THE MAP OF CHROMOSOME 1 OF MAN

PROEFSCHRIFT

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Chapter I. GENERAL INTRODUCTION

Making maps is an essential procedure in the exploration of new territories. In the field of genetics, many basic concepts concerning the structure of a genome and the regulation of gene activity have emerged from regional mapping studies on the chromosomes of e.g. Escherichia coli and Drosophila melanogaster. The studies on Drosophila owe much of their significance to the suitability of this organism for correlation of data on gene linkage and gene expression with data on morphological and biochemical differentiation along chromosomes.

Whereas chromosome mapping in Drosophila has a history of more than half a century, the history of regional mapping of human chromosomes covers slightly more than half a decade. The experimental approaches employed within that short period, although, show a striking diversity. This development was possible because several important techniques became available almost simultaneously.

In 1970, Caspersson et al. published the first complete description of a quinacrine-stained human karyotype. Since then, many other differential staining techniques have been developed; for reviews, see Pearson (1972) and Dutrillaux & Lejeune (1975). Application of these techniques allows identification of chromosomes and of structural rearrangements within a particular chromosome based upon banding patterns which provide the landmarks necessary to define chromosomal regions (Paris conference, 1971). In addition, the reproducibility of the staining patterns suggests an underlying linear structural differentiation of the metaphase chromosome. Elucidation of the mechanisms of differential staining might therefore be an approach to investigate chromosome structure.

A second important advance was the development of the somatic cell hybrid system. The significance of this system to human genetics has recently been reviewed by Ruddle & Creagan (1975). Man-rodent somatic cell hybrid

lines carrying aberrant human chromosomes are being used for regional assignment of genes (see II-1 and II-3).

Application of the somatic cell hybrid system for gene localization demands procedures for in vitro detection of human gene products. At the First Conference on Human Gene Mapping in 1973 data on some 50 loci determined in man-rodent hybrid cells were presented. That number was nearly doubled at the Third Conference in 1975. Most of these genes code for enzymes separable from rodent isoenzymes by electrophoretical procedures. Other genes are coding for e.g. regulatory factors, surface antigens and toxin sensitivity.

Finally, the recently employed techniques of DNA-DNA or DNA-RNA reannealing should be mentioned. Here the binding of radioactively labeled DNA or RNA probes to DNA in fixed denatured metaphase chromosomes (in situ hybridization) followed by autoradiography allows for the direct localization of complementary DNA in the chromosomes (Gall & Pardue, 1969). Alternatively, presence of human DNA complementary to a specific RNA or DNA probe in a man-rodent hybrid cell can be determined by studying renaturation kinetics (Di Cioccio et al., 1975). The import of these techniques is determined by nature and specificity of DNA and RNA probes which can be obtained (see II 1-4 and II 2).

The present thesis deals with methods and achievements in regional mapping of human chromosomes. The term "mapping" is used in a broad sense: it refers not only to the regional assignment of genes, but also to the linear distribution of chromosomal features such as types of DNA, bands and breaks. Chapter II gives a survey of present approaches and achievements in chromosome mapping, with special reference to chromosome 1. The significance of the achievements is discussed in chapter III. In conclusion, chapter IV deals with prospects of future research within this field of human genetics.

Chapter II. APPROACHES IN REGIONAL MAPPING OF CHROMOSOMES

The concepts of "linkage group" and "chromosome" date back to the infancy of genetics. Still it has long been obscure that both refer to the same physical entity (cf. Sturtevant, 1966). Gradually, elucidation of the nature of units of genetical function and of chromosome structure has led to the discovery of the genetic code and the postulation of mechanisms of genetical processes at morphological and biochemical levels. In the practice of human chromosome mapping, however, mapping of genes and mapping of structures are methodologically distinct, although several approaches tend to bridge the gap between structure and function by relating positions of genes to chromosomal substructures.

An efficient way to observe connexions between results of different ways of chromosome mapping is to concentrate on one relatively well-mapped chromosome. Of the 24 different chromosomes of man, no. 1 is the most suitable for this purpose. This may be partially caused through chance in that although regional assignments may be revealed elsewhere, chromosome 1, being the largest, has a statistical advantage over the other chromosomes. But chromosome 1 has also been an intentionally chosen subject of investigation, principally because of the characteristic banding patterns produced by differential staining which allow for identification of small segments and accurate localization of breakpoints (Latt, 1974a; Jongsma & Burgerhout, in press).

In a vast majority of studies, chromosomal regions to which assignments were made have been defined by reference to patterns of differential staining. The Paris Conference (1971) brought forth a nomenclature to describe the characteristical pattern of each chromosome, and thus to indicate individual bands. This nomenclature, which has been applied almost universally since its publication, will also be used in the present paper. With "G", "Q", "R", "C" and "T" bands 1 will refer to chromosomal regions which

stain positively with G, Q, R, C and T banding techniques respectively.

1. Regional localization of genes

1.1 Linkage studies

With the term "linkage studies" I will refer to studies employing methods by which the relative distance of gene loci is established by measuring frequencies of concordant segregation, either in sibships or in cultured somatic cells. Since these methods deal with the presence or absence of phenotypical traits only, they do not provide information about the actual position of genes relative to morphological chromosome markers, unless the traits include chromosomal polymorphisms or aberrations.

A study of linkage relationships within families is the classical way to obtain a gene map. The distance between two loci is measured as the probability of unequal numbers of crossovers between the loci at meiosis. The methodology of the "family study" approach has been reviewed by Renwick (1971).

Two groups of linked markers established by family studies have been assigned independently to chromosome 1. They will respectively be referred to as the Rhesus (Rh) and Duffy (Fy) linkage groups. No significant linkage has been demonstrated between any marker of the former group and any of the latter.

The Rh linkage group was assigned indirectly to chromosome 1 by somatic cell genetic localization of the Rh-linked locus PGM1 (Ruddle et al. 1972, Jongsma et al. 1973). In 1975 (Baltimore Conference), the following markers were found to be significantly linked to Rh: PGM1, PGD, PPH1, UMPK and EI1. A map based on the cumulative recombination data in males and females as presented at the Baltimore Conference (1975) is given in Fig. 1. Linkage analysis in families carrying chromosome polymorphisms and structural rearrangements has indicated that the linkage group is probably located on the p arm with Rh lying distal to PGM1 (Cook et al., 1974;

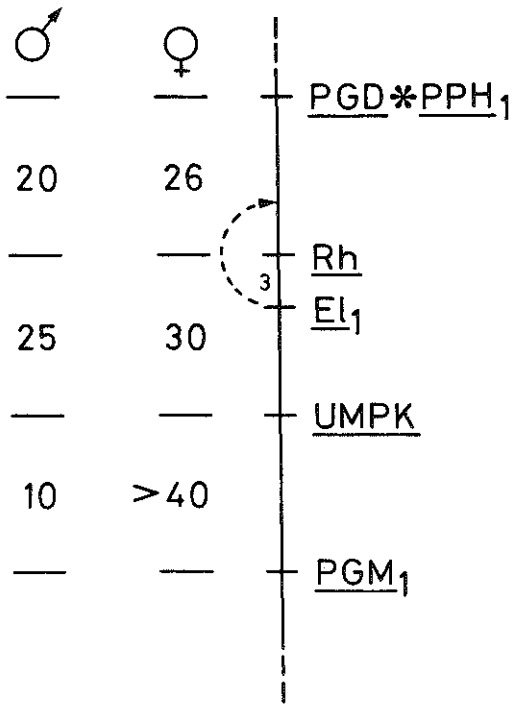

FIG. 1

Map of the Rh linkage group as derived from family studies. Figures indicate distances in centi-Morgans.

Syrett et al., 1975). 1)

Fy was assigned directly to chromosome 1 by Donahue et al. (1968), who found concordant segregation of Fy and a 1q heterochromatin polymorphism (originally indicated as the "uncoiler" locus). In 1975 this linkage group consisted of the loci Fy, Amy 1, Amy 2 and Cae and the chromosome region 1qh (Paris Conference band 1q12). Amy 1/Amy 2 and Fy are probably located on either side of 1qh (Rivas et al., 1976). The polarity with respect to the chromosome is not known.

1) A list of abbreviations is given at page 42.

Ott et al. (1976) have recently introduced a new linkage approach. Their method makes use of benign teratomas which may originate by suppression of the second meiotic division or by fusion of the nucleus of an ovum with its second polar body. If the mother is heterozygous for a gene, the teratoma will be homozygous, unless crossing over has occurred between the gene locus and the centromere of the chromosome on which the locus is situated. By investigating a number of teratomas, the frequency of crossing over between a gene and a centromere can be measured. Ott et al. have used this method for the mapping of the PGM3 locus relative to the centromere of chromosome 6.

In man-rodent somatic cell hybrids, human genes located on the same chromosome should be present or absent simultaneously, unless a chromosome has been broken and partially lost. In that case, only genes located on the retained chromosome segment will be expressed. Without chromosome analysis, this phenomenon can be used for mapping purposes by observing patterns of simultaneous presence or absence of human genes known to be located on the same chromosome. The distance between two genes is expressed by the relative frequency of breakage in the chromosome segment between the loci (Miller et al., 1971). Although de novo breakage of human chromosomes in hybrid cell lines is a common phenomenon (Burgerhout et al., in press a), the number of hybrid clones needed to calculate relative distances with any reliability would make this approach a very laborious one if no breakage-enhancing treatment is applied. The latter has been performed by Goss & Harris (1975). Human cells were treated with several doses of X-rays and subsequently fused with HPRT-deficient Chinese hamster cells. After selection of HPRT-positive hybrid clones, the relative position of HPRT and three other human x-linked markers was calculated from the observed patterns of presence and absence. Recently, the same authors (Goss & Harris, in press) have demonstrated that the presence of a selectable marker on the chromosome under study is not imperative for this type of mapping. In a study using man-mouse hybrids on the relative position of eight genes located on

chromosome 1 (see II-1.3), the gene order FH-Pep-C-UGPP-PGM1-UMPK-AK2-αFUC-PPH1 was found to be most likely.

A similar way of mapping might be achieved by transfer of isolated chromosome material (Mc Bride & Ozer, 1973). By incubating rodent HPRT- or TK-deficient cells with isolated human chromosomes and subsequent selection in HAT medium, stable cell lines showing expression of human HPRT or TK have been obtained. In several clones carrying human TK, human GaK was also expressed (Willecke et al., 1976; Wullems et al., in press). Both TK and GaK are known to be located on chromosome 17. The absence of detectable human chromosome material in any of the latter clones indicates a close association of TK and GaK on the chromosome. Though the involvement of chromosome analysis places these particular observations beyond the scope of true linkage studies, the findings suggest the possibility to express the distance between genes as the frequency of co-transfer in this system.

Translated into morphological terms, a distance between markers established by any "linkage" method represents a relative probability of chromosome breakage. When interpreting the results, one should consider that the relative probabilities of e.g. crossing over during meiosis, de novo breakage during culture and radiation-induced breakage within a particular chromosome segment may not be the same for different breakage systems.

1.2 Mapping by aneuploidy

The presence of a partial deletion or duplication of an autosome in a human cell implies single or triple presence of the genes located on the deleted or duplicated chromosome segment. The deletion or duplication of such a gene may be discovered by a dosage effect, i.e. a significant decrease or increase of the amount of gene product relative to the "normal" level. If a gene is polymorphic, its involvement may be discovered by qualitative phenotype analysis if the theoretical (diploid) phenotype of the individual or cell line carrying an unbalanced chromosome aberration is known.

Mapping by aneuploidy can be performed with cell material from human individuals with an unbalanced chromosome aberration as well as with cultured cells which have lost or duplicated chromosome material in vitro. Gene dose effects have been utilized, amongst others, by Mayeda et al., 1974 (assignment of LDH-B to 12p by use of a deletion) and by Magenis et al., 1975 (assignment of the ACP1 locus to 2p23→pter). An aberrant phenotype of a polymorphic marker was first utilized by Ferguson-Smith et al. (1973). An individual having a partial deletion of chromosome 2 was found to show a homozygous phenotype of acid phosphatase, although pedigree analysis had predicted a heterozygous phenotype. This was interpreted as hemizygosity due to the deletion. In accordance, quantitative analysis showed a relatively low acid phosphatase activity. The qualitative method can also be applied in a reversed way: if a person with a chromosomal deletion shows heterozygosity for a particular locus, then the locus cannot be situated on the deleted chromosome segment. Using this principle, Ferguson-Smith (1975) and others have excluded considerable portions of the human genome as possible locations of highly polymorphic loci such as HLA and MNSs.

One case of mosaicism and two familial chromosomes aberrations have allowed the association of a partial deletion of chromosome 1 with hemi- or heterozygosity for genes assigned to that chromosome. Marsh et al. (1974) have placed Rh and PGM1 respectively distal and proximal to a break in the 1p32 → p34 segment. Turner et al. (1975) have localized Rh distal to a band which corresponds to 1p31 (the authors did not use the Paris nomenclature in their paper). Ferguson-Smith (1975) has excluded band 1q31 as a possible location of Rh, Fy and PGM1.

Mapping by aneuploidy in cultured cells has been achieved by Arthur et al. (1975). In clonal populations derived from an apparently diploid human lymphoblastoid cell line which had originally shown peptidase A heterozygosity, absence of one form of Pep-A was found to be associated with possible deletions involving chromosome 18. These clones also showed a decreased Pep-A activity relative to the parental population and heterozygous sister clones.

1.3 Regional assignments by using somatic cell hybrids

Man-rodent somatic cell hybrid lines tend to lose human chromosomes. If a part of a human chromosome is lost from a hybrid cell population, regional localization of the genes on that chromosome relative to the break point is possible by correlating presence or apparent absence of the chromosome fragment with presence or absence of gene products. Hybrid cell lines carrying broken human chromosomes have been obtained by fusion of cells from human carriers of reciprocal translocations with rodent cells (Grzeschik et al. 1972), by irradiation of either hybrid populations or human parental cells (Burgerhout et al. 1973 ; Burgerhout et al., in press) and by making use of de novo chromosome breakage during cultivation of hybrids (Douglas et al., 1973; Jongsma et al., 1973). The methodology of this approach to chromosome mapping has been discussed elsewhere (Burgerhout et al., in press b).

Ten loci, all coding for enzymes, have at present (Baltimore Conference 1975) been assigned to chromosome 1 by use of man-mouse or man-Chinese hamster hybrids: PGM1, Pep-C, PPH1, UMPK, αFUC, UGPP, FH, GuK, PGD, and AK2. The assignment of UGPP and FUC should be considered as provisional since they have each been reported by one group of investigators only. A combination of data showing the position of loci relative to points of chromosome breakage (Hamerton, 1976; Burgerhout et al. in press a, b) leads to the map displayed in Fig. 2. The location of each gene is confined to the region between the most distal break in a chromosome arm which places the locus at its distal side and the most proximal break which places it proximal. Since no successful attempt has been made to localize a break at a level more accurately than a single band, the whole band in which a break has occurred should be considered as a possible position of the locus and not just one side of the breakpoint. In consequence, a locus can be assigned to one particular band only if it is situated between two breaks within that band. This applies to UMPK, PGM1, and Pep-C (Burgerhout et al., in press a, b; Jongsma & Burgerhout, in press).

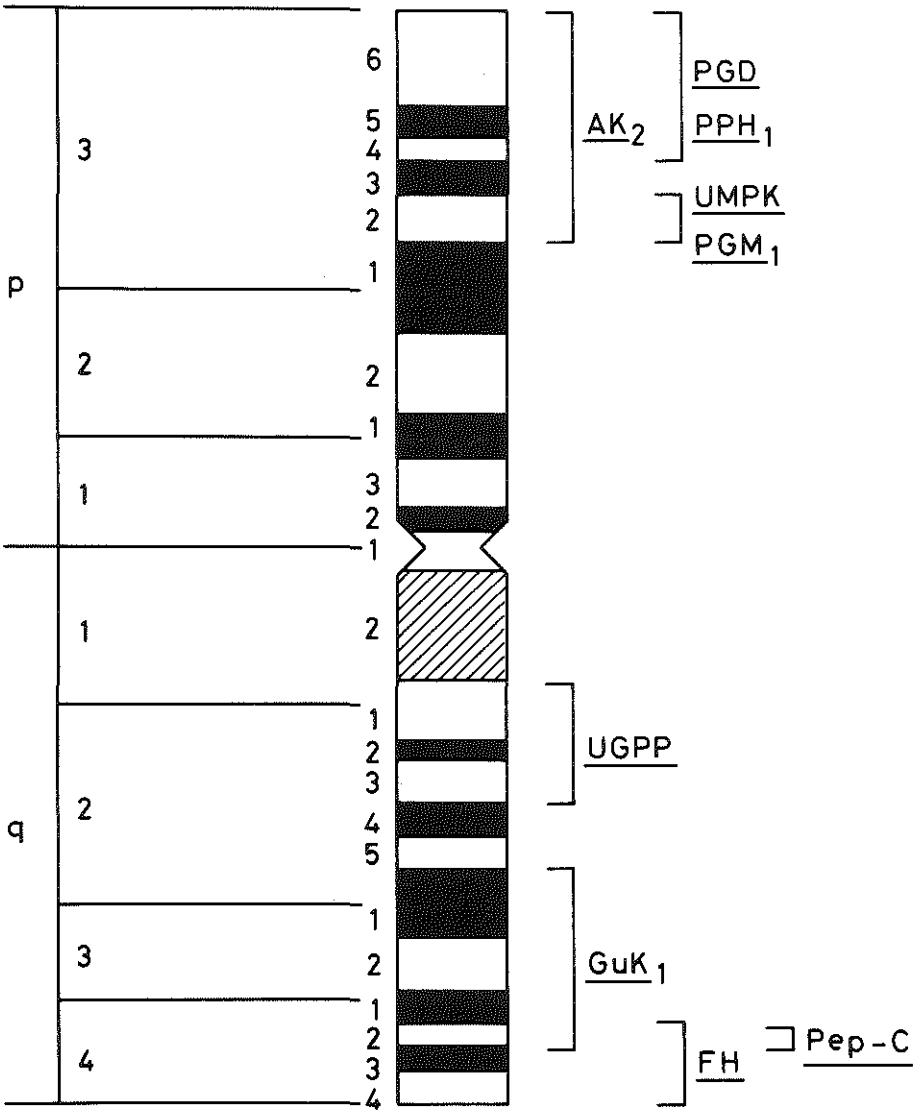

FIG. 2

Map of chromosome 1 as derived from regional assignments by somatic cell hybridization studies.

The relative positions of loci which share one band as a possible location are determined if a break within that band results in segregation of the loci. This yields the gene order PGD/PPH1 - UMPK - PGM1 - UGPP - GuK1 - Pep-C - FH (Burgerhout et al., in press a, b). AK2 is located distal to PGM1 and UMPK (Grzeschik, 1975; Burgerhout, in press b). The position of AK2 relative to PGD and PPH1, cannot be given more accurately since potentially informative breaks in the pter→p34 region have only been investigated in man-Chinese hamster hybrids in which the electrophoretic mobilities of human and Chinese hamster AK2 are equal. For similar reasons, the location of PGD and GuK1 cannot be studied in man-mouse hybrids.

1.4 In situ RNA-DNA annealing

The chromosomal localization of templates for specific RNA or DNA fractions is feasible by molecular hybridization of radioactively labeled copies with denatured but visually intact metaphase chromosomes (Gall & Pardue, 1969). Application of this technique for gene localization demands a highly purified RNA transcript which yields (possibly following re-transcription with a reverse transcriptase to cDNA) a detectable amount of isotope desintegration after annealing over the part of the chromosome coding for the gene product under investigation. These demands seem to confine the scope of this approach to genes of high multiplicity, e.g. those coding for ribosomal RNAs (Henderson et al., 1972). However, results in the localization of low-multiplicity genes coding for globins, which have been considered as impossible on theoretical grounds, have been proved to be reproducible (Atwood et al., 1975).

Genes coding for 5S and 18S/28S ribosomal RNA are known to be present in several hundred copies in a haploid human genome (Jeanteur & Attardi, 1969; Hattlen & Attardi, 1971). In situ hybridization studies have yielded evidence for the location of copies of both genes on chromosome 1.

Steffensen et al. (1974a) have localized a "major" 5S RNA locus on 1q. By using balanced translocations and chromosomes carrying Adenovirus 12 - induced gaps as templates for hybridization, the locus has subsequently been more precisely assigned to 1q42-43 (Steffensen et al., 1974b, 1976).

A "minor locus" for 18S/28S RNS has been assigned to the centromeric region of chromosome 1 by Pardo et al. (1975). However, earlier studies by Henderson et al. (1972) and Evans et al. (1974) do not confirm this assignment.

2. Studies on linear differentiation of human chromosomes

Data on the linear differentiation of human chromosomes have almost exclusively been obtained from chromosomes at metaphase. Interpretation of these data should take into account the process of folding and coiling by which a stretched DNA molecule is transformed into a structure which is a tenthousandfold shorter in length. The structural organization of human mitotic chromosomes has recently been reviewed by Schwarzacher (1976).

Most studies on chromosome morphology are dealing with banding patterns induced by differential staining techniques. The literature concerning the nature of chromosomal bands displays a great variety of explanatory models, indicating differences in morphology as well as in biochemical composition of chromatin as underlying factors (see e.g. Comings et al., 1973, 1975; Dutrillaux & Lejeune, 1975; Schwarzacher, 1976). From a theoretical point of view, one would expect any aspect of linear differentiation of a chromosome, including the presence of non-DNA components and tertiary structures, to be dependent on the base composition of the DNA in chromosomal regions. At the level of chromosome segments large enough to be visualized at metaphase, it should be possible to reduce the characteristics of DNA to two parameters: relative content of base pairs (AT versus GC) and repetitiveness of sequences. Variation of these parameters might allow for any pattern of differential staining. The validity of this statement can be tested by investigating the distribution of bases and repetitive sequences within a chromosome.

