it is noteworthy that the gene *TK* for mitochondrial TK has been assigned to chromosome 16 (*178*) and that for soluble TK to chromosome 17 (*179*).

King and Wilson (*14*) proposed that the amino acid structure of proteins is so similar in humans and chimpanzees that the basis for their very considerable organismal differences must be sought in regulatory mutations. These may be of at least two types: (i) point mutations in regulatory genes; and (ii) changes in the order of genes through inversion, translocation, insertion, deletion. Hence, a scientific usefulness of comparative mapping.

Clustering of genes with similar functions suggests a biologic significance of the association. The genes for three enzymes (*98*) involved in the Embden-Meyerhof glycolytic pathway, *GAPD*, *TPI*, and LDH_B, are on chromosome 12 (*180*). The tight linkage of *TK* and *GalK* is found not only in man and chimpanzee

(*171*) but also in the Chinese hamster and in *Mus musculus* (*181*). The genes for *GOT-1* and *GSS*, enzymes involved in proline metabolism, are on chromosome 10 (*182*). Another example is the tight linkage between the fourth component of complement and the major histocompatibility complex in man, mouse, and guinea pig (*183*). The precise reason for the conservation of these linkages is unknown.

In some other cases, the basis for clustering is clearer. A considerable body of evidence indicates that gene duplication and subsequent divergent evolution played a role, and probably a significant one, in the evolution of the human genome (*176*). Linkage persists between loci that determine closely similar proteins, probably because they arose by gene duplication. Examples are the amylase loci [in drosophila, mouse, and man (*177*)], the loci for Ag lipoprotein allotypes (*178*), the loci for three different unlinked clusters of immunoglobulin genes, the genes for β-, δ-, and γ-polypeptide chains of hemoglobin, and the components of HLA. The LDH_B and LDH_C (testicular LDH, or LDH-X) loci are linked in the pigeon (*179*); it has not been possible to test this linkage in man because of no known polymorphism of LDH_C in man and no expression in cultured somatic cells. In contrast, the three PGM isozymes are determined by three loci, each on a separate human chromosome (Fig. 6).

Although per se the phenotype of trisomic states and deletion syndromes tells us little about the gene content of the chromosomes involved, the opposite may be quite different. As the specific genetic constitution of the chromosomes becomes better known, the mechanism of the phenotypic changes associated with chromosomal aberrations may become clear, including the basis for neoplasia associated with chromosomal changes, for example, the Philadelphia chromosome (22q−; 9q+) in myeloid leukemia (*144*). Already, plausible suggestions concerning the phenotype in the Down syndrome have been made; the information that this phenotype results from trisomy of the terminal segment of the long arm of chromosome 21 (*184*) and that the locus for soluble superoxide dismutase is situated there suggests that a disturbance of superoxide metabolism may be an important pathogenetic factor (*150*). In ataxia-telangiectasia (*185*), lymphoreticular malignancy accompanies microscopically demonstrable alteration in chromosome 14 at a site (14q12) close to that for nucleoside phosphorylase, an enzyme involved in nucleic acid metabolism. Similarly, the oncogenic adenovirus 12 causes changes in chromosome 17 near the TK locus (*87*), on chromosome 1q near the guanylate kinase loci (*185a*), and on chromosome 1p near an adenylate kinase locus (*185a*).

Application of Information on the Map

Mapping the human genome, like any uncharted terrain, is a challenge to the human intellect. Inevitably, information on the detailed gene anatomy of the chromosomes will also have usefulness. Practical value of the information need not and should not be a primary concern. However, an example of practical application is now provided by diagnosis in utero of Mendelian disorders that are not per se biochemically identifiable (*186*). These potentially include a large number of conditions such as hemophilia and the muscular dystrophies. If loci closely linked to the disease locus are identified and if allelic variation at the given locus can be scored in amniotic cells or amniotic fluid, there is a chance of prenatal diagnosis of the disease by the linkage principle. Confirmation of theory has been achieved in the case of at least one autosomal and one X-linked disease. The autosomal disorder is myotonic muscular dystrophy (*187*), which is known to be closely linked to the locus for secretor trait (which can be determined for the fetus by study of amniotic fluid). The X-linked disorder is classic hemophilia (*188*), whose gene locus is situated close to that for G6PD. The G6PD type can be determined in amniotic cells. Thus, if the genetic makeup of the parents is sufficiently clear and is of a type that will give information on linkage (as indicated earlier, there are often difficulties here), then one can determine, with a high probability of accuracy, whether the as-yet-unborn baby would have hemophilia or myotonic dystrophy, by testing for G6PD or secretor, respectively. What has been said about prenatal diagnosis applies also to diagnosis of conditions such as Huntington chorea (*143*) before clinical manifestation.