The most widely used approach to investigate biochemical differentiation along a human chromosome has been fractionation of DNA followed by characterization and regional localization of fractions. Fractionation has been based on differences in buoyant density (Corneo et al., 1971) renaturation kinetics (Sanchez & Yunis, 1974) and condensation in interphase nuclei (Gosden & Mitchell, 1975). By their very mechanisms, the former two principles of fractionation are also suitable to characterize DNA with regard to base composition and repetitiveness. Regional localization is usually performed by in situ annealing of labeled cRNAs transcribed from isolated DNA fractions by a bacterial RNA polymerase (Jones & Corneo, 1971).

Fractions of DNA which can be separated from the bulk of DNA by isopycnic gradient centrifugation in dense salt solutions are called "satellite DNA". The buoyant density of a satellite fraction is lower as the relative AT content is higher. All satellite DNAs have a highly repetitive nature. Properties of satellite DNA have been reviewed by Jones (1973).

In man, four satellite fractions (I to IV), together comprising about 6,5% of total DNA, have been localized on chromosomes by use of in situ hybridization. Satellites I, III and IV are relatively AT-rich, whereas satellite II is relatively GC-rich in relation to the bulk of human DNA. Only chromosomal regions of constitutive heterochromatin were found to contain significant amounts of satellite DNA (Jones & Corneo, 1971; Jones et al., 1974; Gosden et al., 1975). The 1qh region was found to be a major location of satellite II with satellites III and IV being present in small amounts.

Sanchez & Yunis (1974) have isolated four arbitrary fractions of human repetitive DNA on hydroxyapatite. The reassociation (Cot) values of the fractions varied from 10^{-3} (fraction I, highly repetitive) to between 1 and 5 (fraction IV, moderately repetitive). Localization of the fractions was carried out by in situ hybridization of labeled cRNA. On chromosome 1, fraction 1 annealed specifically to the 1qh region. The other fractions annealed to 1qh, all Q bands and the telomeric R band 1p36. This pattern of annealing (C bands+ Q bands + prominent T bands) was observed throughout the genome.

The distribution of DNA species within a metaphase chromosome has also been studied by investigation of de- and renaturation kinetics in situ (de la Chapelle et al., 1973), application of antibodies against specific bases (Dev et al., 1972; Miller et al., 1974), and differential staining by dyes of known specificity (Distèche & Bontemps, 1974; Kim, 1975). Based on a sequential in situ denaturation and reassociation detected by acridine orange fluorescence, de la Chapelle et al. (1973) suggest a decreasing AT content in the sequence C bands→Q bands → R bands → T bands. This suggestion is sustained by the pattern observed after staining of chromosome with 2,7-di-t-butyl proflavine, said to be an AT-binding dye (Distèche & Bontemps, 1974).

Application of fluorescent antibodies against specific nucleosides has not been informative since anti-adenosine, anti-guanosine and anti-cytidine yielded similar patterns on the chromosomes, roughly corresponding to Q bands (Dev et al., 1972). Immunofluorescent staining of 5-methyl-cytosine (5-MeC) has revealed that 5-MeC is located principally in regions of constitutive heterochromatin, 1q being a prominent site (Miller et al., 1974; Lubit et al., 1974).

3. Replication pattern analysis

The sequence of DNA replication within a chromosome can be studied by allowing a synchronized population of cells to take up a labeled DNA precursor during a defined part of the S-phase. When the cells are fixed during the next mitosis, the position of the label relative to the banding pattern indicates which parts of a chromosome were being replicated during the presence of the label in the medium.

Originally this procedure was carried out using autoradiography on tritium-labeled thymidine or deoxycytidine in chromosomes (Calderon & Schnedl, 1973; Schnedel, 1973; Ganner & Evans, 1971). Recently, the method of BUdR incorporation followed by staining with the dye "33258 Hoechst" has come into use (Epplen et al., 1975; Grzeschik et al., 1975). The latter

technique surpasses the former one in both sensitivity and resolving power and is much faster.

All available data on DNA replication patterns in human chromosomes agree in establishing that late replicating segments generally coincide with Q/G bands (Ganner & Evans, 1971; Calderon & Schnedl, 1973; Epplen et al., 1975; Grzeschik et al., 1975). Replication starts in R bands, band 1p36 being the first part of the human genome to have replication completed (Epplen et al., 1976).

Some discordance exists with regard to the replication time of 1qh. According to Ganner & Evans (1971) and Epplen et al. (1975), 1qh is the latest replicating region in chromosome 1. According to Calderon & Schnedl (1973) and Grzeschik et al. (1975), 1qh is replicated earlier than the prominent G bands 1p31 and 1q31, although later replication of 1qh is found occasionally. Grzeschik et al. suggest a hereditarily determined variance between individuals in replication time of heterochromatic regions.

4. Localization of chromosome breaks

Chromosome breakage may occur following exposure of cells to various mutagenic agents which can be physical, chemical or biological in nature. Differences between agents in the mechanism of breakage induction may result in differences in the types of aberrations produced and in different distributions of breaks along a chromosome. Establishment of these parameters may yield information on mechanisms of breakage induction and repair as well as on structural differentiation along chromosomes.

Unless stated otherwise, the data on the distribution of chromosome breaks to be mentioned have been obtained by analysis of preparations of cultured lymphocytes. The term "break", if not further specified, refers to a disruption leading to any cytologically detectable deletion or rearrangement, including sister chromatid exchange (SCE; cf. Latt, 1974a). Chiasma formation during the first meiotic division is also a manifestation of chromosome breakage. Therefore, establishment of chiasma distribution at diakinesis (Hultén, 1974) is included on this class of approach to chromosome mapping.

4.1 Familial rearrangements

Surveys of data on the chromosome break points involved in familial rearrangements have been given by Jacobs et al. (1974), Nielsen & Rasmussen (1976) and Nagakome & Chizo (1976). The combined data, which are mainly based on analyses by Q or G banding techniques, indicate a preferential location of breaks in R bands. Jacobs et al. and Nagakome & Chizo report a relatively enhanced involvement of terminal and centromeric regions in reciprocal translocations. This finding is not sustained by Nielsen & Rasmussen. The three papers yield a total number of 21 independent breaks in chromosome 1. Of these breaks, 14 are located in R bands, 3 in Q/G bands and 4 in the paracentric region (bands p11, q11 and q12).

4.2 De novo breaks in cultured lymphocytes

The data of Aymé et al. (1976), obtained by the use of R banding, show a preferential location of breaks in Q/G bands. Of the 22 breaks observed in chromosome 1, 16 are located in Q/G bands, 3 in R bands and 3 in the paracentric region. In a similar study on G banded chromosomes Aula & von Koskull (1976) find a preferential location of breaks in R bands.

4.3 De novo sister chromatid exchange

The distribution of sites of de novo SCE has been studied by Latt, 1974a (BUdR-Hoechst technique + Q banding) and Smyth & Evans, 1976 (^{3}H-TdR autoradiography + G banding). When analysing exchanges in chromosome 1 in particular, Latt finds a preferential location of break points in R bands and at R/Q interfaces. Smyth & Evans, on the other hand, report an overrepresentation of areas rich in G band material over the whole complement, including chromosome 1.

4.4 De novo breakge in chromosome instability syndromes

Fanconi's anemia is characterized by frequent occurrence of chromo-

some breakage resulting mainly in terminal deletions. Von Koskull & Aula (1973) have found sites of breakage to be located exclusively in R bands of G-banded chromosomes.

A characteristic of Bloom's syndrome is the occurrence of symmetrical exchanges between homologues ("mitotic chiasmata"). Meyer Kuhn (1976) has found a preferential location of involved break points in R bands (except terminal bands) of Q banded chromosomes. In contrast to the findings of German et al. (1974) based on the analysis of orcein-stained chromosomes, no specific involvement of constitutive heterochromatin could be demonstrated by Meyer Kuhn.

4.5 Chromosome breakage by irradiation

Investigating patterns of breakage after X-irradiation of lymphocytes, Caspersson et al. (1972), Seabright (1973) Holmerg & Jonasson (1973), San Roman & Bobrow (1973) and Cooke et al. (1975) all found an excess of breaks in the R bands of Q- or G-banded chromosomes.

Buckton (1976) has studied the pattern of X-ray induced breakage in chromosomes by separate and sequential application of both G- and R-banding. In metaphases first analyzed after G and subsequently after R banding no preferential location of breaks in G or R bands could be demonstrated, but 30% of all breaks were located at G-R band junctions. Terminal and centromeric regions appeared to be overrepresented. Application of G or R banding only yielded a relative overrepresentation of non-stained ("pale") regions with either technique.

After irradiation of lymphocytes with UV and X-rays in sequence, Holmberg & Jonasson (1974) find a random distribution of breaks over G and R bands in G-banded chromosomes.

The pattern of breakage in chromosome 1 followed the general pattern of the whole chromosome complement in all investigations cited.

4.6 Chromosome breakage by chemical inducters

In agreement with data obtained in the "pre-banding" era (see e.g. Cohen and Shaw, 1964), Morad et al. (1973) and Bourgeois (1974) have found that the antibiotical drug Mitomycin C specifically induces interchanges between regions of heterochromatin with exception of the y chromosome. Especially the regions 1qh, 9qh and 16qh are overrepresented as sites of breakage. The distribution of breaks associated with other aberration types shows the same pattern of preference, but far less markedly.

Latt (1974b) has investigated the pattern of SCE induced by Mitomycin C. At a given dose, the frequency of chromosome breakage is reported to be only "a few per cent" of the frequency of SCE. Breaks involved in exchanges, as localized in Q-banded chromosomes, occur preferentially in R bands or at R/Q interfaces. No preferential involvement of heterochromatic regions could be detected.

Ayraud et al. (1976) have localized sites of breakage by arabinosyl cytosine, which is considered to inhibit DNA synthesis by blocking dCDP production. In R banded chromosomes, breaks were found to be preferentially located in Q/G bands.

4.7 Chromosome breakage by Adenovirus 12

Adenovirus 12 has been found to induce uncoiling and breakage in two specific chromosome regions of man, namely 1q41-43 and 17q21-22. A third site of uncoiling, at which no preferential breakage has yet been detected, is situated in 1p36 (Mc Dougall et al., 1973; Stich et al., 1974; Steffensen et al., 1976).

4.8 Chiasma distribution

Hultén (1974) has investigated the distribution of chiasmata at diakinesis in human testicular biopsy material. Slides were stained sequentially with quinacrine, orcein and a C-banding technique. The preferential posit-

ion of chiasmata was found to be dependent on the number of chiasmata per chromosome arm. Terminal arm regions were clearly overrepresented. In addition, Hultén has the impression that chiasmata are often located at Q/R junctions; the p31-p32 junction in chromosome 1 is mentioned as an example. The mean chiasma frequencies (± 1SD) in 1p and 1q are respectively 2.00 ± 0, 35 and 1,91 ± 0,63.

Chapter III. THE MAP OF CHROMOSOME 1 : SIGNIFICANCE OF THE ACHIEVEMENTS

Of the genes located on chromosome 1, PGD, PPH1, UMPK, PGM1 and Pep-C have been investigated both by family studies and by somatic cell hybridization. Data on the former four loci, indicate that all genes belonging to the Rh linkage group are located on the segment 1p32→pter, the order being PGM1 - UMPK - Rh/ E11 - PGD/PPH1 - telomere. This conclusion is consistent with aneuploidy mapping data on Rh and PGM1 (cf. 11-1.2). Family studies did not reveal a significant linkage between Pep-C and either Rh or Fy (Cook et al., 1974). Assuming the position of Pep-C to be in 1q42, far from Rh or the Fy-linked marker 1qh, it is not surprising that linkage has not been demonstrated if one takes into account that chromosome 1 probably has a total meiotic length of 200 cM in human males (Cook et al., 1974; Hultén, 1974).

The Baltimore Conference map (Fig. 1) shows a distance of 55 cM for PGD/PPH1 - PGM1 in the human male. Hybrid cell data (Fig. 2) yield a distance of at least the length of band p33 and at most the length of the pter→p32 segment between these markers. The latter segment comprises about one quarter of the total length of chromosome 1 at metaphase. The recombination frequencies between PGM1 and UMPK of 10 in males (data from 5 individuals; see Hamerton, 1976) and of more than 40 in females (data from 3 individuals) are based on very few aberrations and thus do not allow any conclusions to be made about the relationship between bands and centiMorgans.

The relative sequence of FH, Pep-C, UGPP, PGM1, UMPK, AK2 and PPH1 as determined by Goss & Harris (see II -1.1) and by somatic cell hybrid studies using chromosome analysis are fully compatible. The position of αFUC as claimed by Goss & Harris should be checked by use of other methods to see whether this gene maps into the 1p3 segment as predicted on

the basis of its relative position.

Assignments of human genes coding for enzymes which catalyze consecutive steps in a particular metabolic pathway has so far not yielded evidence for the existence of clusters which might represent an operon-like configuration. Of the genes located in chromosome 1, only UGPP and PGM1 code for enzymes which may act sequentually (by catalyzing the respective reactions UDP-glucose + pyrophosphate $\rightleftharpoons$ glucose-1-phosphate + UTP and glucose-1-phosphate $\rightleftharpoons$ glucose-6-phosphate). However, the distance between these genes is about one third of the length of the chromosome (Fig. 2).

It has been postulated (Ohno, 1970) that new genetic information may be formed by tandem duplication of an ancestral gene followed by divergence of the originally identical copies by mutation and selection. Because of a general evolutionary conservation of chromosome structure (Ohno, 1973), this process can be expected to yield a preferential clustering of genes coding for proteins which are related in structure and function. The map of chromosome 1 provides two possible examples of such a clustering. The first is the close linkage of the genes which are respectively coding for the salivary and pancreatic forms of α-amylase, Amy1 and Amy2 (Meritt et al., 1972). The second example, a more questionable one, comprises the location of three genes coding for nucleoside monophosphate kinases on chromosome 1. The distinct enzymes AK, UMPK and GuK in man may have evolved from an ancestral ATP : nucleoside monophosphate phosphotransferase acting on several nucleoside substrates; a similar broad specifity enzyme has been demonstrated in E.coli (Bello & Bessmann, 1963). However, the value of this argument in favour of the tandem duplication hypothesis is restricted by the following facts: (1) GuK1 is located at a large distance from AK2 and UMPK; (2) AK1 and AK3 have been assigned to another chromosome, no. 9 (Westerveld et al., 1976; Povey et al., 1976); (3) preliminary results obtained in our laboratory indicate that at least one gene coding for

TMPK is not located on either chromosome 1 or 9 (Burgerhout, unpublished data).

Three genes (UMPK, PGM1 and Pep-C) have each been assigned to one particular band of chromosome 1; in each case, this is an R band. Five more genes have been assigned to particular bands in the human genome (Baltimore Conference, 1976): ACP1 to 2p23 (Hamerton et al., 1975) Pep-B to 12q21; α-Hp to 16q22; Pep-A to 18q23 (Arthur et al., 1975) and β-gal to 22q13 (de Wit et al., in press). This makes up a total of eight genes, of which only one (Pep-B) has been assigned to a Q/G band. Although the sample is small and several factors may have biassed the distribution (Burgerhout et al., in press a), the data suggest that the chance of a gene being located in an R band is greater than the 40% which has been claimed to be the relative amount of R band material in the human genome (Holmberg & Jonasson, 1973).

Do biochemical data on the linear differentiation of chromosomes support a preferential location of genes in R band material? Before considering this question, it should be noted that bands as defined at the Paris Conference represent chromosomal structures in a statistical sense only. No staining technique produced an all-or-none pattern : in fact, staining intensities show a continuous scale from "strong Q/G" to "strong R". The relative dimensions of staining areas in a chromosome can alter during the condensation process and individual bands disappear by fusion. In particular "large" bands at metaphase appear to be composed of distinct "Q/G" and "R" bands if early metaphase chromosomes are investigated (cf Yunis & Sanchez, 1975). Finally, the similarity of patterns evoked in one particular chromosome by different techniques or by one particular technique under variable conditions does not necessarily indicate that it is the same material which is actually stained. For example, different techniques classified as C-banding (Dutrillaux & Lejeune, 1975) do not stain segments of the same size in the 1qh region.

When these restrictions are taken into account, the following gross conclusions may be drawn from the data mentioned in II-2:

- DNA in R bands (except terminal bands) is nonrepetitive and not particularly rich in either AT or, GC base pairs.
- DNA in Q/G bands is moderately repetitive, moderately AT rich
- DNA in C bands (including 1qh) is highly repetitive, highly AT rich
- DNA in T bands (1p36 being prominent) is moderately repetitive, GC rich

Repetitiveness and unequal base composition are common properties of genetically inactive DNA (Comings, 1973). As DNA in R bands is neither repetitive nor rich in particular bases, R bands may indeed be expected to be preferential locations of genetically active material. This hypothesis is further supported by Hoehn (1975). Reviewing the involvement of particular chromosome material in reported cases of aneuploidy, Hoehn states that the chance of a chromosome or chromosome segment to be involved in an imbalance compatible with human viability shows an inverse relationship with its content of R band material rather than with its content of DNA. For example, the chromosomes 18 and 21, which are rich in Q/G band material, are far more frequently involved in trisomies than the chromosomes 17, 19, 20 and 22 which predominantly consist of R band material.

As expected, imbalance with regard to C band material is most frequent and in vast majority of cases such imbalances ("polymorphisms") occur without phenotypical abnormalities. The association of particular symptoms with 1qh+ as reported by Gardner et al.(1974)and Halbrecht & Shabtay (1976) is dubious since only a minority of individuals having a particular 1qh variant within a family did show the symptoms. If 1qh polymorphism has any phenotypic effect at all, then their expression is highly dependent on genetic or environmental backgrounds.

Combined data on the nature and behaviour of constitutive heterochromatin in man, as well as in other eukaryotes, do not yet provide conclusive evidence for any particular function. Current theories on the significance of heterochromatin have been discussed by Hsu (1975). One of the characteristics of heterochromatin is its late replication. Combined data on chromosome composition and replication pattern indicate that this characteristic is associated with base composition rather than with repetitiveness: band 1p36, which is a particular carrier of GC-rich repetitive DNA, is the first part of the genome to complete replication.

The most striking feature of the data concerning the distribution of chromosome breaks as listed in III-4 is the dependence of the observed pattern on the staining technique applied. Breaks generally seem to be preferentially located in negative chromosomal bands, i.e. in R bands after Q or G staining and in Q/G bands after R staining. Probably this phenomenon is due to breakage event which have occurred in positive bands close to the edge of a negative band but are interpreted as having taken place in the negative band. If a break is located in the proximal section of a positive band relative to the centromere, the retained positive material may not be seen as distinct from the adjacent negative band; if a break is located in the distal section, the loss of positively staining material from the band may not be observed. Errors of this kind can be eliminated by using both Q/G and R banding to localize a particular break point (cf. Buckton, 1976). This procedure should be considered as indispensable in any situation where precise localization of breaks is necessary, including the analysis of chromosome aberrations which are utilezed in gene localization studies.

With regard to the localization of sites of sister chromatid exchange, the methodological problems are different. Morphologically intact chromosomes are consecutively stained with a banding technique, destained, and treated for SCE vizualization (i.e. autoradiography or 33258 Hoechst staining). The pattern of SCE is thus projected over the banding pattern of the same chromosome. Still, the results obtained by Latt (1974; 33258

Hoechst staining) and Smyth & Evans (1976; ^{3}H-autoradiography) are discordant with regard to the relationship between de novo SCE break points and chromosomal bands. It should be considered, however, that both BUdR and tritium may induce SCE (Smyth & Evans, 1976). Differences in pattern and/or frequency of SCE induction may have been caused by the methods of detection themselves.

Regarding the spatial distribution of breaks over the chromosome, a prevalence of centromeric and terminal regions compared to median sections of chromosome arms has been reported by Jacobs et al. (1974) and Nagakome & Chiyo (1976) for familial rearrangements and by San Roman & Bobrow (1973) and Buckton (1976) for X-ray induced breaks. The latter observations might be of significance with regard to a comparison of the chromosome maps obtained by Goss & Harris (1975; in press) based on frequencies of X-ray induced breakage between loci with the regional maps obtained from studies on somatic cell hybrids carrying cytologically identified aberrant chromosomes.

Chiasmata at diakinesis are preferentially located in terminal chromosome regions and, perhaps, at Q/G- R band junctions. Wagner & Radman (1975) have postulated a mechanism for initiation of genetic recombination which involves pairing, nicking and cross-annealing of palindromic DNA sequences. Palindromic sequences (stretches of inverted, self-complementary DNA) occur abundantly in eukaryotic DNA (Cavalier-Smith, 1976). It would be interesting to investigate whether the distribution of palindromic sequences in human chromosomes resembles the chiasma pattern. Cavalier-Smith (1974) has proposed a model of telomere replication which implies that chromosome ends consist of palindromic sequences. Although this model does not demand particularly large amounts of palindromic DNA, the presence of palindromes at telomeres would be compatible with a preferential location of chiasmata.

Chapter IV. PROSPECTS FOR FUTURE RESEARCH

Working on the map of a particular chromosome of man becomes both more efficient and more rewarding as data accumulate. This holds especially for the regional localization of genes.

In sibship analysis, the addition of a marker to a linkage group may imply a more exact determination of the relative position of established markers and an extension of the chromosome length which is within linkage distance of any marker of the group. In addition, the chance that at least one marker within the group is polymorphic in an individual is enhanced. The latter two factors will facilitate still further extension of the linkage group.

In studies on somatic cell hybrids, the utilization of an increasing number of breaks in a particular chromosome refines the localization of genes relative to the banding pattern. In our laboratory, investigation of gene segregation following 17 different chromosome breakage events has yielded assignments to the "single" bands 1p32 and 1q42 (Burgerhout et al., in press a). Apart from the centromeric area, these bands were the only ones in which more than one break could be localized. Taking into account that the present map of chromosome 1 has been established on the basis of about 25 breaks (Baltimore Conference, 1975), the introduction of material containing some thirty more breaks in selected chromosome areas would in principle permit a regional assignment to any of the Paris Conference bands. Additional breaks in the region 1pter $\longrightarrow$ p32 are of special interest, since these will extend the scope of comparing the map of that region with the map of the Rh linkage group as inferred from family studies. Regional localization of new markers assigned to chromosome 1 can be carried out efficiently by testing the presence of the phenotype under test in a panel of hybrid clones carrying different products of chromosome breakage (Burgerhout et al., in press b).

Because of its size and staining patterns, chromosome 1 is a suitable subject also in the investigation of the karyological evolution of man and related primates. The karyotypes of chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla) and orang utan (Pongo pygmaeus) each display a chromosome which is apparently homologous to the no. 1 of Homo sapiens, although each of the four presumed homologues is slightly different from any other according to centromere index and banding pattern. Recent investigations have provided the first evidence with regard to homology at a functional level. For example, Henderson et al. (1976) have localized a major site of 5S RNA loci in the distal third of 1p (which is homologous to 1q in man) of the three ape species. Data presented at the Baltimore Conference (Warburton and Pearson, 1976), on the other hand, indicate that no FH locus is situated on chromosome 1 of Gorilla. These data suggest that whereas a region homologous to the section in 1q42-43 of man carrying the 5S RNA loci is present in corresponding parts of chromosome 1 in the ape species, a region homologous to the part of 1q42 → qter in man carrying FH is not present in at least chromosome 1 of Gorilla. This example shows the suitability of aiming regional mapping studies in primates at a chromosome which is homologous to a relatively well-mapped chromosome in man.

The scope of any approach to chromosome mapping is limited by the number and nature of genetic or structural markers which can be mapped and by the resolution of the method which is used to determine the position of a marker.

Linkage analysis in sibships is a powerful method for mapping loci which are highly polymorphic. A fair achievement is the map of the histocompatibility complex on chromosome 6 (Bodmer, 1976). For may enzyme loci, the frequency of polymorphism is low, which makes the search for informative sibships a very laborious one. Therefore it may be recommended to give priority to loci of particular interest, e.g. loci which are potential-

ly informative with regard to the nature of particular birth defects. A potentially efficient method to perform linkage analysis on segregation resulting from meiosis could be the fusion of germ cells (spermatozoa or spermatids) from males who are heterozygous for at least two syntenic markers in a known phase (coupling or repulsion) with rodent somatic cells (Ruddle & Creagan, 1975). If viable hybrids are formed at random with regard to a prior recombination event, the recombination frequency could be established by analysis of hybrid clones.

The potential of the man-rodent somatic cell hybrid system is limited by two demands: a genetic marker should be expressed in cultured hybrid cells and its product should be distinguishable from products of homologous rodent genes. Mehtods employing in situ annealing of RNAs are not subject to these limitations, provided that a specific messenger RNA can be isolated from differentiated human cells in a suitable quantity and that a system is available to detect annealing to unique sequences. The isolation of specific mRNAs may be facilitated by application of specific immunoprecipitation of polysomes synthesizing a particular protein (Shapiro & Schimke, 1975).

The possibilities to isolate specific clones of DNA have been greatly extended by the discovery of bacterial sequence-specific endonucleases "restriction enzymes"). Properties and significance of restriction enzymes have been reviewed by Nathans & Smith (1975). Following digestion of DNA by a restriction enzyme, DNA fragments of different molecular weight can be separated by gel electrophoresis. Digestion of repetitive DNAs which do contain recognition sites for specific endonucleases will result in formation of distinct bands after electrophoresis, and these can be cut out for further analysis. By this procedure, Cooke (1976) has demonstrated the existence of repeated sequences which occur in the human male but not in the female. The sequences are presumed to exist in the y chromosome, of

which they comprise about 50%. Chromosomal localization of any DNA fraction which can be isolated by application of restriction enzymes should be possible by in situ annealing of labeled cRNAs.

At still finer levels, restriction enzymes are being used for regional mapping of viral genomes, isolation of genes, and determination of base sequence within specific DNAs (Nathans & Smith, 1975; Kolata, 1976). The technique utilized in these investigations will probably become of great significance for the more detailed mapping of the human genome.

The precision of localization in many approaches to chromosome mapping is determined by the resolution of chromosome banding techniques. As noted in chapter III, application of differential staining is most profitable when different techniques are combined. Further refinement of banding patterns can be achieved by employing chromosomes from early stages of mitosis. Yunis (1976) has reported the appearance of up to 106 distinct bands in chromosome 1 at prophase; for comparison, the Paris Conference diagram shows 24 bands. Alternatively, detailed banding patterns can be obtained by making use of the phenomenon of premature chromosome condensation in interphase nuclei after fusion of interphase cells with mitotic cells (Unakul et al., 1973). A common problem with these methods is the identification of individual chromosomes in highly intertwined configurations. In cases where no necessity exists to keep chromosomes from one cell together, it should be possible to avoid this problem by utilizing suspensions of isolated chromosomes to achieve a greater spreading.

A number of 106 bands ("G" +'R") in chromosome 1 of man is still far from the pattern one might expect in a one band-one gene situation as is claimed to exist in dipteran salivary gland chromosomes. Regarding the enormous potentialities of the polytene chromosome system in studies on gene localization and regulation, the possibility of inducing polytenization in human chromosomes should be investigated.

Methods employing linkage analysis are not bound to any theoretical limitation of resolution. In practice, however, serious restrictions follow from the need for extensive screening of individuals or clones in order to state frequences of recombination or segregation with a sufficient statistical accuracy. In vitro approaches such as the one developed by Goss & Harris (1975, see II - I) permit reduction of this restriction by selection of informative clones and the choice of the mechanism of chromosome breakage induction.

The first, sketchy outline maps of human chromosomes have now been drawn. With such a wide range of tools available to the tophographers, details will soon follow.

REFERENCES

Arthur, E.; Steel, C.M.; Evans, H.J.; Povey, S.; Watson, B. and Harris, H.: Localization of human peptidase-A structural locus from studies on a cultured lymphoblastoid line. Nature, Lond. 257: 308-310 (1975).

Atwood, K.C.; Henderson, A.S.; Kacian, D. and Eicher, E.M.: On the feasability of mapping low-multiplicity genes by in situ hybridization. In Rotterdam Conference (1975): Second international workshop on human gene mapping. Birth Defects: Original article series, Vol. 11, No. 3 (The National Foundation, New York, 1976, pp. 59-61).

Aula, P. and von Koskull, H.: Distribution of spontaneous chromosome breaks in human chromosomes. Hum. Genet. 32, 143-148 (1976).

Aymé, S.; Mattei, J.F.; Mattei, M.G.; Aurran, Y. and Garaud, F.: Nonrandom distribution of chromosome breaks in cultured lymphocytes of normal subjects. Hum. Genet. 31, 161-175 (1976).

Ayraud, N.; Cantrelle, C. and Lloyd, M.: Distribution des points de cassure chromosomique induit par l'arabinosyl cytosine sur les lymphocytes humains in vitro. Ann. Génét. 19: 29-35 (1976).

Baltimore Conference (1975): Third international workshop on human gene mapping. Birth Defects: Original article series, Vol. 12, No. 7 (The National Foundation, New York, 1976).

Bello, A.J. and Bessman, M.J. J. Biol. Chem. 238:1777

Bodmer, W.F.: Report of the committee on the genetic constitution of chromosome 6. In Baltimore Conference (1975): Third international workshop on human gene mapping. Birth Defects: Original article series, Vol. 12, no. 7 (The National Foundation, New York, 1976).

Bourgeois, C.A.: Distribution of mitomycin C-induced damage in human chromosomes with special reference to regions of repetitive DNA. Chromosoma (Berl.) 48: 203-211 (1974).

Buckton, K.E.: Identification with G and R banding of the position of breakage points induced in human chromosomes by in vitro X-irradiation. Int. J. Radiat. Biol. 29: 475-488 (1976).

Burgerhout, W.G.; Someren, H. van and Bootsma, D.: Cytological mapping of the genes assigned to the human A1 chromosome by use of radiation-induced chromosome breakage in a human-Chinese hamster hybrid cell line. Humangenetik 20: 159-162 (1973).

Burgerhout, W.G.; Leupe-de Smit, S. and Jongsma, A.P.M.: Regional assignment of seven genes on chromosome 1 of man by use of man-Chinese hamster somatic cell hybrids. II. Results obtained after induction of breaks in chromosome 1 by X-irradiation (in press)(A).

Burgerhout, W.G.; Leupe-de Smit, S. and Jongsma, A.P.M.: Regional localization of a gene coding for Uridine Monophosphate Kinase in band p32 of chromosome 1 of man by application of a standardized assignment procedure (in press) (B).

Calderon, D. and Schnedl, W.A.: A comparison between quinacrine fluorescence banding and 3H thymidine incorporation patterns in human chromosomes. Humangenetik 18: 63-70 (1973).

Caspersson, T. Zech, L., Johansson, C. and Modest, E.J.: Identification of human chromosomes by DNA-binding fluorescent agents. Chromosoma 30: 215-227 (1970).

Caspersson, T.; Haglund, U.; Lindell, B. and Zech, L.: Radiation-induced non-random chromosome breakage. Exptl. Cell Res. 75, 541-546 (1972).

Cavalier-Smith, T.: Palindromic base sequences and replication of eukaryotic chromosome ends. Nature (Lond.) 250, 467-470 (1974).

Cavalier-Smith, T.: Long palindromes in eukaryotic DNA. Nature (Lond.) 262, 255-256 (1976).

de la Chapelle, A.; Schröder, J.; Selander, R.K. and Stenstrand, K.: Differences in DNA composition along mammalian metaphase chromosomes. Chromosoma (Berl.) 42: 365-382 (1973).

Di Cioccio, R.A.; Voss, R.; Krim, M.; Grzeschik, K.H. and Siniscalco, M.: Identification of human RNA transcripts among heterogeneous nuclear RNA from man-mouse somatic cell hybrids. Proc. Nat. Acad. Sci. (Wash.) 72: 1868-1872 (1975).

Cohen, M.M. and Shaw, M.W.: Effects of mitomycin C on human chromosomes. J. Cell Biol. 23: 386-395 (1964).

Comings, D.E.: The structure and function of chromatin. Adv. Hum. Genet. 3, 237-431 (1972).

Comings, D.E.; Avelino, E.; Okada, T.A.; Wynandt, H.E.: The mechanism of C and G-banding of chromosomes. Exptl. Cell Res. 77: 469-493 (1973).

Comings, D.E.; Kovacs, B.W.; Avelino, E. and Harris, D.C.: Mechanisms of chromosome banding. V. Quinacrine banding. Chromosoma (Berl.) 50: 111-145 (1975).

Cook, H.: Repeated sequence specific to human males. Nature (Lond.) 262: 182-186 (1976).

Cook, P.J.L.; Robson, E.B.; Buckton, K.E.; Jacobs, P.A. and Polani, P.E.: Segregation of genetic markers in families with chromosome polymorphisms and structural rearrangements involving chromosome 1. Ann. Hum.Genet. 37: 261-274 (1974).

Cooke, P.; Seabright, M. and Wheeler, M.: The differential distribution of X-ray induced chromosome lesions in trypsin-banded preparations from human subjects. Humangenetik 28: 221-231 (1975).

Corneo, G.; Ginlli, E. and Polli, E.: Renaturation properties and localization in heterochromatin of human satellite DNAs. Biochem. Biophys. Acta 247, 528-534 (1971).

Dev, V.G.; Warburton, D.; Miller, O.J.; Miller, D.A.; Erlanger, B.F., Beiser, S.M.: Consistent pattern of binding of anti-adenosine antibodies to human metaphase chromosomes. Exptl. Cell Res. 74: 288-293 (1972).

Distèche, C. and J. Bontemps: Chromosome regions containing DNAs of known base compositon, specially evidenced by 2.7-di-t-butyl proflavine. Chromosoma (Berl.) 47: 263-281 (1974).

Donahue, R.P., Bias, W.B., Renwick, J.H. and McKusick, V.A.: Probable assignment of the Duffy bloodgroup locus to chromosome 1 in man. Proc. Nat. Acad. Sci. USA, 61: 949-956 (1968).

Douglas, G.R.; McAlpine, P.J. and Hamerton, J.L.: Regional localization of the loci for human PGM and 6PGD on human chromosome one using Chinese hamster-human somatic cell hybrids. Proc. Nat. Acad. Sci. USA, 70: 2737-2740 (1973).

Dutrillaux, B. and Lejeune, J.: New techniques in the study of human chromosomes: methods and application. Adv. Hum. Genet. 5, 119-156 (1975).

Epplen, J.T.; Siebers, J.W. and Vogel, W.: DNA replication patterns of human chromosomes from fibroblasts and amniotic fluid cells revealed by a Giemsa staining technique. Cytogenet. Cell Genet. 15, 177-185 (1975).

Epplen, J.T.; Bauknecht, T. and Vogel, W.: DNA replication patterns of the early S phase from amniotic fluid cells as revealed by a Giemsa staining technique. Hum. Genet. 31, 117-119 (1976).

Evans, H.J.; Buckland, R.A. and Pardue, M.L.: Location of the genes coding for 18S and 28S ribosomal RNA in the human genome. Chromosoma (Berl.) 48: 405-426 (1974).

Ferguson-Smith, M.A.; Newman, B.F.; Ellis, P.M.; Thompson, D.M.G. and Riley, I.D.: Assignment by deletion of human red cell acid phosphatase gene locus to the short arm of chromosome 2. Nature New Biol. 243: 271-274 (1973).

Ferguson-Smith, M.A.: Gene mapping by exclusion. In Rotterdam Conference (1974): Second international workshop on human gene mapping. Birth Defects:Original article series. (The National Foundation New York, 1975, 126-129), Vol. 11, No.

Gall, J.G. and Pardue, M.L.: Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Nat. Acad. Sci. (Wash.) 63: 378-383 (1969).

Ganner, E. and Evans, H.J.: The relationship between patterns of DNA replication and of quinacrine fluorescence in the human chromosome complement. Chromosoma (Berl.) 35: 326-341 (1971).

Gardner, R.J.M.; Mc Creanor, H.R.; Parslow, M.J. and Veale, A.M.O.: Are 1q+ chromosomes harmless? Clin. Genet. 6: 383-393 (1974).

German, J.; Crippa, L.P. and Bloom, D.: Bloom's syndrome. III. Analysis of the chromosome aberration characteristic of this disorder. Chromosoma (Berl.) 48, 361-366 (1974).

Gosden, J.R. and Mitchell, A.R.: Characterization of human DNA from condensed and dispersed human chromatin. Exptl. Cell Res. 92: 131-137 (1975).

Gosden, J.R.; Mitchell, A.R.; Buckland, R.A.; Clayton, R.P. and Evans, H.J.: The location of four human satellite DNAs on human chromosomes. Exptl. Cell Res. 92, 148-158 (1975).

Goss, S.J. and Harris, H.: New method for mapping genes in human chromosomes. Nature (Lond.) 255: 680-684 (1975).

Goss, S.J. and Harris, H.: Gene transfer by means of cell fusion II. The mapping of 8 loci on human chromosome 1 by statistical analysis of gene assortment in somatic cell hybrids (in press).

Grzeschik, K-H.; Allderdice, P.W.; Grzeschik, A.; Opitz, J.M.; Miller, O.J. and Siniscalco, M: Cytological mapping of human X-linked genes by use of somatic cell hybrids involving an X-autosome translocation. Proc. Nat. Acad. Sci. (USA) 69: 69 (1972).

Grzeschik, K-H.: Regional mapping of human chromosomes I and II. In: Rotterdam Conference (1974): Second international workshop on human gene mapping, 172-175. Birth Defects: Original Article Series Vol. 11, No. 3 (The National Foundation, New York 1975).

Grzeschik, K.; Kim, A.M. and Johannsmann, R.: Late replicating bands of human chromosomes demonstrated by fluorochrom and Giemsa staining. Humangenetik 29: 41-59 (1975).

Halbrecht, J. and Shabtay, F.: Human chromosome polymorphism and congenital malformations. Clin. Genet. 10: 113-122 (1976).

Hamerton, J.L.; Mohandas, T.; McAlpine, P.J. and Douglas, G.R.: Localization of human gene loci using spontaneous chromosome rearrangements in human-Chinese hamster somatic cell hybrids. Am.J. Hum. Genet. 27, 595-608 (1975).

Hamerton, J.L.: Report of the committee on the genetic constitution of chromosomes 1 and 2. In Baltimore Conference (1975): Third international workshop on human gene mapping. Birth Defects: Orginal article series, 12, No. 7 (The National Foundation, New York, 1976, pp. 7-23.)

Hatlen, L. and Attardi, G.: Preportion of the HeLa cell genome complementary to transfer RNA and 5S RNA. J. Molec. Biol. 56: 535-553 (1971).

Henderson, A.S.; Atwood, K.C.; Yu, M.T.; and Warburton, D.: The site of 5S RNA genes in primates. I. The great apes. Chromosoma (Berl.) 56: 29-32 (1976).

Henderson, A.S.; Warburton, D. and Atwood, K.C.: Location of ribosomal DNA in the human chromosome complement. Proc. Nat. Acad. Sci. (Wash.) 69, 3394-3398 (1972).

Hoehn, H.: Functional implications of differential chromosome banding. Am. J. Hum. Genet. 27: 676-686 (1975).

Holmberg, M. and Jonasson, J.: Preferential location of X-ray induced chromosome breakage in the R-bands of human chromosomes. Hereditas 74: 57-68 (1973).

Holmberg, M. and Jonasson (1974): Synergistic effect of X-ray and UV irradiation on the frequency of chromosome breakage in human lymphocytes. Mutation Res. 23: 213-221.

Hultén, M: Chiasma distribution at diakinesis in the normal human male. Hereditas 76: 55-78 (1974).

Jacobs, P.; Buckton, K.E.; Cunningham, C. and Newton, M.: An analysis of the break points of structural rearrangements in man. J. Med. Genet. 11: 50-64 (1974).

Jeanteur, P. and Attardi, G.: Relationship between HeLa cell ribosomal RNA and its precursors studied by high resolution RNA-DNA hybridization. J. Molec. Biol. 45, 305-324 (1969).

Jonasson, J. and Holmberg, M.: Evidence for an inverse relationship between X-ray induced chromatid and chromosome breakage in human chromosomes. Hereditas 75: 259-266 (1973).

Jones, K.W. and Corneo, G.: Localization of satellite and homogeneous DNA sequences on human chromosomes. Nature (Lond.) New Biol. 233: (1971).

Jones, K.W.: Satellite DNA. J. Med. Genet. 10: 273-281 (1973).

Jones, K.W.; Purdon, J.F.; Prosser, J. and Corneo, G.: The chromosomal localization of human satellite DNA. Chromosoma (Berl.) 49: 161-171 (1974).

Jongsma, A.P.M.; Someren, H. van.; Westerveld, A.; Hagemeijer, A. and Pearson, P.L.: Localization of genes on human chromosomes by using human-Chinese hamster somatic cell hybrids: assignment of PGM3 to chromosome C6 and mapping of the 6PGD, PGM1 and Pep-C genes on chromosome A1. Humangenetik 20: 195-202 (1973).

Jongsma, A.P.M. and Burgerhout, W.G.: Regional assignment of seven genes on chromosome 1 of man by use of man-Chinese hamster somatic cell hybrids. I. Results obtained after hybridization of human cells carrying reciprocal translocations involving chromosome 1. (in press).

Kim, M.A.: Fluorometrical detection of thymine base differences in complementary strands of satellite DNA in human metaphase chromosomes. Humangenetik 28: 57-63 (1975).

Kolata, G.B.: DNA sequencing: A new era in molecular biology. Science 192: 645-647 (1976).

von Koskull, H. and Aula, P.: Nonrandom distribution of chromosome breaks in Fanconi's anemia. Cytogenet. Cell Genet. 12: 423-434 (1973).

Latt, S.A.: Localization of sister chromatid exchanges in human chromosomes. Science 185: 74-76 (1974)(A).

Latt, S.A.: Sister chromatid exchanges, indices of human chromosome damage and repair: detection by fluorescence and induction by Mitomycin C. Proc. Nat. Acad. Sci. (Wash.) 71: 3162-3166 (1974)(B).

Lubit, B.W.; Schreck, R.R.; Miller, O.J. and Erlanger, B.F.: Human chromosome structure as revealed by an immunoperoxidase staining procedure. Exptl. Cell Res. 89: 426-429.

Magenis, R.E.; Koler, R.D.; Lovrien, E.; Bigley, R.H.; Duval, M.C. and Overton, K.M.: Gene dosage: evidence for assignment of erythrocyte acid phosphatase locus to chromosome 2. Proc. Nat. Acad. Sci. (Wash.) 72: 4526-4530 (1975).

Marsh, W.L.; Chaganti, R.S.K.; Gardner, F.H.; Mager, K.; Nowell, P.C. and German, J.: Mapping human autosomes: evidence supporting assignment of Rhesus to the short of chromosome No. 1. Science 183: 966-968 (1974).