Prospects

Mapping by the method of somatic cell hybridization can be expected to continue apace, limited mainly by the availability of cellular phenotypes for study. Methods for eliciting differentiated functions in cultured cells will be a boon to mapping, as well as to understanding development and to practical matters of prenatal diag-

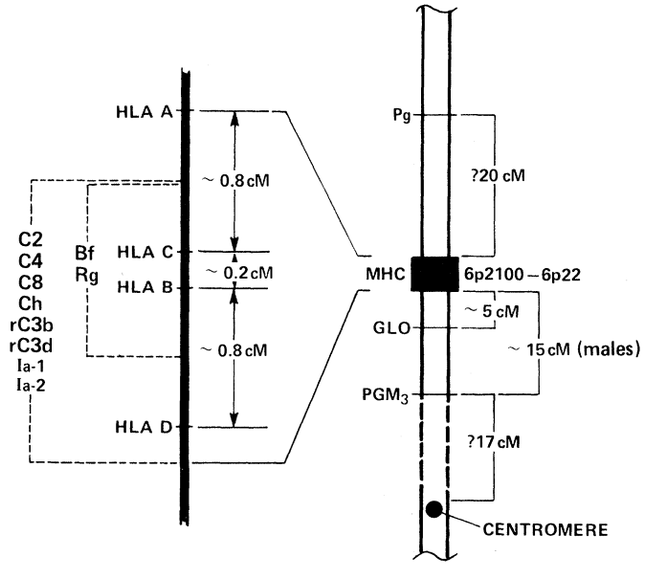


Fig. 7. Tentative genetic map of chromosome 6 based on data derived from family linkage studies and study of cell hybrids. Study of ovarian teratomas tentatively places the PGM_3 locus about 17 cM from the centromere (*164*). Study of hamster-human cell hybrids with a translocation involving human chromosome 6 (*198*) suggests that the major histocompatibility complex (MLC) is on the short arm of that chromosome distal to 6p2100 [proximal limit of the 21 band on the short arm of 6, according to the 1975 supplement to the Paris Conference of 1971 (*71*)]. Earlier work (*199*) indicated that MLC is proximal to 6p22. The gene for a form of spinocerebellar degeneration is also on 6, about 12 cM from MHC (*200*). ME_1 and SOD_2 are on 6, but their position relative to MHC is unknown.

nosis through study of cultured amniotic cells.

Additional parasexual methods for mapping are already being developed. One of these involves the transfer of small numbers of human chromosomes into cells of a nonhuman species by fusing them with human "microcells" formed by treatment of cultured human cells with colchicine and cytochalasin (*189*). A second (*190*) method involves the transfer of a short segment of human DNA (estimated to be no more than 3×10^7 nucleotides in length) into nonhuman cells by adding isolated human metaphase chromosomes (which become broken up in the lysosomes of the recipient cell). Cotransfer of tightly linked loci has been demonstrated. A third method involves fusion of human spermatids (which are haploid) with nonhuman somatic cells (*191*).

Isolation of chromosome-specific segments of DNA has been demonstrated in the case of the Y chromosome (*192*). Similar techniques should make it possible to isolate DNA specific for each of the autosomes and the X chromosome. Direct nucleotide analysis and plasmid techniques (*193*) can then be applied to determine the physical nature and function of the chromosome-specific DNA.

For the foreseeable future, family studies for chromosome mapping will continue to be worthwhile for purposes which include (i) mapping traits for which no phenotype at the cellular level is known, for example, achondroplasia, to cite an abnormal trait, and the several components of complement, to cite normal ones; and (ii) measurement of the distance separating loci shown to be syntenic by parasexual methods.