Mayeda, K.; Weiss, L.; Lindahl, R. and Dully, M.: Localization of the human lactate dehydrogenase B gene on the short arm of chromosome 12. Am. J. hum. Genet. 26: 59-64 (1974).

McBride, W.O. and Ozer, H.L.: Transfer of genetic information by purified metaphase chromosomes. Proc. Nat. Acad. Sci. (Wash.) 70: 1258-1262 (1973).

Mc Carthy, B.J. and Church, R.B.: The specificity of molecular hybridization reactions. Ann. Rev. Biochem. 39: 131-150 (1970).

Mc Dougall, J.K., Kucherlapati, R. and Ruddle, F.: Localization and induction of the human thymidine kinase gene by adenovirus 12. Nature (Lond.) New Biol. 245: 172-175 (1973).

Merritt, A.D.; Lovrien, E.W.; Rivas, M.L. and Conneally, P.M.: Human amylase loci: genetic linkage with the Duffy blood group locus and assignment to linkage group I. Am. J.hum. Genet. 25: 523-528 (1973).

Meyer Kuhn, E.: Localization by Q-banding of mitotic chiasmata in cases of Bloom's syndrome. Chromosoma (Berl.) 57: 1-11 (1976).

Miller, O.J.; Cook, P.R.; Meera Khan, P; Shin, S. and Siniscalco, M.: Mitotic separation of two human X-linked genes in man-mouse somatic cell hybrids. Proc. Nat. Acad. Sci.(Wash.) 68: 116-120 (1971).

Miller, O.J., Schnedl., W., Allen, J. and Erlanger, B.F.: 5-Methylcytosine localized in mammalian constitutive heterochromatin. Nature (Lond.) 251: 636-657 (1974).

Morad, M.; Jonasson, J. and Lindsten, J.: Distribution of mitomycine C induced breaks on human chromosomes. Hereditas 74: 273-282 (1973).

Nakagome, Y. and Chiyo, H.: Nonrandom distribution of exchange points in patients with structural rearrangements. Am. J. Hum. Genet. 28: 31-41 (1976).

Nathans, D. and Smith, H.O.: Restriction endonucleases in the analysis and restructuring of DNA molecules. Ann. Biochem. 44: 273-293 (1975).

New Haven Conference (1973): First international workshop on human gene mapping. Birth Defects: Original article series, Vol. 10, No.3 (The National Foundation, New York, 1974).

Nielsen, J, and Rasmussen, K.: Distribution of break points in reciprocal translocations in children ascertained in population studies. Hereditas 82: 73-78 (1976).

Ohno, S.: Evolution by gene duplication. Springer-Verlag, New York, 1970.

Ohno, S.: Ancient linkage groups and frozen accidents. Nature 244: 259-262 (1973).

Ott, J.; Hecht, F.; Linder, D.; Lovrien, E.W. and Kaiser Mc Caw, B.: Human centromere mapping using teratoma data. In: Baltimore Conference (1975): Third international workshop on human gene mapping. Birth Defects: Original article series, Vol. 12, No.7 (The National Foundation, New York, 1976 pp. 396-398).

Pardo, D.; Luciani, J.M. and Stahl, A.: Localisation, par hybridation in situ, des gènes des ARN 28S et 18S dans les chromosomes somatiques humains. Ann. Génét. 18: 105-109 (1975).

Paris Conference, 1971: Standardization in human cytogenetics. Birth Defects: Original article series, Vol. 8, No. 7 (The National Foundation, New York, 1972).

Pearson, P.L.: The use of new staining techniques for human chromosomes identification. J.med. Genet. 9, 264-275 (1972).

Povey, S.; Slaughter, C.A.; Wilson, D.E.; Gormly, I.P.; Buckton, K.E.; Perry, P. and Bobrow, M: Evidence for the assignment of the loci AK_1, AK_3 and ACONs to chromosome 9 in man. Ann. Hum. Genet. (Lond.) 39: 413-423 (1976).

Renwick, J.H.: The mapping of human chromosomes. In: Annual Review of Genetics pp. 81-120, Vol. 5 (1971).

Ruddle, F.; Ricciuti, F.; McMorris, F.A.; Tischfield, J.; Creagan, R.; Darlington, G. and Chen, T: Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A-1 in man. Science 176: 1429-1431 (1972).

Ruddle, F.H. and Creagan, R.P.: Parasexual approaches to the genetics of man. Ann. Rev. Genet. 9: 407-486 (1975).

San Roman, C. and Bobrow, M.: The sites of radiation-induced breakage in human lymphocyte chromosomes, determined by quinacrine fluorescence. Mutation Res. 18: 325-331 (1973).

Sanchez, P. and Yunis, J.J.: The relationship between repetitive DNA and chromosomal bands in man. Chromosoma (Berl.) 48: 191-202 (1974).

Schnedl, W.: Late DNA replication pattern of human chromosomes determined by means of 3H-deoxycytidine. Humangenetik 20: 55-58 (1973).

Schwarzacher, H.G.: Chromosomes in mitosis and interphase. Handbuch der mikroskopischen Anatomie des Menschen 1/3, Springer-Verlag, Berlin, 1976.

Seabright, M.: High resolution studies on the pattern of induced exchanges in the human karyotype. Chromosoma (Berl.) 40: 333-346 (1973).

Shapiro, D.J. and Schinke, R.T.: Immunochemical isolation and characterization of ovalbumin messenger ribonucleic acid. J. Biol. Chem. 250: 1759-1764 (1975).

Smyth, D.R. and Evans, H.J.: Mapping of sister-chromatid exchanges in human chromosomes using G-banding and autoradiography. Mutation Res. 35: 139-154 (1976).

Steffensen, D.M.; Prensky, W. and Duffy, P.: Localization of the 5S ribosomal RNA genes in the human genome. In: New Haven Conference (1973) First international workshop on human gene mapping. Birth Defects: Original article series, Vol. 10, No. 3 (The National Foundation, New York, 1974[a] p.153).

Steffensen, D.M., Duffey, P. and Prensky, W.: Localization of 5S ribosomal RNA genes on human chromosome 1. Nature (Lond.) 252: 741-743 (1974).

Steffensen, D.M., Szabo, P. and McDougall, J.K.: Adenovirus 12 uncoiler regions of human chromosome 1 in relation to the 5S rRNA genes. Exptl. Cell Res. 100: 436-439 (1976).

Stich, H.F.; Stich, W. and Lam, P.: Susceptability of xeroderma pigmentosum cells to chromosome breakage by adenovirus type 12. Nature (Lond.) 250: 599-601.

Sturtevant, A.H.: A history of genetics. Harper & Row, New York, 1966.

Syrett, J.E.; Gray, J.E.; Grace, E.; Bowser Riley, S.M.; Cook, P.J.A.; Robson, E.B. and Teesdale, P.W.: Two informative 1p translocation families. In: Rotterdam Conference (1974): Second international workshop on human gene mapping. Birth Defects: Original article series, Vol. 11, No. 3 (The National Foundation, New York, 1975, pp. 269-271).

Turner, J.H.; Crawford, M.H. and Leyshon, W.C.: Phenotypic-karyotypic localization of the human Rh-locus on chromosome 1. J. Hered. 66: 97-99 (1975).

Unakul, W.; Johnson, R.T.; Rao, P.N. and Hsu, T.C.: Giemsa banding in prematurely condensed chromosomes obtained by cell fusion. Nature (Lond.) New Biol. 242: 106-107 (1973).

Wagner, R.E. and Radman, M.: A mechanism for initiation of genetic recombination. Proc. Nat. Acad. Sci. (Wash.) 72: 3619-3622.

Warburton, D. and Pearson, P.L.: Report of the committee on comparative mapping. In: Baltimore Conference (1975): Third international workshop on human gene mapping. Birth Defects: Original article series. Vol. 12, No. 7 (The National Foundation, New York, 1976).

Westerveld, A.; Jongsma, A.P.M.; Meera Khan, P.; Someren, H. van and Bootsma, D.: Assignment of the AK-1 : Np : ABO linkage group to chromosome 9. Proc. Natn. Acad. Sci. USA 73: 895-899 (1976).

Willecke, K.; Lange, R.; Krüger, A. and Reber, T.: Cotransfer of two linked human genes into cultured mouse cells. Proc. Nat. Acad. Sci. (Wash.) 73: 1275-1278 (1976).

de Wit, J.; Hoeksema, H.L.; Halley, D.; Hagemeijer, A.; Bootsma, D. and Westerveld, A.: Regional localization of a beta-galactosidase locus on the human chromosome 22 (in press).

Wullems, G.J.; van der Horst, J. and Bootsma, D.: Transfer of the human genes coding for thymidine kinase and galactokinase to Chinese hamster cells and human-Chinese hamster cell hybrids. (in press)

Yunis, J.J.: High resolution of human chromosomes. Science 191: 1268-1270 (1976).

Yunis, J.J. and Sanchez, O.: The G-banded prophase chromosomes of man. Humangenetik 27: 167-172 (1975).

ABBREVIATIONS

ACP	acid phosphatase
AK	adenylate kinase
Amy	α-amylase
ATP	adenosine triphosphate
BUdR	5-bromodeoxyuridine
cRNA	complementary RNA
cM	centi Morgan
dCDP	deoxy-cytidine diphosphate
El	elliptocytosis
FH	fumarate hydratase
αFUC	α-l-fucosidase
GaK	galactokinase
β-Gal	β-galactosidase
HAT medium	medium containing hypoxanthine, aminopterine and thymidine
HLA	human lymphocyte antigens (= major histocompatibility complex)
α-Hp	α-haptoglobin
HPRT	hypoxanthine phosphoribosyltransferase
MNSs	MNSs blood group
Pep	peptidase
PGD	6-phosphogluconate dehydrogenase
PGM	phosphoglucomutase
PPH	phosphopyruvate hydratase (= enolase)
TK	thymidine kinase
TMPK	thymidine monophosphate kinase
UDP	uridine diphosphate
UGPP	UDP glucose pyrophosphorylase
UMPK	uridine monophosphate kinase
UTP	uridine triphosphate

SUMMARY

Somatic cells from man and mouse or Chinese hamster can be fused to form proliferating hybrid cell lines. These lines tend to loose chromosomes of man, which implies the loss of human genes. Correlation of results from determination of the presence of specific chromosomes and gene products of man in hybrid cell lines has lead to the assignment of genes to individual human chromosomes.

The present investigations were initiated in order to determine the position of genes on a human chromosome using a similar method. If a part of a chromosome has been lost from a population of hybrid cells after breakage, regional localization of genes on that chromosome relative to the breakpoint is feasible by correlating presence or apparent absence of chromosome maetrial with presence or absence of gene products. Our investigations were principally aimed at chromosome 1. A motivation is given in Chapters I and II.

Several ways were followed to obtain hybrid cell lines carrying aberrant chromosomes involving human no. 1 material. One method was the induction of chromosome breaks by X-irradiation; this was carried out in two experiments. In the first experiment (Appendix paper I and IV) a hybrid cell line which carried an intact human chromosome 1 was irradiated and subsequently clones, yielding two clones with a broken chromosome 1. In the second experiment (Appendix paper IV) human fibroblasts were irradiated and cloned. Chromosome analysis of the clones led to the discovery of one clone carrying an aberrant chromosome 1. Cells from this clone were fused with Chinese hamster cells and hybrids were isolated.

A second way to obtain informative hybrids was accomplished by fusion of cells from human carriers of reciprocal translocations with Chinese hamster cells. Paper III of the Appendix gives the results of hybridization experiments with cells from three individuals carrying different aberrations involving chromosome 1. Finally, suitable material was provided by the occurrence of de

de novo chromosome breakage during cultivation of hybrids. Products of eight *de novo* breaks in chromosome 1 have been utilized for mapping of genes (Appendix papers III and IV).

The feasibility of regional mapping studies is highly dependent upon the detection and identification of aberrant human chromosomes in hybrid cells. Paper II of the Appendix describes how the Giemsa-11 staining technique can be utilized in identification of interspecific man-Chinese hamster translocation chromosomes.

Appendix paper V deals with the methodology of gene assignment. Gene localization studies have often yielded inconsistencies, which may well be due to the use of incorrect criteria in establishment of the presence or absence of human chromosomes and gene products in hybrid cell lines. In paper V a model procedure for localization of genes is proposed and its application in a study on the location of a gene coding for UMPK is described.

From the material presented in papers I, III, IV and V the following locations of genes on chromosome 1 can be deduced: *PGD* and *PPH1* in p36 → p34; *UMPK* in p32, distal to *PGM1*; *PGM1* in p32, proximal to *UMPK*; *UGPP* in q21 → q23; GuK_1 in q31 → q42; *Pep-C* in q42; *FH* in qter → q42.

The approach followed in the papers mentioned in the preceding paragraph is but one of the ways to make a map of a chromosome. The chapters I - IV of the present thesis are intended to give a general picture of the field of human chromosome mapping, with special reference to chromosome 1. After a general introduction (Chapter I) a survey of present approaches and achievements in human chromosome mapping is given (Chapter II). The significance of the achievements is discussed in Chapter III. In conclusion, Chapter IV deals with prospects of future research within this field of human genetics.

SAMENVATTING

Door fusie van somatische cellen van mens en Chinese hamster kunnen prolifererende hybride cellijnen worden verkregen. Deze lijnen verliezen bij voortkweken chromosomen van de mens; dit impliceert het verlies van menselijke genen. Correlatie van resultaten met betrekking tot de aanwezigheid van specifieke menselijke chromosomen en genprodukten heeft geleid tot het localiseren van genen op menselijke chromosomen.

Het in dit proefschrift beschreven onderzoek werd opgezet met als doel, op analoge wijze de positie van genen op een menselijk chromosoom te bepalen. Als een deel van een menselijk chromosoom na chromosoombreuk verloren is gegaan uit een populatie van hybride cellen, kan de positie van op dat chromosoom gelegen genen t.o.v. het breukpunt worden bepaald door aan- en afwezigheid van een chromosoomgedeelte met die van de genprodukten te correleren. Ons onderzoek was in eerste instantie gericht op chromosoom 1. Het onderzoek wordt gemotiveerd in hoofdstuk I en II.

Voor het verkrijgen van hybride cellijnen met een gebroken menselijk chromosoom no. 1 werden verschillende methoden toegepast. De eerste methode was de inductie van chromosoomafwijkingen m.b.v. Röntgenstraling; dit werd gerealiseerd in twee experimenten. In het eerste experiment (Appendix, artikel I en IV) werd een hybride cellijn, waarin een intact chromosoom 1 voorkwam, bestraald en vervolgens gekloneerd. Dit leverde twee klonen met een gebroken chromosoom 1 op. In het tweede experiment (Appendix, artikel IV) werden menselijke fibroblasten bestraald en gekloneerd. Bij toepassing van chromosoomanalyse op de klonen werd één kloon met een afwijkend chromosoom 1 gevonden. Cellen van deze kloon werden gefuseerd met Chinese hamster cellen; hieruit werden hybriden geisoleerd.

Een tweede methode voor het verkrijgen van informatieve hybriden werd verwezenlijkt door fusie van cellen van menselijke dragers van reciproke translocaties met Chinese hamster cellen. Artikel III van de Appendix geeft

de resultaten van hybridisatie-experimenten met cellen van drie individuen, die drager waren voor een translocatie waarbij chromosoom 1 betrokkken was. Tenslotte werd bruikbaar materiaal verkregen door het optreden van de novo chromosoombreuken in hybriden. De produkten van acht de novo breuken in chromosoom 1 zijn gebruikt voor het in kaart brengen van het chromosoom (Appendix, artikel III en IV).

De uitvoerbaarheid van dit onderzoek is in hoge mate afhankelijk van de mogelijkheid, afwijkende menselijke chromosomen in hybride cellen te herkennen en te determineren. Artikel II van de Appendix beschrijft hoe de Giemsa-11 kleuringstechniek kan worden toegepast bij het identificeren van interspecifieke mens-Chinese hamster translocatiechromosomen.

Artikel V van de Appendix houdt zich bezig met de methodologie van het localiseren van genen. Genlocalisatie-onderzoek heeft dikwijls inconsistente resultaten opgeleverd. Dit is waarschijnlijk in belangrijke mate te wijten aan het hanteren van onjuiste criteria m.b.t. het vaststellen van aan- en afwezigheid van menselijke chromosomen en genprodukten in hybride cellijnen. In artikel V wordt een modelprocedure voor genlokalisatie voorgesteld; deze procedure wordt toegepast in een onderzoek naar de positie van een gen dat codeert voor UMPK.

Combinatie van de gegevens die gepresenteerd worden in de artikelen I, III, IV en V levert de volgende posities van genen op chromosoom 1: PGD en PPH1 in p36→p34; UMPK in p32, distaal van PGM1; PGM1 in p32, proximaal van UMPK; UGPP in q21 → q23; GuK1 in q31 → q42; Pep-C in q42; FH in qter → q42.

De benaderingswijze die in bovenstaande artikelen is gevolgd is slechts één van de vele methoden om een kaart van een chromosoom op te stellen. In dit proefschrift wordt getracht een algemeen beeld van het in kaart brengen van menselijke chromosomen te geven, met speciale aandacht voor chromosoom 1. Na een algemene inleiding (hoofdstuk I) wordt een overzicht ge-

geven van gangbare methodieken en de hiermee verkregen gegevens (hoofdstuk II). De betekenis van de gegevens wordt besproken in hoofdstuk III. Hoofdstuk IV, tenslotte, behandelt vooruitzichten van toekomstig onderzoek binnen dit gebied van de antropogenetica.

CURRICULUM VITAE

Na het behalen van mijn einddiploma HBS-B aan het Caland Lyceum te Rotterdam in 1963 begon ik een studie Biologie aan de Rijksuniversiteit te Utrecht. Het kandidaatsexamen werd afgelegd in november 1967, het doctoraal met als hoofdvak Erfelijkheidsleer en als bijvakken Vergelijkende Fysiologie en Wijsbegeerte der Exacte Natuurwetenschappen in 1970. Bij het vervullen van mijn militaire dienstplicht ben ik van mei 1971 tot september 1972 gedetacheerd geweest op het Medisch Biologisch Laboratorium TNO te Rijswijk (Z.H.); ik werkte daar aan biologische dosimetrie van ioniserende straling, en raakte via het detecteren van chromosoombreuken betrokken bij het genlocalisatie-onderzoek. In september 1972 ben ik aangesteld als wetenschappelijk medewerker bij de afdeling Celbiologie en Genetica van de Medische Faculteit (later Erasmus Universiteit) te Rotterdam. Binnen deze afdeling werd het in dit proefschrift beschreven onderzoek verricht.

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Sinds voorjaar 1975 heeft Mevr. S. Leupe-de Smit mij bijgestaan in het uitvoeren van de experimenten. Zij deed dit vaardig en volhardend, en al snel zeer zelfstandig. Vooral door haar openhartigheid was het plezierig, met haar samen te werken.

Alle medewerkers van de werkgroep Genlocalisatie in Rotterdam en van de met ons samenwerkende groepen op het Medisch Biologisch Laboratorium TNO te Rijswijk en de Rijksuniversiteit te Leiden wil ik hartelijk danken voor de bijdragen, die zij met hoofd en hand hebben geleverd. Deze dank geldt ook een groot aantal andere medewerkers van de afdeling Celbiologie en Genetica te Rotterdam; enkelen wil ik met name noemen. De heer T.M.

van Os heeft veel aandacht besteed aan de moeilijke taak, intensiteitsnuances in gekleurde chromosomen op de foto vast te leggen. Mej. M. van Duuren en de heren W.J. Visser en J.H. Fengler hebben met accuratesse de figuren getekend en gefotografeerd. Mevr. Godijn en Mej. J.A. Bolman zorgden voor koffie en steriel glaswerk, en de heer A.H. Hammer voor kweekmedia en andere essentiële vloeistoffen. De dames R.J. van den Hoek-Boucke, C.M. van Vugt-van Vliet en E.G.L. Quartel-van Wenum hebben het typewerk voor dit proefschrift verricht.

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PAPER I

Cytological Mapping of the Genes Assigned to the Human A 1 Chromosome by Use of Radiation-Induced Chromosome Breakage in a Human-Chinese Hamster Hybrid Cell Line

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Summary. A human-Chinese hamster hybrid cell line was treated with X-rays and subsequently cloned. In two clones, segregation between three markers assigned to the human A1 chromosome (6-phosphogluconate dehydrogenase, phosphoglucomutase 1 and peptidase C) was found. Chromosome analysis revealed an aberrated human A1 chromosome in both clones. The results suggest the following gene sequence on chromosome A1: 6 PGD, PGM_1 (on the short arm), Pep C (on the long arm).

Zusammenfassung. Eine Zell-Linie (Hybride zwischen Zellen vom Menschen und vom Chinesischen Hamster) wurde mit Röntgenstrahlen behandelt und anschließend kloniert. In zwei Klonen wurde eine Aufspaltung bei drei Markern beobachtet, die zum menschlichen A1-Chromosom gehören (6-Phosphogluconat-Dehydrogenase; Phosphoglucomutase 1; Peptidase C). Die Chromosomenanalyse zeigte ein aberrantes menschliches A1-Chromosom in beiden Klonen. Die Ergebnisse legen die folgende Reihenfolge nahe: 6 PGD; PGM_1 (auf dem kurzen Arm); Pep C (auf dem langen Arm).

Introduction

By use of somatic cell hybridization, electrophoretic techniques and chromosome analysis, an increasing number of human genes is assigned to chromosomes (for a review, see Ruddle, 1973). The next step in these investigations is the mapping of genes on a chromosome relative to the banding pattern. This can be achieved *in vitro* by the study of hybrid cell lines in which broken human chromosomes are present. Retention or loss of a gene can be correlated with retention or loss of a part of a chromosome.

Hybrid cell lines suitable for this kind of study have been obtained by hybridizing cells from patients carrying chromosome aberrations (Grzeschik *et al.*, 1972a; Ricciuti and Ruddle, 1973) and from spontaneous chromosome breakage in hybrids in culture (Ruddle *et al.*, 1972; Jongsma *et al.*, in press). An alternative way is the induction of chromosome breaks by physical or chemical agents. These breaks can be introduced either in parental human cells, which can subsequently be cloned and hybridized, or by treating hybrid cell lines. In this study, we followed the latter approach.

At present, the two largest human linkage groups which are known are assigned to the chromosomes A1 and X, respectively (Ruddle *et al.*, 1972; Grzeschik *et al.*, 1972b). Therefore, a hybrid line was irradiated in which both these chromosomes were retained. In order to facilitate chromosome analysis after irradiation, a line was selected which had lost most other human chromosomes.

Materials and Methods

The hybrid cell line originated from a fusion between normal human leucocytes and the wg3-h line, a hypoxanthine-guanine phosphoribosyl transferase (HGPRT)-deficient Chinese hamster cell line derived from the Don line.

Hybridization and culture techniques have been described by Westerveld *et al.* (1971). Chromosome analysis of the line had shown the retention of the human chromosomes A1, C8, C11, F20, G21, G22, X and Y.

Irradiation was carried out with a Philips Müller MG300 X-ray apparatus, filter 0.5 mm Al, operated at 250 kV 10 mA, dose rate 100 rad/min. Cells were irradiated in monolayer with a total dose of 600 rad. After irradiation, clones were isolated either directly from the irradiated monolayer (HRA clones) or after seeding a diluted suspension of irradiated cells (HRB clones). For chromosome preparations, mitotic cells were obtained by shaking a nearly full-grown Roux bottle; no metaphase-arresting treatment was applied. The cells were treated with a 0.075 M KCl solution for 15 min and fixed in a 3:1 mixture of methanol and acetic acid. Chromosome preparations were made by the air-drying method. Slides were treated with a balanced salt solution containing 0.005% trypsin (NBC) for 2—4 min, rinsed in balanced salt solution and stained in a 1:10 diluted Giemsa solution (Gurr) at pH 6.8 for 3 min. After rinsing briefly in deionized water, slides were dried quickly and mounted in Depex (Gurr). From each clone, at least 15 metaphases were analysed.

After harvesting the mitotic cells, the remaining cells in a bottle were trypsinized and prepared for electrophoresis. Preparation of cell lysates and procedures for Cellogel electrophoresis were carried out as described by Meera Khan (1971).

The following enzymes were tested: 6-phosphogluconate dehydrogenase (6 PGD), phosphoglucomutase 1 (PGM_1), peptidase C (Pep C), HGPRT, glucose-6-phosphate dehydrogenase (G6PD), phosphoglycerate kinase (PGK) and α-galactosidase (α-Gal.).

Results

From the irradiated populations, 9 HRA and 28 HRB clones have been isolated. Segregation between the X-linked markers (HGPRT, PGK, G6PD and α-Gal.) was not observed: 36 clones had retained the four markers, whereas one had lost them all. Two clones showed segregation of the markers, known to be located on chromosome A1. The clone HRA11 had retained 6PGD and PGM_1 and lost Pep C, whereas in HRB2 PGM_1 and Pep C were present and 6PGD was absent. All other clones were positive for all A1 markers tested.

Chromosome analysis of HRA11 and HRB2 revealed a break in A1 in both clones. In HRA11, a translocation had occurred between the human A1 and the Chinese hamster chromosome No. 1, the break in A1 being located in the interband region below the secondary constriction in the long arm (Fig. 1). In HRB2, the distal part of the short arm of A1 was deleted (Fig. 1). In both clones, a normal A1 chromosome was not found. No chromosome resembling the aberrant chromosomes was found in any of 20 sister clones (8 HRA and 12 HRB) analysed so far.

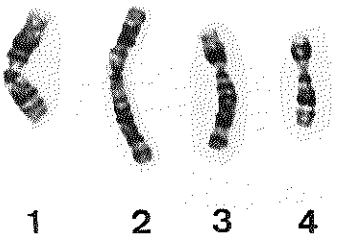

Fig. 1. Giemsa-stained normal and aberrant chromosomes. *1* Human chromosome A1. *2* Chinese hamster chromosome No. 1. *3* Translocation chromosome t (human 1, Chinese hamster 1) from HRA11. *4* Human chromosome 1p— from HRB2

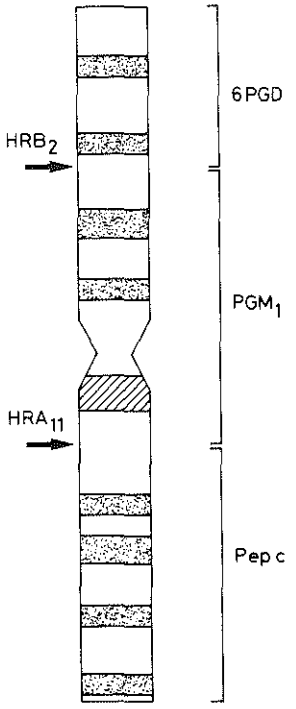

Fig. 2. Diagram of human chromosome A1. Position of breaks in HRA11 and HRB2 and postulated location of markers are indicated. In terms of the Paris nomenclature, the HRA11 and HRB2 breakage points may be localized in the 1q12 and 1p32 regions, respectively

Recloning of HRA11 and HRB2 yielded 5 HRA11 and 10 HRB2 subclones. All showed retention of both the segregation pattern of the A1 markers and the aberrant A1 chromosomes of their parental clone.

Discussion

The results suggest the following gene sequence on chromosome A1: 6PGD-PGM_1-Pep C. Fig. 2 presents the localization of the genes relative to the banding pattern. In combination with the results obtained by Jongsma *et al.* (in press), the localization of PGM_1 can be restricted to the region between the secondary constriction and the HRB2 breakage point. However, the possibility cannot be ruled out that the deletion in the A1 chromosome of HRB2 is not terminal, but interstitial. In the latter case, PGM_1 may be located near the telomere of the short arm. This would be in accordance with the gene order proposed by Robson *et al.* (1973), based on data from family studies.

The subclones are not very informative, since in no case was the aberrant A1 chromosome lost. As reported, complete loss of the human A1 did not occur in any of the HRA and HRB clones and subclones; this suggests the presence of selective powers which promote its retention in this particular hybrid cell line.

Acknowledgements. We are very grateful to Drs. A. Jongsma, P. Meera Khan and A. Westerveld for encouragement and criticism. We also wish to thank Mr. H. M. A. Beijersbergen van Henegouwen and Mrs. M. J. Nijman Custers for their technical assistance. This work was supported in part by the Netherlands Foundation for Medical Research.

Note Added in Proof. Very recently, Douglas *et al.* (1973), using a spontaneous chromosome break in a human-Chinese hamster hybrid cell line, have presented results which suggest the localization of 6PGD and PGM_1 on the most distal quart of the short arm of the human A1 chromosome.

References

Douglas, G. R., Mc Alpine, Ph. J., Hamerton, J. L.: Regional localization of the loci for human PGM1 and 6PGD on human chromosome one using chinese hamster-human somatic cell hybrids. Proc. nat. Acad. Sci. (Wash.) (in press)

Grzeschik, K. H., Allderdice, P. W., Grzeschik, A., Opitz, J. M., Miller, O. J., Siniscalco, M.: Cytological mapping of human X-linked genes by use of somatic cell hybrids involving an X-autosome translocation. Proc. nat. Acad. Sci. (Wash.) **69**, 69—73 (1972a)

Grzeschik, K. H., Romeo, G., Grzeschik, A. M., Banhof, S., Siniscalco, M., van Someren, H., Meera Khan, P., Westerveld, A., Bootsma, D.: X-linkage of human α-galactosidase. Nature (Lond.) New Biol. **240**, 48—50 (1972b)

Jongsma, A. P. M., van Someren, H., Westerveld, A., Hagemeijer, A., Pearson, P. L.: Localization of genes on human chromosomes by using human-Chinese hamster somatic cell hybrids. Assigment of PGM3 to chromosome C6 and mapping of the 6 PGD, PGM_1 and Pep C genes on chromosome A1. Humangenetik (in press)

Meera Khan, P.: Enzyme electrophoresis on cellulose acetate gel: Zymogram patterns in man-mouse and man-Chinese hamster somatic cell hybrids. Arch. Biochem. Biophys. **145**, 470—483 (1971)

Ricciuti, F., Ruddle, F. H.: Assignment of nucleoside phosphorylase to D-14 and localization of X-linked loci in man by somatic cell genetics. Nature (Lond.) New Biol. **241**, 180—182 (1973)

Robson, E. B., Cook, P. J. L., Corney, G., Hopkinson, D. A., Noades, J., Cleghorn, T. E.: Linkage data on Rh, PGM_1, PGD, Peptidase C and Fy from family studies. Ann. hum. Genet. **36**, 393—399 (1973)

Ruddle, F. H.: Linkage analysis in man by somatic cell genetics. Nature (Lond.) **242**, 165—169 (1973)

Ruddle, F. H., Ricciuti, F., McMorris, F. A., Tischfield, J., Creagan, R., Darlington, G., Chen, T.: Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A1 in man. Science **176**, 1429—1431 (1972)

Westerveld, A., Visser, R. P. L. S., Meera Khan, P., Bootsma, D.: Loss of human genetic markers in man-Chinese hamster somatic cell hybrids. Nature (Lond.) New Biol. **234**, 20—24 (1971)

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PAPER II

Identification of Interspecific Translocation Chromosomes in Human-Chinese Hamster Hybrid Cells

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Summary. Chromosome material of human and Chinese hamster origin can be stained differentially in human-Chinese hamster translocation chromosomes by use of the Giemsa-11 staining technique.

Introduction

The Giemsa-11 technique (Bobrow *et al.*, 1972) is a specific staining procedure for several heterochromatical regions in human chromosomes. With the same technique, chromosomes of human and murine origin can be stained differentially in human-mouse hybrid cells (Bobrow and Cross, 1974). In studies on human-Chinese hamster hybrids, differential staining has also been observed for human and Chinese hamster chromosomes (Westerveld *et al.*, in press).

In our laboratory, studies concerning regional mapping of human chromosomes are performed by the use of human-Chinese hamster hybrid cell lines carrying aberrated human chromosomes (Burgerhout *et al.*, 1973, 1974; Jongsma *et al.*, 1973). In these studies, presence or absence of parts of a human chromosome in a hybrid cell line are correlated with presence or absence of the expression of genes which have been assigned to that chromosome. Therefore, recognition of interspecific human-Chinese hamster translocation chromosomes and identification of the human-derived parts are of great importance. If the differential staining properties of the Giemsa-11 technique hold in human-Chinese hamster translocation chromosomes, that technique will be a valuable tool for recognition of human material. The applicability of the Giemsa-11 technique for this aim is investigated in the underlying study.

Two hybrid lines (HRA11 and HRB2L) were selected in which the presence of a translocation involving the human chromosome No. 1 has been detected by the use of a trypsin-Giemsa technique (Fig. 1, upper row). In HRA11, the break in chromosome 1 has occured just below the 1qh region (in band q21 to q23). The remaining part of the translocation chromosome has been identified as a part of the Chinese hamster chromosome No. 1. In the HRB2L translocation chromosome, one arm is similar to the q arm of the human chromosome 1. The origin of the other arm could not be determined with certainty, though the banding pattern of the proximal part is identical with the pattern in the human 1p. The presence of the human heterochromatic 1q region, which is stained brightly red by the

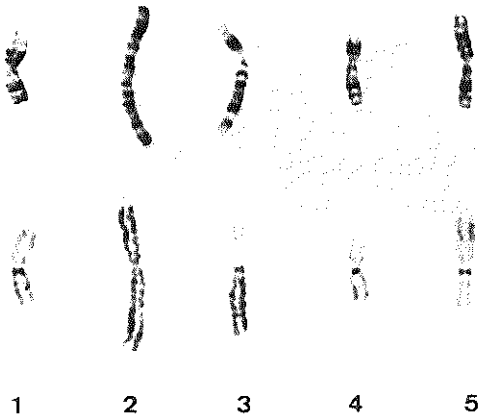

Fig. 1. Chromosomes stained by trypsin-Giemsa (upper row) and Giemsa-11 (lower row) techniques. *1* Human chromosome No. 1. *2* Chinese hamster chromosome No. 1. *3* Human-Chinese hamster translocation chromosome from HRA11. *4* Human 1p— chromosome from HRB2. *5* Human-Chinese hamster translocation chromosome from HRB2L

Giemsa-11 technique, provides a marker for identification in both translocation chromosomes.

Materials and Methods

The human-Chinese hamster hybrid cell lines HRA11 and HRB2 have been described previously (Burgerhout *et al.*, 1973). HRB2 contains a del(1)p32→pter chromosome (Fig. 1). The translocation chromosome in HRB2L, which is a clonal population derived from HRB2, is a *de novo* derivate from the deletion chromosome in HRB2.

Preparation techniques and trypsin-Giemsa staining procedure have been described (Burgerhout *et al.*, 1973). In applying the Giemsa-11 technique, the procedure of Bobrow *et al.* (1972) was followed.

Results

In well-stained slides, human chromosomes are blue, with red dots at sites mentioned by Bobrow *et al.* (1972), while Chinese hamster chromosomes are violet or dark magenta. As a rule, optimal staining time for the red differentiation of human paracentric heterochromatin is a few minutes shorter than the time for optimal differentiation between human and Chinese hamster chromosomes. A suitable compromise must be established empirically for each batch of slides. Using air-dried preparations stored for 3—7 days at room temperature, optimal staining time is usually between 15 and 25 min.

The HRA11 and HRB2L translocation chromosomes could be identified easily by their morphology and the presence of red-staining heterochromatin. Both chromosomes consist of two parts which are stained blue and magenta, respectively; in a black- and-white photograph, the respective areas are displayed as light and dark grey (Fig. 1, lower row). In the HRA11 chromosome, the blue and magenta areas coincide with the parts which have respectively been identified as being human and hamster-derived. In the HRB2L chromosome, the arm bearing the red paracentric region stains blue, as well as the proximal part of the other arm. The distal part of the latter arm stains magenta.

Discussion

The results show that the Giemsa-11 technique can be used as a differential stain for human and Chinese hamster-derived material in interspecific translocation chromosomes. In HRA11, the staining pattern confirms the nature of the translocation chromosome as it was established after trypsin-Giemsa staining. In HRB2L, the pattern confirms the presence of the human 1q arm, while it indicates the human origin of the proximal part of the other arm of the translocation chromosome. Regarding the trypsin-Giemsa banding pattern, this part is likely to be identical with the proximal part of the human 1p arm, the breakpoint being situated in band p32.

The emergence of *de novo* translocations involving human chromosomes in hybrid cell lines is considered to be an important source of exceptional segregation patterns in studies on assignment of genes to human chromosomes (Boone *et al.*, 1972; Jongsma *et al.*, 1973). Application of the Giemsa-11 technique in combination with other staining procedures might facilitate the explanantion of exceptions in terms of translocations, thus converting sources of disturbance into useful material for regional mapping of the genes on the chromosome.

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References

Bobrow, M., Madan, K., Pearson, P. L.: Staining of some specific regions of human chromosomes, particularly the secondary constriction of no. 9. Nature (Lond.) New Biol. **238**, 122—124 (1972)

Bobrow, M., Cross, J.: Differential staining of human and mouse chromosomes in interspecific cell hybrids. Nature (Lond.) **251**, 77—79 (1974)

Boone, C., Chen, T. R., Ruddle, F. H.: Assignment of three human genes to chromosomes (LDH-A to 11, TK to 17, and IDH to 20) and evidence for translocation between human and mouse chromosomes in somatic cell hybrids. Proc. nat. Acad. Sci. (Wash.) **69**, 510—514 (1972)

Burgerhout, W. G., Jongsma, A. P. M., Meera Khan, P.: Regional assignments of seven enzyme loci on the A1 chromosome of man. In: New Haven Conference (1973). First International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. 10, No. 3, pp. 73—75. The National Foundation, 1974

Burgerhout, W. G., van Someren, H., Bootsma, D.: Cytological mapping of the genes assigned to the human A1 chromosome by use of radiation-induced chromosome breakage in a human-Chinese hamster hybrid cell line. Humangenetik **20**, 159—162 (1973)

Jongsma, A. P. M., van Someren, H., Westerveld, A., Hagemeijer, A., Pearson, P. L.: Localization of genes on human chromosomes by using human-Chinese hamster somatic cell hybrids. Assignment of PGM_3 to chromosome C6 and mapping of the 6PGD, PGM_1 and Pep C genes on chromosome A1. Humangenetik **20**, 195—202 (1973)

Westerveld, A., Jongsma, A. P. M., Meera Khan, P., van Someren, H., Bootsma, D.: Assignment of the AK_1:Np:ABO linkage group to chromosome 9. (in press 1975)

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REGIONAL ASSIGNMENT OF SEVEN GENES ON CHROMOSOME 1 OF MAN BY USE OF MAN-CHINESE HAMSTER SOMATIC CELL HYBRIDS

I. RESULTS OBTAINED AFTER HYBRIDIZATION OF HUMAN CELLS CARRYING RECIPROCAL TRANSLOCATIONS INVOLVING CHROMOSOME 1

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ABSTRACT

Regional localization studies on genes coding for human PGD, PPH_1, PGM_1, UGPP, GuK_1, Pep-C and FH which have been assigned to chromosome 1 were performed by using man-Chinese hamster somatic cell hybrids. Informative hybrids which retained fragments of the human chromosome 1 were produced by fusion of hamster cells with human cells carrying reciprocal translocations involving chromosome 1. Analysis of the hybrids which retained one of the translocation chromosomes or de novo rearrangements involving the human 1 revealed the following gene positions: PGD and PPH_1 in 1pter ⟶ 1p32, PGM_1 in 1 p32 ⟶ 1p22, UGPP and GuK_1 in 1q21 ⟶ 1q42, FH in 1qter ⟶ 1q42 and Pep-C probably in 1q42.

INTRODUCTION

Biochemical and cytological analyses of man-rodent cell hybrids have elucidated the localization of a large number of genes to human chromosomes (Ruddle, 1973). Regional localization studies have been performed by use of

hybrid cells carrying broken human chromosomes (Grzeschik, 1972). By integration of such data obtained from hybrid cell lines which have lost different pieces of a particular chromosome a genetic map of that chromosome can be composed.

A prerequisite for such studies is the identification of human chromosomes or chromosome fragments which is possible by using differential staining techniques. The recognition of a particular chromosome or chromosome fragment is facilitated by the presence of polymorphic regions.

The discrimination between chromosomes of human and rodent origin is facilitated by the G-11 technique (Bobrow and Cross, 1974; Westerveld et al., 1976). The observed difference in staining between human and Chinese hamster chromosome material is retained in interspecies translocation chromosomes (Burgerhout, 1975).

In this and an accompanying paper (Burgerhout et al. in press) the regional map of the human chromosome 1 is studied by use of man-Chinese hamster somatic cell hybrids. Chromosome 1 was chosen because of its diversity of properties revealed by different staining procedures and the relatively large number of genes which have already been assigned to it. The chromosome shows a remarkable banding pattern after application of Q- and G-staining procedures. It also has unique regions which can be stained intensively as for instance the distal half of the short arm following T-staining and the heterochromatin region 1qh after C-staining or by applying the G-11 technique. A relatively large number of enzyme markers has been assigned to chromosome 1 using somatic cell hybrids by the direct association of enzyme phenotypes with the presence of human chromosomes as well as by synteny studies including markers which have already been assigned to chromosome 1. The seven enzymes which are studied are 6-phosphogluconate dehydrogenase (PGD : EC 1.1.1.44), phosphoglucomutase 1 (PGM_1 : EC 2.7.5.1) peptidase C (Pep-C : EC 3.4.3-) (van Cong et al., 1971; Westerveld et al., 1972; Ruddle et al., 1972, Hamerton et al., 1972; Jongsma et al., 1973),

phosphopyruvate hydratase 1 (PPH_1, EC 4.2.1.11), guanylate kinase 1 (GuK_1 : EC 2.7.48) (Meera Khan et al., 1974[a]); Meera Khan et al., 1974[b]; Weil et al., 1974), fumarate hydratase (FH : EC 4.2.1.2) and UDPG pyrophosphorylase (UGPP : EC 2.7.7.9) (van Someren et al., 1974).

Inferential assignment of some genes which are not detectable in cultured cells have been performed by combination of both family studies and studies of somatic cell hybrids. The Rh linkage group including PGD (Weitkamp et al., 1971) and PGM_1 (Renwick, 1971) could be assigned to chromosome 1 (Ruddle, 1972) since the latter markers can be studied in both approaches. Evidence for linkage between the Rh and PPH_1 loci placed PPH_1 on chromosome 1 (Giblett, 1975) which has been confirmed by the linkage relationship of PPH_1 and other chromosome 1 markers. In these combined studies the relation between recombination frequency and length expressed in bands as shown in metaphase chromosomes may also be elucidated.

In the present study man-Chinese hamster cell hybrids carrying parts of human chromosomes were produced by fusion of hamster cells with human cells derived from carriers of translocations involving chromosome 1. The second paper (Burgerhout et al., in press) deals with chromosome breaks induced by X-irradiation. De novo break events involving the human chromosome 1 which also occurred in the hybrids were used to extend the genetic map of this chromosome as well.