Correlation of the gene map with the banding map or other morphology-based map of the chromosome (for example, an integration of maps such as those given in Fig. 4, A and B) will bring added precision to clinical cytogenetics. By testing for the presence of particular genes in cells or in patients carrying a chromosomal deletion or duplication, for example, it should be possible to define the limits of the aberration in finer detail than is now the case. Aberrations will be described in terms of gene loci rather than in terms of chromosomal arms and bands.

Summary

In man, the specific chromosome that carries each of about 210 gene loci is known. These loci include at least one assigned to each chromosome (including the Y), about 110 assigned to specific autosomes, and about 100 to the X chromosome. For many loci, information on regional chromosomal localization is also available. The information comes mainly from studies in families and somatic cell hybrids, as well as an integration of results from the two methods.

Knowledge of the chromosome map gives insight into evolution, chromosomal organization in relation to genetic control mechanisms, and the pathogenesis of neoplasms and malformations. Furthermore, it is useful in prenatal or premorbid diagnosis of hereditary diseases.

References and Notes

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7. Rotterdam Conference, (1974), *Birth Defects, Orig. Artic. Ser.* **11** (3), 1 (1975); also found in *Cytogenet. Cell Genet.* **14**, 162 (1975).
8. Baltimore Conference, (1975), *Birth Defects, Orig. Artic. Ser.* **12** (7), 1 (1976); also found in *Cytogenet. Cell Genet.* **16**, 1 (1976).
9. For information on mapping of individual loci, with bibliographic references, see McKusick (*10*).
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NEWS AND COMMENT

Carter's New Plutonium Policy: Maybe Less Than Meets the Eye

The fate of the Clinch River breeder reactor was called into question by President Carter's decision to defer indefinitely the use of plutonium fuel in the country's nuclear reactors, but reports of the demise of the $2.7 billion project—planned as a one-third size version of a commercial breeder that would produce 1000 megawatts of power and 1.5 tons of plutonium per year—may be greatly exaggerated.

The Carter Administration has not yet made a final decision on the fate or the form of the Clinch River project, and Washington insiders suggest it may take several months before the office of James Schlesinger, Carter's special adviser on energy, chooses among the various possible options. The Clinch River program may "never be the same," as one official said, but it is not yet clear whether the policy will be to slightly modify it, drastically alter it, or cancel it.

The liquid metal fast breeder reactor (LMFBR) program was raised to national preeminence by Richard Nixon in 1971, when he called it "our best hope" for future energy, and the Clinch River demonstration project has been the centerpiece of the country's energy R & D strategy throughout the 1970's. Any move to alter the project can be expected to meet stiff resistance from the Energy Research and Development Administration (ERDA), from the nuclear industry, and from Capitol Hill, where many districts are affected by the project's funding.

For 5 years critics of the breeder have said that the technology was too great a proliferation risk, was eating up R & D funds that could be better spent on other alternatives, and was being pursued with unnecessary urgency. But the program has survived numerous governmental reviews—the latest being a 5-week study performed by a 12-member LMFBR Steering Committee in response to Carter's call for "an intensive review" when the fiscal 1978 budget was announced. According to one Washington official, the latest breeder crisis is "another chapter in a saga that would make a Russian novel look like a throwaway paperback."

In his prepared statement on 7 April, Carter announced that he would restructure the breeder program to stress alternatives and slow the program's rush toward commercialization, but in response to a question he said that the Clinch River project "will not be terminated as such." Other Administration comments suggest it might be built as an R & D plant rather than a commercial prototype. What this might mean is difficult to discern, because the program already has a large R & D reactor nearing completion—the $1.1 billion Fast Flux Test Facility being built near Richland, Washington. But in the press conference, Carter indicated that he would like to ensure some continuation of the jobs and income that the Clinch River project would provide. Cancellation would affect not only east Tennessee, but also California, Pennsylvania, New Jersey, New York, and 17 other states.

With so much political and institutional momentum behind the project, near-heroic efforts are being made to save it and "there are so many ideas it is anybody's guess what they will settle out on," said one observer. But a leading contender is to emphasize alternative fuel cycles, particularly thorium.

At the end of the latest review, ERDA's acting assistant administrator for nuclear energy, Robert Thorne, wrote that the use of alternate fuel cycles "offers the possibility of improvement in proliferation aspects while maintaining maximum utilization of the LMFBR concept." In a memo to the head of ERDA, Thorne laid out four alternatives for the