MATERIALS AND METHODS

Hybrid cell lines were obtained by fusing thymidine kinase (TK : EC 2.7.1.75) deficient hamster a3 cells with human lymphocytes or skin fibroblasts as described elsewhere (Westerveld et al., 1971). Parental human cells were obtained from three carriers of reciprocal translocations involving chromosome 1 (Fig. 1, A, B and C). The white blood cells 1/15 UT have been karyotyped 46, XX, t(1 ;15)(p22; q26) (van Hemel et al., 1975) (Fig. 1A). A primary fibroblast culture 1/17 HE with the karyotype 47, XX, 21+,

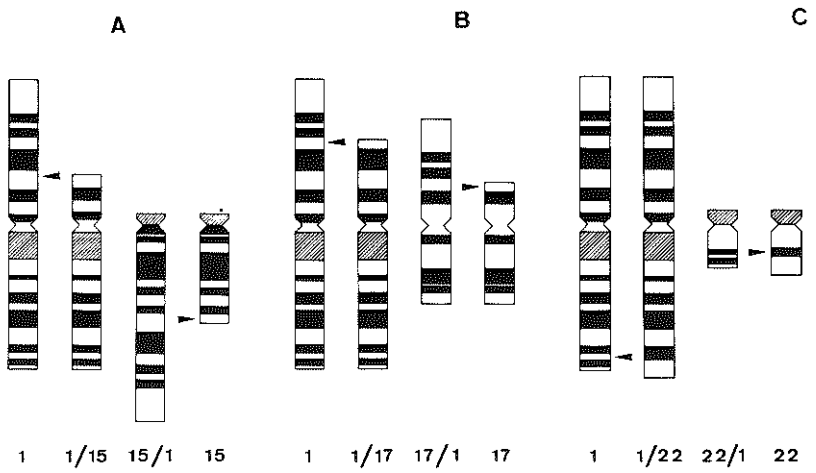

FIG. 1A, B, C

Diagrams of the translocations as observed in the human parental cells 1/15 UT (A), 1/17 HE (B) and 1/22 AM (C) used in hybridization experiments. The positions of the breakpoints are indicated in the normal chromosomes. The nomenclature of the translocation products refers to the chromosomes involved in the translocation event. The chromosome from which the centromere has originated is mentioned first.

t(1; 17)(p32; p31) (de la Chapelle et al., 1975) was used in the second hybridization experiment (Fig. 1B). In the third experiment white blood cells 1/22 AM from a clinically healthy donor carrying a reciprocal translocation involving the chromosomes 1 and 22 were fused with a3 cells. These cells were karyotyped 46, XY, t(1; 22)(q42; q12) (Fig. 1C).

Metaphase preparations were fixed, stained with quinacrine dihydrochloride (Q-banding) and analyzed as described previously (Jongsma et al., 1973). The trypsin-Giemsa technique (Seabright, 1971 with slight modifications of Burgerhout et al., 1973) was also applied to the human parental cells to confirm the position of the breakpoints.

Cellogel electrophoresis was performed on cell lysates to detect the following enzymes: PGD, PPH_1, PGM_1, UGPP, GuK_1, Pep-C and FH. The methods to prepare cell lysates and to characterize these isoenzymes have been described by van Someren et al. (1974[a]) and Meera Khan et al.(1971).

RESULTS

The data from chromosome analysis and enzyme electrophoresis of the man-Chinese hamster hybrid cell lines are presented in the Tables I, II and III. The cell lines are classified according to the presence of human chromosome 1 material. Class I represents hybrid cell lines in which at least one normal chromosome 1 or both translocation chromosomes were detected. Clones in which no chromosome 1 material was observed were assigned in class II. The hybrid character of the class II clones was established by the detection of at least one human chromosome or enzyme marker. Class III represents clones in which only one of the translocation products which were observed in the human parental cells was recognized. Class IV shows clones in which de novo breaks involving chromosome 1 were observed.

1/15 UT x a3 Hybridization Experiment

In the 8 clones belonging to class I all the tested markers were present

TABLE 1

CLASS	N	1	1/15	15/1	T	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH
I	3	+	-	-	-	+	+	+	+	+	+	+
	1	+	+	+	-	+	+	+	+	+	+	+
	2	+	-	+	-	+	+	+	+	+	+	+
	2	-	+	+	-	+	+	+	+	+	+	+
II	6	-	-	-	-	-	-	-	-	-	-	-
III	2	-	+	-	-	-	-	-	+	+	+	+
	2	-	-	+	-	+	+	+	-	-	-	-
IV	1*	-	-	-	S_1	+	+	+	-	-	-	-
	1**	-	-	-	S_2	+	+	+	+	+	+	+

Presence of human chromosome 1 material and enzymes in primary hybrid cell lines produced by hybridization of 1/15 UT x a3 cells (+) = present, (-) = absent, T = de novo translocation product involving chromosome 1. * = Clone 1/15 UT-17, S_1 = del(1)(q21)

** = Clone 1/15 UT-34, S_2 = rea(1 ; ?)(1qcen : : ?)

whereas no marker could be detected in any of the 6 clones of class II (Table I).

Class III shows two clones in which the translocation chromosome 1/15 was present together with the enzymes UGPP, GuK_1, FH and Pep-C while the enzymes PGD, PPH_1 and PGM_1 were absent. The reverse situation is found in the remaining two clones of this class in which 15/1 was observed and only PGD, PPH_1 and PGM_1 could be detected.

In clone 1/15 UT-17 a de novo chromosome 1 aberration del (1)(q21) was identified (S_1)(Fig. 2). In this clone only PGD, PPH_1 and PGM_1 were expressed. In the second clone of this class a marker chromosome S_2 was observed including a part of the short arm of the human 1 (Fig. 2). All chromosome 1 markers were present in this clone.

TABLE II

CLASS	N	1	1/17	17/1	T	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH
I	5	+	-	-	-	+	+	+	+	+	+	+
	3	+	+	-	-	+	+	+	+	+	+	+
	3	+	+	+	-	+	+	+	+	+	+	+
	3	+	-	+	-	+	+	+	+	+	+	+
	3	-	+	+	-	+	+	+	+	+	+	+
	1	-	+	+	-	+	+	+	+	-	+	+
II	11	-	-	-	-	-	-	-	-	-	-	-
	1	-	-	-	-	+	+	+	+	+	+	+
III	2	-	+	-	-	-	-	+	+	+	+	+
	3	-	-	+	-	+	+	-	-	-	-	-
IV	1*	-	-	-	S_3	+	+	+	+	+	-	-

Presence of human chromosome 1 material and enzymes in primary hybrid cell lines produced by hybridization of 1/17 HE x a3 cells.

(+) = present, (-) = absent, T = de novo rearrangement involving chromosome 1.

* = Clone 1/17 HE-21, S_3 = del(1)(1q42).

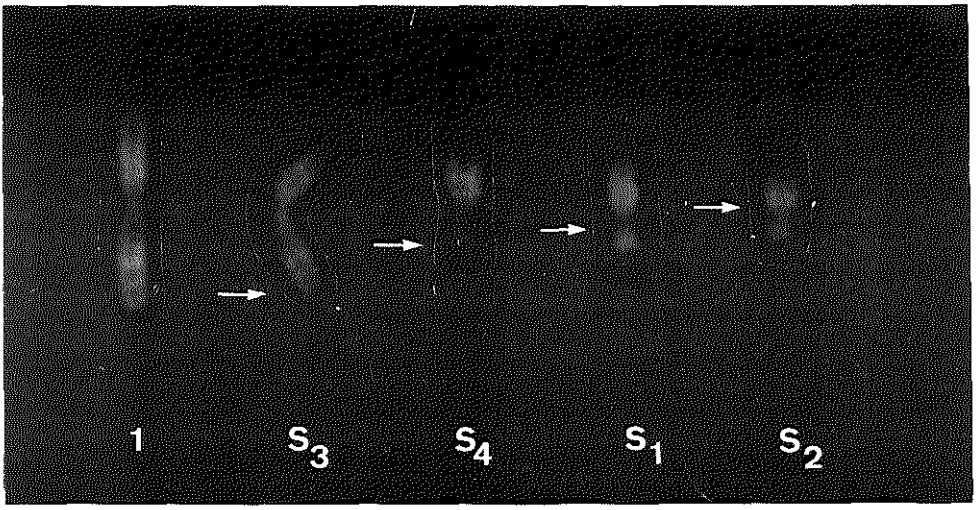

FIG. 2

De novo rearrangements involving the human chromosome 1 observed in the clones 1/15 UT-17 (S_1); 1/15 UT-17 (S_2); 1/17 HE-21 (S_3) and 1/22 AM-15 (S_4) in comparison with a normal human 1.

TABLE III

CLASS	N	1	1/22	22/1	T	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH
I	3	+	-	-	-	+	+	+	+	+	+	+
	1	+	+	-	-	+	+	+	+	+	+	+
	1	+	+	+	-	+	+	+	+	+	+	+
	2	-	+	+	-	+	+	+	+	+	+	+
II	7	-	-	-	-	-	-	-	-	-	-	-
III	1	-	+	-	-	+	+	+	+	+	+	-
	5	-	-	+	-	-	-	-	-	-	-	+
IV	1*	-	-	-	S_4	+	+	+	-	-	-	-

Presence of human chromosome 1 material and enzymes in primary hybrid cell lines produced by hybridization of 1/22 AM x a3 cells. (+) = present; (-) = absent, T = de novo chromosome rearrangement involving chromosome 1.

* = Clone 1/22 AM-15, S_4 = del(1)(q21)

1/17 HE x a3 Hybridization Experiment

Out of the 18 clones of class 1 17 clones expressed all chromosome 1 markers whereas one clone showed all markers except GuK_1 (Table II). In this clone the translocation chromosome 1/17 was observed in only one of 30 analyzed mitoses. Class II includes 11 clones without any of the chromosome 1 markers while in one clone the markers were all present.

In the two clones which retained 1/17 (class III) PGM_1, UGPP, GuK_1, FH and Pep-C were present. The missing chromosome 1 markers PGD and PPH were present in the other three clones of this class in which 17/1 was observed.

A different dissociation pattern of the markers was found in clone 1/17 HE-21 (class IV). In this clone the observed de novo marker chromosome S_3 has been characterized as del (1)(q42) (Fig.2). All chromosome 1 markers except FH and Pep-C were expressed.

1/22 AM x a3 Hybridization Experiment

The seven clones of class I (Table III) retained all chromosome 1 markers whereas the seven clones belonging to class II did not show any activity of the chromosome 1 markers.

Class III presents one clone in which 1/22 was observed as well as PGD, PPH_1, PGM_1, UGPP, GuK_1 and Pep-C. FH, which was not detected in the clone having 1/22, was the only chromosome 1 marker found in the five clones retaining 22/1.

Clone 1/22 AM-15 in class IV contained a newly formed aberrant chromosome S_4 which has been characterized as del (1)(q21). In this clone only human PGD, PPH_1 and PGM_1 were detected.

DISCUSSION

Several procedures have been performed to produce hybrid cells carrying broken human chromosomes: 1) fusion of human cells obtained from individuals carrying reciprocal chromosome translocations (Grzeschik et al., 1972;

Ricciuti and Ruddle, 1973); 2) induction of chromosome breaks by X-irradiation (Burgerhout et al. 1973) and 3) utilization of cell lines in which chromosome breakages occurred during culturing (Boone et al., 1972; Douglas et al. 1973; Jongsma et al., 1973). The first approach has several advantages with respect to identification of the translocation chromosomes which can be performed in the parental human cells before hybridization. In the diploid situation exchange of relatively small parts of chromosome material can be recognized. The identification of these portions is facilitated since the origin is known. Moreover, in cells of a balanced donor all material of the original chromosomes is present in the translocation products. Chromosome pieces originated by de novo rearrangements can only be identified if they are characterized by unique staining properties.

The absence of human chromosome material can only be proven by the detection and recognition of all human material which is present in the hybrid cells. In our opinion, such a proof is still beyond technical possibilities. Though aberrated human chromosomes and man-rodent chromosome rearrangements can be detected in hybrid cells by use of the Giemsa-11 technique (Bobrow et al., 1974; Burgerhout, 1975) this technique may fail to demonstrate small pieces of human chromosome material (Wullems et al., 1976). Consequently, claims of assignment should be based on correlation of the presence and absence of marker enzyme phenotypes with the presence of chromosomes or chromosome parts. If a deleted chromosome has been observed in a hybrid, one can conclude from the absence of markers, which have been assigned to that chromosome, that their loci are not located on the observed chromosome portion. The presence of the other markers does not prove their location on the deleted chromosome since the presence of small pieces of the relevant chromosome can not be excluded.

If cells carrying reciprocal translocation chromosomes are utilized in hybridization experiments, hybrid clones can be expected which are complementary with regard to the presence of translocation chromosomes (Tables I, II & III, class III). Phenotypic markers assigned to the chromosomes which

have been involved in the translocation event, will be absent either in clones having one of the translocation products or in clones having the other one. Thus regional assignment of all phenotypic markers is possible in such experiments. The data presented in the tables also include confirmation of the assignment of the seven studied markers to chromosome 1 (classes I and II).

The segregation pattern of the chromosome 1 markers in the four clones of the 1/15 UT x a3 hybridization experiment carrying only one of the translocation products (class III) places PGD, PPH_1 and PGM_1 distal to the break in 1p22 and UGPP, GuK_1, Pep-C and FH proximal to this break or in the long arm. From the two clones of class IV only clone 1/15 UT-17 is informative since a number of chromosome 1 markers could not be detected. From data of this clone it is concluded that UGPP, GuK_1, Pep-C and FH are not located on the marker chromosome. These loci are thus situated on 1qter $\longrightarrow$ 1q21.

The absence of GuK_1 in the clone derived from the 1/17 HE x a3 hybridization experiment in the presence of the other chromosome 1 markers is probably due to the low frequency of cells which retained chromosome 1/17 (Jongsma et al., 1973; de Wit et al., 1975). The presence of all markers in one clone of class II suggests the presence of chromosome 1 material which could not be detected cytologically. The data from the five clones belonging to class III in this experiment indicate the position of PGD and PPH_1 on 1pter $\longrightarrow$ 1p32 and place the loci for the remaining chromosome 1 markers PGM_1, UGPP, GuK_1, Pep-C and FH on 1qter $\longrightarrow$ 1p32. The absence of Pep-C and FH in the clone carrying a de novo chromosome rearrangement S_2 indicates the position of these loci distal to the breakpoint in 1q42.

The data from the clones isolated from the 1/22 AM x a3 fusion presented in class III provide strong evidence that FH is situated in 1qter $\longrightarrow$ 1q42 while PGD, PPH_1, PGM_1, UGPP, GuK_1 and Pep-C are located in the region 1pter $\longrightarrow$ 1q42.

TABLE IV

N	CHROMOSOME	MISSING 1 MATERIAL	MISSING MARKERS
2	1/17	1pter ⟶ 1p32	PGD, PPH_1
2	1/15	1pter ⟶ 1p22	PGD, PPH_1, PGM_1
5	22/1	1pter ⟶ 1q42	PGD, PPH_1, PGM_1 UGPP, GuK_1, Pep-C
1	S_3	1qter ⟶ 1q42	FH, Pep-C
1	1/22	1qter ⟶ 1q42	FH
1	S_1	1qter ⟶ 1q21	UGPP, GuK_1, Pep-C, FH
1	S_4	1qter ⟶ 1q21	UGPP, GuK_1, Pep-C, FH
2	15/1	1qter ⟶ 1p22	UGPP, GuK_1, Pep-C, FH
3	17/1	1qter ⟶ 1p32	PGM_1, UGPP, GuK_1, Pep-C, FH

Location of human markers on chromosome 1 regions.
The number of independent primary clones in which the indicated marker chromosomes were observed is shown in the first column.

Analyses of clone 1/22 AM-15 which has retained S_4 confirm the assignment of UGPP, GuK_1, Pep-C and FH to 1qter⟶1q21 as was concluded from 1/15 UT-17.

The regional assignments which can be deduced from these data are presented in Table IV. This table shows that PGD and PPH_1 are located in 1pter⟶1p32; PGM_1 is situated within the region 1p32⟶1p22; the positions of UGPP and GuK_1 are in 1q21⟶1q42. Pep-C is situated in 1q42 and the position of FH is in 1qter⟶1q42.

These data are consistent with other data obtained in our laboratory concerning the map of chromosome 1 which will be discussed in the accompanying paper by Burgerhout et al. (in press). The present interpretation of the 1/22 AM data on the position of Pep-C and FH replaces the interpretat-

ion which had been presented at the Baltimore Conference (Burgerhout and Jongsma, 1975) in which the position of Pep-C was claimed to be more distal than the position of FH.

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REFERENCES

Bobrow, M. and Cross, J.: Differential staining of human and mouse chromosomes in interspecific cell hybrids. Nature 251: 77-79 (1974).

Boone, C., Chen, T.R. and Ruddle, F.H.: Assignment of three human genes to chromosomes (LDH-A to 11, Tk to 17 and IDH to 20) and evidence for translocation between human and mouse chromosomes in somatic cell hybrids. Proc. Nat. Acad. Sci., Wash. 69: 510-514 (1972).

Burgerhout, W.: Identification of interspecific translocation chromosomes in human-Chinese hamster hybrid cells. Humangenetik 29: 229-231 (1975).

Burgerhout, W.G. and Jongsma, A.P.M.: The regional map of chromosome 1 of man. In Baltimore Conference (1975): Third International Workshop on Human Gene Mapping. Birth Defects: Original Article Series (The National Foundation, New York) (in press).

Burgerhout, W., Leupe-de Smit, S. and Jongsma, A.: Regional assignment of seven genes on chromosome 1 of man by use of man-Chinese hamster somatic cell hybrids. II. Results obtained after induction of breaks in chromosome 1 by X-irradiation. (in press).

Burgerhout, W.G., Someren, H. van and Bootsma, D.: Cytological mapping of the genes assigned to the human A1 chromosome by use of radiation-induced chromosome breakage in a human-Chinese hamster hybrid cell line. Humangenetik 20: 159-162 (1973).

Chapelle, A. de la., Miller, R.C., Greene, A.E. and Coriell, L.L.: A (1 ; 17) translocation, balanced, plus trisomy 21, 47 chromosomes. Repository identification No. GM-201. Cytogenet. Cellgenet. 14: 82-83 (1975).

Cong, N.V., Billardon, C., Picard, J.Y., Feingold, J. and Frézal, J.: Liaison probable (linkage) entre les locus PGM_1 et Peptidase C chez l'homme. C.R. Acad. Sci. (Paris) 272: 485-487 (1971).

Douglas, G.R., McAlpine, Ph.J. and Hamerton, J.L.: Regional localization of loci for human PGM_1, 6PGD on human chromosome one by use of hybrids of Chinese-hamster human somatic cell hybrids. Proc.Nat. Acad. Sci. Wash. 70 : 2737-2740 (1973).

Giblett, E.R., Chen, S.-H., Anderson, J.E. and Lewis, M.: A family study suggesting genetic linkage of phosphopyruvate hydratase (enolase) to the Rh bloodgroup system. In New Haven Conference (1973): First International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. 10, no. 3, p91-93. (The National Foundation, New York, 1974).

Grzeschik, K.-H, Allderdice, P.W., Grzeschik, A., Opitz, J.M., Miller, O.J. and Siniscalco, M.: Cytological mapping of human X-linked genes by use of somatic cell hybrids involving an X-autosome translocation. Proc. Nat. Acad. Sci. Wash. 69: 69-73 (1972).

Hamerton, J.L., Gee, P.A., Richardson, B.J., Douglas, G.R. and Ray, M.: Further evidence on the assignment of the 6-phosphogluconate dehydro-

genase (6PGD) and phosphoglucomutase-1 (PGM_1) syntenic group in the human genome. Genetics 71(suppl.): 23-24 (1972).

Hemel, J.O. van, Biervliet, J.P. van and Jager-van der Grift, P.W. de: A girl with 46, XX, t(1 ; 15) karyotype. Cytogenetic and clinical observations. Clin. Genet. 8: 213-217 (1975).

Jongsma, A., Someren, H. van, Westerveld, A., Hagemeijer, A. and Pearson, P.: Localization of genes on human chromosomes by studies of human-Chinese hamster somatic cell hybrids. Assignment of PGM_3 to chromosome C6 and regional mapping of the PGD, PGM_1 and Pep-C genes on chromosome A1. Humangenetik 20: 195-202 (1973).

Meera Khan, P.: Enzyme electrophoresis on cellulose acetate gel: Zymogram patterns in man-mouse and man-Chinese hamster somatic cell hybrids. Arch. Biochem. Biophys. 145: 470-483 (1971).

Meera Khan, P., Doppert, B.A., Hagemeijer, A. and Westerveld, A.: The human loci for phosphopyruvate hydratase and guanylate kinase are syntenic with the PGD-PGM_1 linkage group in man-Chinese hamster somatic cell hybrids. In New Haven Conference (1973): First International Workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. 10 (3): 130-132. (The National Foundation, New York 1974[a]).

Meera Khan, P., Los, W.R.T., Pearson, P.L., Westerveld, A. and Bootsma, D.: Genetical studies on the multiple forms of human guanylate kinase in man-Chinese hamster somatic cell hybrids. Human Heredity 24: 415-423 (1974[b]).

Renwick, J.H.: The Rhesus syntenic group in man. Nature, Lond. 234: 475-476 (1971).

Ricciuti, F.C. and Ruddle, F.H.: Assignment of three gene loci (PGK, HGPRT, G6PD) to the long arm of the human X-chromosome by somatic cell genetics. Genetics 74: 661-678 (1973).

Robson, E.B., Cook, P.J.L., Corney, G., Hopkinson, D.A., Noades, J. and Cleghorn, T.E.: Linkage data on Rh, PGM_1, PGD, Peptidase-C

and Fy from family studies. Ann. Hum. Genet. (Lond.) 36: 393-399 (1973).

Ruddle, F.H., Ricciuti, F., Mc Morris, F.A., Tischfield, J., Creagan, R., Darlington, G. and Chen, T.: Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A-1 in man. Science 176: 1429-1431 (1972).

Ruddle, F.H.: Linkage analysis in man by somatic cell genetics. Nature 242: 165-168 (1973).

Seabright, M.: A rapid banding technique for human chromosomes. The Lancet, 30: 971-972 (1971).

Someren, H. van, Beijersbergen van Henegouwen, H., Loss, W., Wurzer-Figurelli, E., Doppert, B., Vervloet, M. and Meera Khan, P.: Enzyme electrophoresis on cellulose acetate gel. II. Zymogram patterns in man-Chinese hamster somatic cell hybrids. Humangenetik 25: 189-201 (1974[a]).

Someren, H. van, Beijerbergen van Henegouwen, H., Westerveld, A., and Bootsma, D.: Synteny of the human loci for fumarate hydratase and UDPG pyrophosphorylase with chromosome 1 markers in somatic cell hybrids. Cytogenet. Cell Genet. 13: 551-557 (1974).

Weil, D., Cong, N.V., Billardon, C., Rebourcet, R., Blanchard, M.-G. and Frézal, J.: Synténie de l'énolase (PPH) et de marqueurs assignés au chromosome 1. (Confirmation par l'étude d'hybrids cellulaires homme-hamster et homme-souris). Ann. Génét. 17: 55-57 (1974).

Weitkamp, L.R., Guttormsen, S.A. and Greendyke, R.M.: Genetic linkage between a locus for 6-PGD and the Rh locus: Evaluation of possible heterogeneity in the recombination fraction between sexes and among families. Am. J. Hum. Genet. 23: 462-471 (1971).

Westerveld, A., Jongsma, A.P.M., Meera Khan, P., van Someren, H. and Bootsma, D.: Assignment of the Ak_1 : Np : ABO linkage group to human chromosome 9. Proc. Nat. Acad. Sci. Wash. 73: 895-899 (1976).

Westerveld, A. and Meera Khan, P.: Evidence for linkage between human loci for 6-phosphogluconate dehydrogenase and phosphoglucomutase 1

in man-Chinese hamster somatic cell hybrids. Nature, Lond. 236: 30-32 (1972).

Westerveld, A., Meera Khan, P., Visser, R.P.L.S. and Bootsma, D.: Loss of human genetic markers in man-Chinese hamster somatic cell hybrids. Nature New Biol. 234: 20-24 (1971).

Wit, J. de, Bootsma, D., Pearson, P.L. and Westerveld, A. : Regional mapping of the human no. 1 and X chromosome in interspecific cell hybrids using an X/1 translocation. Cytogenet. Cell Genet. 15: 129-137 (1975).

Wullems, G.J., Horst, J. van der and Bootsma, D.: Expression of human hypoxanthine phosphoribosyl transferase in Chinese hamster cells treated with isolated human chromosomes. Somat. Cell Genet. 2: 155-164 (1976).

PAPER IV

REGIONAL ASSIGNMENT OF SEVEN GENES ON CHROMOSOME 1 OF MAN BY USE OF MAN-CHINESE HAMSTER SOMATIC CELL HYBRIDS

II. RESULTS OBTAINED AFTER INDUCTION OF BREAKS IN CHROMOSOME 1 BY X-IRRADIATION

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ABSTRACT

The position of genes coding for PGD, PPH_1, UGPP, GuK_1, Pep-C and FH on the human chromosome 1 was investigated by analysis of karyotype and enzyme phenotypes in man-Chinese hamster somatic cell hybrids carrying aberrations involving chromosome 1.
Suitable hybrid cell lines were obtained by X-irradiation of hybrid cells carrying an intact chromosome 1 and by fusion of human cells from a clonal population carrying a translocation involving chromosome 1 with Chinese hamster cells. The latter human cell population had been isolated following X-irradiation of primary Lesch-Nyhan fibroblasts. In addition, products of de novo chromosome breakage in the investigated hybrid lines were utilized. By integrating the results of these analyses with earlier findings in our laboratory, the following positions of genes are deduced: PGD and PPH_1 in 1p36 ⟶ 1p34; PGM_1 in 1p32; UGPP in 1q21 ⟶ 1q23; GuK_1 in 1q31 ⟶ 1q42; Pep-C in 1q42; FH in 1qter ⟶ 1q42.

INTRODUCTION

Simultaneous analyses of karyotype and human phenotypes on human-rodent hybrid cell lines carrying a partially deleted human chromosome can yield information on the position of genes on that chromosome relative to the break point (Grzeschik et al., 1972). Integration of data obtained following different breakage events in a particular chromosome leads to a cytological map of that chromosome (Burgerhout et al., 1973).

This paper and a preceding one (Jongsma et al., in press) are concerned with the position of seven genes on chromosome 1 of man. In the preceding paper data were presented which were obtained by analysis of hybrids produced by fusion of cells from human carriers of reciprocal translocations involving chromosome 1 with Chinese hamster cells. The present paper deals with data from cell lines in which breaks in chromosome 1 were induced by X-irradiation. For this purpose cells were irradiated with 600 rad of 250kV X-rays. Two experiments were performed; these will be referred to as Exp. 1 and Exp. 2.

In Exp. 1, human-Chinese hamster hybrid cells which were known to carry an intact human chromosome 1 were irradiated and subsequently cloned. The clones were analyzed for the presence of human chromosome 1 material and for the expression of genes assigned to chromosome 1. A preliminary report on this experiment has been published earlier (Burgerhout et al., 1973).

In Exp. 2, human Lesch-Nyhan fibroblasts were irradiated and subsequently cloned. Karyotyping revealed the presence of a translocation involving chromosome 1 in one clone.

Cells of this clone were fused with Chinese hamster cells. Hybrid clones were analyzed for relevant chromosomes and gene products.

In both experiments, products of de novo breaks in chromosome 1 were observed in hybrid cells. Several of these additional breaks were utilized for gene mapping purposes.

MATERIALS AND METHODS

In Exp. 1 a hybrid cell line was used which had been isolated after fusion of cytologically normal human male lymphocytes with hypoxanthine phosphoribosyl transferase (HPRT, EC 2.4.2.8) -deficient Chinese hamster cells of the Wg3-h line. Chromosome analysis had revealed the retention of the human chromosomes 1,8, 11,20,21,22, X and Y in this line.

Exp. 2 was performed using human diploid primary HPRT-deficient fibroblasts obtained from a patient suffering from the Lesch-Nyhan syndrome (cell line 170LAD). Cells of the clone RIM9 derived from this strain after irradiation were fused with thymidine kinase (TK; EC 2.7.1.75)-deficient Chinese hamster cells of the a3 line. Cloning of irradiated primary fibroblasts was performed on a feeder layer of homologous cells. Characteristics of Chinese hamster parental cell lines and procedures of cell culture and production of hybrids have been published elsewhere (Westerveld et al., 1971).

Equipment and procedure for irradiation have been described in a previous publication (Burgerhout et al., 1973).

Cellogel electrophoresis was performed on lysates from parental and hybrid cell lines to characterize the following enzymes: 6-phosphogluconate dehydrogenase (PGD; EC 1.1.1.44), phosphoglucomutase 1 (PGM_1; EC 2.7.5.1), peptidase C (Pep-C; EC 3.4.3.-), phosphopyruvate hydratase 1 or enolase 1 (PPH_1; EC 4.2.1.11), UDPG pyrophosphorylase (UGPP; EC 2.7.7.9); guanylate kinase 1 (GuK_1; EC 2.7.4.8) and fumarate hydratase (FH; EC 4.2.1.2). Techniques of electrophoresis and preparation of cell lysates have been described by Meera Khan (1971) and Van Someren et al.(1974).

For characterization of aberrant chromosomes the following differential staining techniques were used: trypsin-Giemsa (Burgerhout et. al., 1973, modified after Seabright, 1971); atebrin fluorescence (Jongsma et al., 1973); R banding (Dutrillaux et al., 1971), T banding (Dutrillaux, 1973) and Giemsa-11 (Bobrow et al., 1972). Chromosome preparations were made as described previously (Burgerhout et al., 1973).Routine chromosome analyses of hybrid lines were done on trypsin-Giemsa stained slides. At least 20 metaphases were analyzed from each clone. The Chinese hamster chromosome nomenclature used in this publication is based on the proposal made by the committee for chromosome markers at the Third International Workshop on Human Gene Mapping in Baltimore, 1975. The nomenclature concerning man-Chinese hamster interspecific translocation chromosomes is in accordance with the Paris Conference (1971), supplement (1975).

RESULTS

EXPERIMENT NO. 1

Thirty-seven clones have been isolated from irradiated hybrid cell populations (Burgerhout et al., 1973). Chromosome analysis revealed the presence of an aberrant human chromosome 1 in two of these clones. In clone HRA11 a human-Chinese hamster translocation chromosome rea (HSA. + CGR.) (1; 1) (1pter ⟶ 1q21, 22 or 23 :: 1q22 ⟶ 1qter) was present. Clone HRB2 carried a human del (1)(p32) terminal deletion chromosome. No other human chromosome 1 material was found in either clone. A cytological description of the translocation chromosomes has been published earlier (Burgerhout et al., 1973; Burgerhout, 1975).

Isoenzyme analysis showed segregation of human chromosome 1 markers in both clones. In HRA11, UGPP, GuK_1, Pep-C and FH had been lost, while PGD, PPH_1 and PGM_1 were retained. In HRB2, absence of PGD and PPH_1 was observed together with presence of PGM_1, UGPP, Pep-C, GuK_1 and FH.

Both HRA11 and HRB2 were recloned, respectively yielding 5 and 11 subclones. With exception of one HRB2 subclone (HRB2L), all clones showed retention of both the aberrant chromosome and the pattern of gene segregation of their parental clone.

Cytological investigation of HRB2L revealed the presence of two populations of cells. In about 40% of the metaphases, the original del (1)(p32) chromosome was found, while in the remaining ones a de novo translocation chromosome was detected. The two chromosomes were never found together in one mitosis. By applying the Giemsa-11 technique in

combination with trypsin-Giemsa staining, the de novo rearrangement could be identified as a man-Chinese hamster translocation, rea (HSA.+CGR.)(1;?) (qter⟶ p32,?) (Burgerhout, 1975). The segregation of chromosome 1 marker genes in this clone followed the pattern of HRB2. Re-cloning of HRB2L yielded 19 subclones. All these clones were found to have retained the de novo translocation chromosome, while the original del(1) chromosome had been lost. In every clone, human PGD, PPH_1 and PGM_1 were found to be absent, while UGPP, GuK_1, Pep-C and FH were present.

EXPERIMENT NO. 2

Fig. 1 shows a karyogram of the clone RIM9 which was isolated from a population of irradiated 170LAD fibroblasts. This karyogram was found consistently in all 30 metaphases of RIM9 cells which were analyzed. Aberrations are present involving one of the homologues of chromosomes no. 1, 3 and 11, respectively. The karyotype suggests a translocation of the 3p23 ⟶3pter segment onto band p36 of chromosome 1. Reciprocity could neither be proved nor excluded. Dimensions and banding pattern of the aberrant chromosome 11 do not indicate the presence of chromosome 1 material. The 1p+ and 3p- chromosomes will be referred to as T1 and T2, respectively.

Fusion of RIM9 cells with Chinese hamster a3 cells yielded 24 primary hybrid clones (R9A3 clones). All clones were investigated for the presence of human chromosome 1 marker genes and of the RIM9 chromosomes 1, T1 and T2. The results are listed in Table 1. In one clone, T1 was present while the normal no. 1 and T2 were absent; in this clone, all seven markers were expressed. The two clones carrying T2 did not express any chromosome 1 marker.

Fig. 1. Karyogram of the RIM9 cell line. Arrows indicate chromosome aberration sites. Trypsin-Giemsa staining.

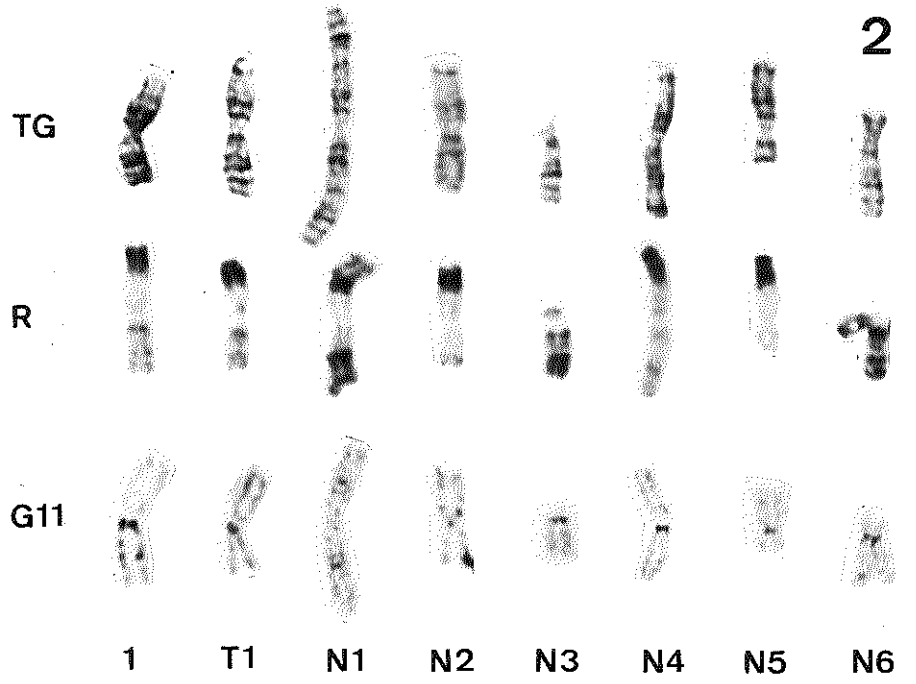

Fig. 2. Rearrangements involving chromosome 1 in R9A3 hybrid lines. The chromosomes are described in the text and in Table 3. Respective staining procedures: TG = trypsin-Giemsa, R = reversed banding, G11 = Giemsa-11 staining.

Table 1. Presence of human enzyme phenotypes and relevant chromosomes in primary clones of R9A3 hybrids

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	1	T1	T2
2	+	+	+	+	+	+	+	+	-	-
1	+	+	+	+	+	+	+	-	+	-
2	-	-	-	-	-	-	-	-	-	+
6	-	-	-	-	-	-	-	-	-	-
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	-	-	-	-	-	-	-
1	-	-	+	+	+	+	+	-	-	-
1	-	-	-	+	+	+	+	-	-	-
1	-	-	-	+	+	-	+	-	-	-
1	-	-	-	-	+	-	-	-	-	-

Thirteen clones expressed chromosome 1 markers while none of the chromosomes 1, T1 and T2 was detected. Karyotype analysis has revealed the presence of human chromosome 1 material in four of these clones (Table 2A). The de novo rearranged chromosomes carrying human No. 1 material will be refered to as N chromosomes. The clones R9A3/4 (chromosome N1) and R9A3/12 (N4) each carried one human 1- derived de novo rearranged chromosome, whereas in the clones R9A3/5 (N2, N3) and R9A3/31 (N5, N6) two chromosomes of this kind were detected. Photographs of all de novo chromosomes as stained by different techniques are shown in Fig. 2; cytological descriptions are given in Table 2B. Regarding the banding patterns, the chromosomes N2, N4 and N5 can be regarded as derived from T1. The descriptions follow this interpretation.

Recloning was carried out with cells of the one clone carrying T1 (R9A3/34) and of the clones carrying de novo chromosome 1 aberrations. Secondary clones were analyzed for the presence of relevant chromosomes and enzyme phenotypes. The results are presented in Table 3. No additional aberration involving chromosome 1 material was detected in

Table 2A. Enzyme phenotypes of clones carrying *de novo* chromosome 1 aberrations

Clone	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH
R9A3/4	-	-	+	+	+	+	+
R9A3/5	+	+	+	+	+	+	+
R9A3/12	+	+	+	-	-	-	-
R9A3/31	+	+	+	+	+	+	+

Table 2B. De novo aberration chromosomes detected in R9A3 hybrid clones

Clone	chromosome code	chromosome description	deleted chromosome 1 material
R9A3/4	N1	i inv(1)(q11q21) inv(1)(p34q11) del(1)(p34)	pter→p34
R9A3/5	N2	rea(1;3;6)(3pter→3p23::1p36→cen→6qter)	qter→cen
R9A3/5	N3	rea(1;?)(qter → cen → ?)	p36 →cen
R9A3/12	N4	rea(1;3;5)(3pter→3p23::1p36→1q12 or 21::5q11 → 5qter)	qter→q12 or 21
R9A3/31	N5	rea(1;3;?)(3pter→3p23::1p36→cen→?)	qter→cen
R9A3/31	N6	rea(1;?)(qter→cen→?)	p36 →cen

The question mark indicates the involvement of human chromosome material of unknown origin in rearrangements

any secondary clone. In each recloning experiment except the one involving R9A3/5 retention or loss of a particular human 1- derived chromosome is associated with retention or loss of a particular set of human chromosome 1 marker genes. In R9A3/5 subclones (Table 3C), loss of chromosome N3 is associated with three different phenotypical classes: one clone has lost $\underline{GuK_1}$ only; two clones have lost both UGPP and $\underline{GuK_1}$; one clone has lost UGPP, $\underline{GuK_1}$, Pep-C and FH. The only subclone in which neither N2 nor N3 has been detected shows loss of all chromosome 1 markers.

Table 3A. R9A3/34 SUBCLONES

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	T1
5	+	+	+	+	+	+	+	+
2	-	-	-	-	-	-	-	-

Table 3B. R9A3/4 SUBCLONES

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	N1
11	-	-	+	+	+	+	+	+
1	-	-	-	-	-	-	-	-

Table 3C. R9A3/5 SUBCLONES

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	N2	N3
7	+	+	+	+	+	+	+	+	+
1	+	+	+	+	-	+	+	+	-
2	+	+	+	-	-	+	+	+	-
1	+	+	+	-	-	-	-	+	-
1	-	-	-	-	-	-	-	-	-

Table 3D. R9A3/12 SUBCLONES

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	N4
8	+	+	+	-	-	-	-	+
2	-	-	-	-	-	-	-	-

Table 3E. R9A3/31 SUBCLONES

No. of clones	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH	N5	N6
5	+	+	+	+	+	+	+	+	+
5	-	-	-	+	+	+	+	-	+
1	-	-	-	-	-	-	-	-	-

Table 3. Segregation patterns in secondary R9A3 clones. T1 and N1-6 indicate aberrant human No. 1 chromosomes (see text and Table 2).

DISCUSSION

If only a part of a particular chromosome has been detected in a hybrid line, the absence of a gene which has been assigned to that chromosome indicates its location on the part which has not been detected. As present techniques of chromosome identification do not allow detection of small parts of a particular chromosome in every case, a claim of regional assignment based on simultaneous retention of a gene and of part of a chromosome is less convincing. The claim can be reinforced, however, by observation of simultaneous loss of the gene and the aberrant chromosome in secondary clones.

In Exp. 1, all secondary clones derived from HRA11 and HRB2 (with exception of the mixed population of HRB2L) showed the pattern of presence of chromosome 1 material and marker segregation which had been observed in the parental populations. Regarding these patterns, HRA11 yields a strong indication for the assignment of UGPP, GuK_1, Pep-C and FH to 1qter⟶1q21 and a weaker one for the assignment of PGD, PPH_1 and PGM_1 to 1pter⟶1q23, whereas HRB2 yields assignments of the same respective strength for PGD and PPH_1 to 1pter⟶1p32 and for PGM_1, UGPP, GuK_1, Pep-C and FH to 1qter⟶1p32. In HRB2L subclones, the correlation between absence of human PGM_1 and presence of a de novo rea (HSA.+CGR.)(1;?)(qter⟶p32;?) chromosome which must have been derived from the del(1)(p32) chromosome in HRB2 gives a strong indication for the location of PGM_1 in 1p32. The absence of human PGM_1 activity may be caused either by loss of the PGM_1 locus following a secondary break in 1p32 or by inactivation of the gene caused by the proximity of Chinese hamster chromosome material (Hamerton et al., 1975). With regard to the

other human chromosome 1 markers, the HRB2L subclones do not provide new independant information apart from their position relative to $\underline{PGM}_1$.

In Exp. 2, the enzyme phenotypes of the three primary clones carrying a parental translocation chromosome (T1 or T2) suggest that all seven markers are located proximal to the supposed break point in 1p36 (Table 1). Based on analysis of primary clones only, this claim is a weak one because of the lack of evidence for reciprocity of the (1;3) translocation and the existence of 13 other primary clones which do not carry a complete chromosome 1, T1 or T2, but do express human chromosome 1 markers. However, reinforcement is provided by data obtained after recloning of the T1-carrying clone R9A3/34 (Table 3A) and of the R9A3/clones 5, 12 and 31 which carry a T1-derived de novo aberration (Table 3C, D and E).

The gene segregation patterns in R9A3/4 and 12 (Table 2) indicate that PGD and $\underline{PPH}_1$ are not located on N1, whereas UGPP, $\underline{GuK}_1$, Pep-C and FH are not located on N4. The patterns in secondary clones derived from R9A3/4 and 12 (Table 3B and D) are in accordance with these indications; moreover, the absence of any chromosome 1 enzyme marker in clones having lost either N1 or N4 suggest the respective assignments of $\underline{PGM}_1$, UGPP, $\underline{GuK}_1$, Pep-C and FH to N1 and of PGD, $\underline{PPH}_1$ and $\underline{PGM}_1$ to N4.

The data from R9A3/31 secondary clones (Table 3E) indicate that PGD, $\underline{PPH}_1$ and $\underline{PGM}_1$ are not located on N6. In addition, the absence of any enzyme marker in a clone which has lost both N5 and N6 suggest the assignment of UGPP, $\underline{GuK}_1$, Pep-C and FH to N6.

Table 4. Regional assignments based on data presented in this publication

Cell line	Segment of chromosome 1	Genes located on the segment
HRA11	qter ⟶ q21	UGPP, GuK_1, Pep-C, FH
HRB2	pter ⟶ p32	PGD, PPH_1
HRB2L	pter ⟶ p32	PGD, PPH_1, PGM_1
RIM9	qter ⟶ p36	All markers
R9A3/4	pter ⟶ p34	PGD, PPH_1
	qter ⟶ p34	PGM_1, UGPP, GuK_1, Pep-C, FH
R9A3/5	qter ⟶ cen	UGPP, GuK_1, Pep-C, FH
R9A3/12	p36 ⟶ q21	PGD, PPH_1, PGM_1
	qter ⟶ q12	UGPP, GuK_1, Pep-C, FH
R9A3/31	p36 ⟶ cen	PGD, PPH_1, PGM_1
	qter ⟶ cen	UGPP, GuK_1, Pep-C, FH

With regard to R9A3/5 subclones (Table 3C), the absence of UGPP, GuK_1, Pep-C and FH in a clone carrying N2 indicates that these markers are not located on N2. Though all chromosome 1 markers are absent in one clone in which neither N2 nor N3 was detected, the existence of three different phenotypical classes of clones which had lost N3 while N2 was retained, does not justify any positive assignment to either N2 or N3.

A summary of all regional assignments which can be deduced from data presented in this paper is given in Table 4.

By integrating the data presented here with earlier results obtained in our laboratory (Jongsma et al., 1973; Burgerhout et al., 1974; De Wit et al., 1975; Jongsma et al., in press) a map of chromosome 1 can be composed (Fig. 3), which is based on segregation patterns following 17 different breakage events. All data are mutually concordant. The relative position of different break points in the bands p32 and q42 and in the q21 ⟶ q23 region as indicated in Fig. 3 is not based on cytological evidence but reflects the respective segregation patterns

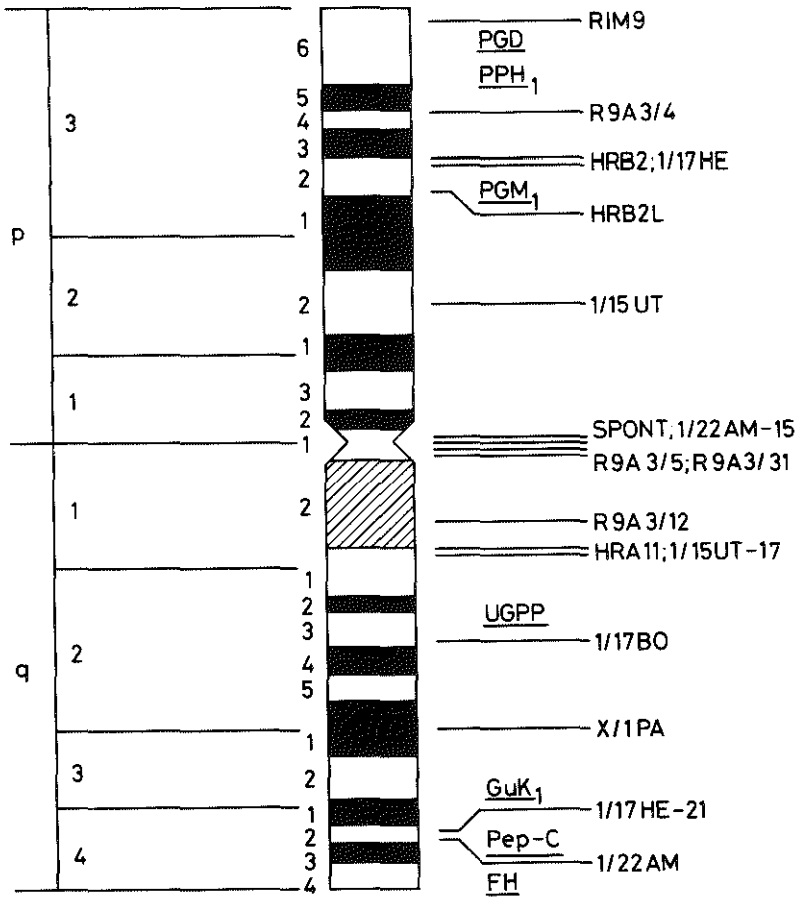

Fig. 3. Regional map of chromosome 1. The positions of the genes relative to the break points are indicated. The breaks are labeled by the names of the cell lines in which they have been detected.

involving PGM_1, Pep-C and UGPP. Of the three breaks located in p32, the ones in 1/17HE and HRB2 yielded a segregation of PGM_1 with UGPP, GuK_1, Pep-C and FH, whereas in HRB2L, PGM_1 segregates with PGD and PPH_1. Analogous situations exist in q21⟶q23 with regard to the chromosome breaks in HRA11, 1/15UT-17 and 1/17BO and in q42 with regard to those in 1/22AM and 1/17HE-21. As we were

not able to locate breaks within q21⟶q23 in a single band, the three patterns involved assign UGPP to that region. The positions of the seven loci are thus as follows: PGD and PPH_1 in 1p34⟶1p36; PGM_1 in 1p32; UGPP in 1q21⟶1q23; GuK_1 in 1q31⟶1q42; Pep-C in 1q42; FH in 1qter⟶1q42.

Studies on man-rodent hybrid cells by other investigators (Douglas et al., 1973; McAlpine et al., 1974; Grzeschik, 1975; Shows & Brown, 1975) concerning the map of chromosome 1 have yielded data on all markers investigated by us except UGPP and GuK_1. The localization of PGM_1 in 1pter ⟶1p33 by Douglas et al. is discordant with our results and with those obtained by Grzeschik. This discordance may be caused by inaccurate establishment of breakpoints in the 1p32-33 region in any study, though a majority of evidence is in favor of localizing PGM_1 proximal to 1p33. All other data are mutually concordant. They can be summarized as follows: PGD and PPH_1 in 1pter⟶1p32; PGM_1 in 1p21⟶1p32; Pep-C in 1qter⟶1q41; FH in 1qter⟶ 1p21. These localizations are in agreement with our data.

As gene polymorphism has been detected for PGD, PPH_1, PGM_1 and Pep-C in human populations, these loci have been investigated in linkage studies. PGD, PPH_1 and PGM_1 have been found to be linked to the Rhesus blood group locus (Rh) (Robson et al., 1973; Giblett et al., 1974). A recently published compiled linkage map comprising Rh, PGD, PPH_1 and PGM_1 (Hamerton & Cook, 1975) gives PGD/PPH_1 - $Rh-PGM_1$ as the most probable gene order. Considering our data, this linkage group can be localized in the 1pter⟶ 1p32 region, PGM_1 being closest to the centromere. This polarity is also suggested by deletion mapping data of Marsh et al. (1974) which place PGM_1 and Rh respectively proximal and distal to a break point in the region 1p32⟶ 1p34.

A first and crude attempt can be made to compare distances between loci as inferred from linkage studies and from somatic cell hybrids. Hamerton & Cook (1975) give maximal lod scores for combined male and female recombination frequencies of 22 between PGD and Rh and of 35 between Rh and PGM_1. This results in a PGD-PGM_1 distance of 57 centiMorgan. As can be seen in Fig. 3 of the present paper, this distance equals a segment of the metaphase chromosome of at least the length of band p33 and at most the length of the whole 1pter⟶p32 part. More studies on hybrids carrying breaks in the distal half of chromosome 1 and refinement of cytogenetical techniques will be needed to make a more exact estimate of the distance.

Investigations on the mechanisms of differential chromosome staining have revealed that Q and G bands are associated with the presence of DNA which is relatively rich in AT base pairs (De la Chapelle et al., 1973) and in repetitions sequences (Sanchez & Yunis, 1974). As these qualities are believed to be characteristics of genetically inactive DNA (Comings, 1972) one may expect a relative accumulation of genes in the R bands. This hypothesis is supported by Hoehn (1975) in a survey on the involvement of specific chromosome material in duplications and deletions which are compatible with human life. Hoehn found the chance of a chromosome or chromosome segment being involved in an inbalance to be related to its amount of R band material rather than to its total amount of DNA. Assuming an imbalance to be more harmful as more genetically relevant information is involved, one may conclude that this information is principally present in R bands.

According to this theory, it may be expected that if regional mapping studies lead to the localization of a gene in a "single" band of a chromosome, the chance of this band for being an R band will be greater than the

40% which has been claimed to be the relative amount of R band material in the human genome (Holmberg & Jonasson, 1973).

In our investigations $\underline{PGM_1}$ and Pep-C have been assigned to the respective R bands 1p32 and 1q42. More regional assignments of genes to one band have been presented at the Second and Third Conferences on Human Gene Mapping (1975; 1976): Uridine Monophosphate kinase (UMPK) to 1p32; Acid Phosphatase-1 ($\underline{ACP_1}$) to 2p23 (Hamerton et al., 1975); Peptidase-B (Pep-B) to 12q21; Haptoglobin-α ($\underline{\alpha\text{-Hp}}$) to 16q22; Peptidase-A (Pep-A) to 18q23 (Arthur et al., 1975). This makes up a total of six genes located in R bands, whereas one locus (Pep-B) has been assigned to a Q/G band. However, it must be considered that (1) all these assignments have a provisional status, (2) the chance of making an assignment to a Q/B band is reduced if an excess of breaks in R bands of relevant chromosomes is present as was the case in the cell material used in this study and (3) breaks in Q/G bands may be misinterpreted as breaks in R bands if only Q and G banding techniques are used to localize a break point (San Roman & Bobrow, 1973).

Regarding the human chromosome complements of the primary clones in Exp. 2 of the present study, the probable occurrence of de novo aberrations involving chromosome 1 in 13 out of 24 clones is striking. As far as aberrations were detected cytologically, the abnormal chromosomes were present in a majority of mitoses of each primary clone. None of the seven de novo aberration chromosomes carries detectable Chinese hamster material. These observations suggest a frequent occurrence of chromosome breakage at or shortly after cell fusion, principally

affecting the human complement. A possible mechanism of this process is the phenomenon of "fragmentation" of chromosomes during premature condensation of S phase chromatin in multinuclear cells (Johnson & Rao, 1970). Premature chromosome condensation (PCC) has been observed to occur in chromatin of an interphase nucleus in a multinuclear cell if another nucleus in the same cell enters mitosis. Since the mitotic cycle of Chinese hamster a3 cells has a duration of 10 to 14 hours (Westerveld, pers. comm.), whereas proliferating human fibroblasts have a cycle of at least 16-18 h (Maciera-Coelho et al., 1966), the a3 nucleus in man-Chinese hamster binuclear cells can be expected to enter mitosis first in a majority of cases, thus causing PCC of human chromatin. The fused RIM9 cells had a long population doubling time (1-2 weeks), probably due to radiation damage and long subsequent culturing. If this reflects a prolonged cell cycle of proliferating cells, it may have contributed to the high incidence of human chromosome breakage in the RIM9 x a3 fusion experiment.

The position of de novo breaks as observed in chromosome 1 (of Table 3) suggests a preferential location at or near the centromere and 1q heterochromatin. This suggestion is sustained by observations of Jongsma et al. (1973; in press). If de novo breaks are induced as a consequence of PCC of S phase chromatin, preferential breakage in the centromere -1qh region may be attributed to the late replication of this region in S phase (Ganner & Evans, 1971; Epplen et al., 1975). PCC of early S phase chromatin may cause chromosome breakage at many sites; its products, if retained in hybrid clones, will probably be too small to be recognized.

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REFERENCES

ARTHUR, E., STEEL, C.M., EVANS, H.J., POVEY, S., WATSON, B. and HARRIS, H. : Localization of human peptidase-A structural locus from studies on a cultured lymphoblastoid line. Nature (Lond.) 257: 308-310 (1975)

BALTIMORE CONFERENCE (1975): Third international workshop on Human Gene Mapping, Birth Defects: Original Article Series, XII, 7 (The National Foundation, New York, 1976)

BOBROW, M., MADAN, K. and PEARSON, P.L.: Staining of some specific regions of human chromosomes, particularly the secondary constriction of No. 9. Nature (Lond.) New Biol. 238: 122-124 (1972)

BURGERHOUT, W.G., SOMEREN, H. VAN, and BOOTSMA, D.: Cytological mapping of the genes assigned to the human A1 chromosome by use of radiation-induced chromosome breakage in a human-Chinese hamster hybrid cell line. Humangenetik 20: 159-162 (1973)

BURGERHOUT, W.G., JONGSMA, A.P.M. and MEERA KHAN, P.: Regional assignments of seven enzyme loci on chromosome 1 of man, pp. 73-75. In: New Haven Conference (1973): First International workshop on Human Gene Mapping. Birth Defects. Original Article Series, vol. 10, no. 3 (The National Foundation, New York, 1974)

BURGERHOUT, W.G.: Identification of interspecific translocation chromosomes in human-Chinese hamster hybrid cells. Humangenetik 29: 229-231 (1975)

DE LA CHAPELLE, A., SCHRÖDER, J., SELANDER, R.-K. and STENSTRAND, K.: Differences in DNA composition along mammalian metaphase chromosomes. Chromosoma (Berl.) 42: 365-382 (1973)

COMINGS, D.E.: The structure and function of chromatin. Adv. Hum. Genet. 3: 237-431 (1972)

DOUGLAS, G.R., McALPINE, P.J. and HAMERTON, J.L.: Regional localization of the loci for human PGM_1 and 6PGD on human chromosome one using Chinese

hamster-human somatic cell hybrids. Proc. Natn. Acad. Sci. USA 70: 2737-2740 (1973)

DUTRILLAUX, B. and LEJEUNE, J.: Sur une nouvelle technique d'analyse du caryotype humain. C.R. Acad. Sci., Paris, 272: 2638-2640 (1971)

DUTRILLAUX, B.: Nouveau système de marquage chromosomique: les bandes T. Chromosoma (Berl.) 41: 395-402 (1973)

EPPLEN, J.T., SIEBERS, J.-W. and VOGEL, W.: DNA replication patterns of human chromosomes from fibroblasts and amniotic fluid cells revealed by a Giemsa staining technique. Cytogenet. Cell Genet. 15: 177-185 (1975)

GANNER, E. and EVANS, H.J.: The relationship between patterns of DNA replication and of quinacrine fluorescence in the human chromosome complement. Chromosoma (Berl.) 35: 326-341 (1971)

GIBLETT, E.R., CHEN, S.-H., ANDERSON, J.E. and LEWIS, M.: A family study suggesting genetic linkage of phosphopyruvate hydratase (enolase) to the Rh bloodgroup system. In: New Haven Conference (1973): First International Workshop on Human Gene Mapping, p. 91. Birth Defects: Original Article Series, Vol. 10, no. 3 (The National Foundation, 1974)

GRZESCHIK, K.-H., ALDERDICE, P.W., GRZESCHIK, A., OPITZ, J.M.,MILLER, O.J. and SINISCALCO, M.: Cytological mapping of human X-linked genes by use of somatic cell hybrids involving an X-autosome translocation. Proc. Natn. Acad.Sci. USA 69: 69-73 (1972)

GRZESCHIK, K.-H. Regional mapping of human chromosomes 1 and 11. In: Rotterdam Conference (1974). Second international workshop on Human Gene Mapping, pp. 172-175. Birth Defects: Original Article Series, Vol. 11, No. 3 (The National Foundation, New York, 1975)

HAMERTON, J.L. and COOK, P.J.L.: Genetic constitution of chromosomes 1 and 2, pp. 3-12. In: Rotterdam Conference (1974): Second international workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. XI, no. 3 (The National Foundation, New York, 1975)

HAMERTON, J.L., MOHANDAS, T., McALPINE, P.J. and DOUGLAS, G.R.. Localization of human gene loci using spontaneous chromosome rearrangements in human-Chinese hamster somatic cell hybrids. Am. J. Hum. Genet. 27: 595-608 (1975)

HOEHN, H.: Functional implications of differential chromosome banding. Am. J. Hum. Genet. 27: 676-686 (1975)

HOLMBERG, M. and JONASSON, J.: Preferential location of X-ray induced chromosome breakage in the R-bands of human chromosomes. Hereditas 74: 57-68 (1973)

JOHNSON, R.T. and RAO, P.N.: Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature 226: 717-722 (1970)

JONGSMA, A.P.M., SOMEREN, H. VAN, WESTERVELD, A., HAGEMEIJER, A. and PEARSON, P.L.: Localization of genes on human chromosomes by using

human-Chinese hamster somatic cell hybrids: assignment of PGM_3 to chromosome C6 and mapping of the 6PGD, PGM_1 and Pep-C genes on chromosome A1. Humangenetik 20: 195-202 (1973)

JONGSMA, A.P.M. and BURGERHOUT, W.G.: Regional assignment of seven genes on chromosome 1 of man by use of man-Chinese hamster somatic cell hybrids. I. Results obtained after hybridization of human cells carrying reciprocal translocations involving chromosome 1. (In press)

MACIERA-COELHO, A., PONTEN, J. and PHILIPSON, L.: The division cycle and RNA synthesis in diploid human cells at different passage levels in vitro. Exptl. Cell Res. 42: 673-684 (1966)

MARSH, W.L., CHAGANTI, R.S.K., GARDNER, F.H., MAGER, K., NOVELL, P.C. and GERMAN, J.: Mapping human autosomes: evidence supporting assignment of Rhesus to the short arm of chromosome no. 1. Science 183: 966-968(1974)

McALPINE, P.J., MOHANDAS, T. and HAMERTON, J.L.: Isozyme analysis of somatic cell hybrids: assignment of the phosphoglucomutase 2 (PGM_2) gene locus to chromosome 4 in man with data on the molecular structure and human chromosome assignment of six additional markers. In: C.L. Markert, ed.: Isozymes. IV. Genetics and evolution, pp. 147-167 (Academic Press, N.Y. 1975)

MEERA KHAN, P.: Enzyme electrophoresis on cellulose acetate gel: zymogram patterns in man-mouse and man-Chinese hamster somatic cell hybrids. Arch. Biochem. Biophys. 145: 470-483 (1971)

PARIS CONFERENCE (1971): Supplement (1975): Standardization in human cytogenetics. Birth Defects: Original Article Series, Vol. XI, no. 9 (The National Foundation, New York, 1975)

ROBSON, E.B., COOK, P.J.L., CORNEY, G., HOPKINSON, D.A., NOADES, J. and CLEGHORN, T.E.: Linkage data on Rh, PGM_1, PGD, Peptidase C and Fy from family studies. Ann. Hum. Genet. (Lond.) 36: 393-399 (1973)

ROTTERDAM CONFERENCE (1974): Second International workshop on Human Gene Mapping. Birth Defects: Original Article Series, Vol. 11, no. 3 (The National Foundation, New York, 1975)

SAN ROMAN, C. and BOBROW, M.. The sites of radiation induced breakage in human lymphocyte chromosomes, determined by quinacrine fluorescence. Mutation Res. 18: 325-331 (1973)

SANCHEZ, O. and YUNIS, J.J.: The relationship between repetitive DNA and chromosomal bands in man. Chromosoma (Berl.) 48: 191-202 (1974)

SEABRIGHT, M.: A rapid banding technique for human chromosomes. Lancet ii: 971-972 (1971)

SHOWS, T.B. and BROWN, J.A.: Mapping chromosomes 1 and 2 employing a 1/2 translocation in somatic cell hybrids. In: Rotterdam Conference (1974). Second International workshop on Human Gene Mapping, pp. 251-255. Birth Defects: Original Article Series, Vol. 11, no. 3 (The National Foundation, New York, 1975)

SOMEREN, H. VAN, BEIJERSBERGEN VAN HENEGOUWEN, H., LOS, W., WURZER-FIGURELLI, E., DOPPERT, B., VERVLOET, M. and MEERA KHAN, P.: Enzyme electrophoresis on cellulose acetate gel. II. Zymogram patterns in man-Chinese hamster somatic cell hybrids. Humangenetik 25: 189-201 (1974)

WESTERVELD, A., MEERA KHAN, P., VISSER, R.P.L.S. and BOOTSMA, D.: Loss of human genetic markers in man-Chinese hamster somatic cell hybrids. Nature (Lond.). New Biol. 234: 20-24 (1971)

WIT, J. DE., BOOTSMA, D., PEARSON, P.L. and WESTERVELD, A.: Regional mapping of the human no. 1 and X-chromosome in interspecific cell hybrids using an X/1 translocation. Cytogenet. Cell Genet. 15: 129-137 (1975)

PAPER V

REGIONAL ASSIGNMENT OF A GENE CODING FOR URIDINE MONOPHOSPHATE KINASE TO BAND p32 OF CHROMOSOME 1 OF MAN BY APPLICATION OF A STANDARDIZED ASSIGNMENT PROCEDURE

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ABSTRACT

The methodology of assignment of genes to chromosomes of man by use of man-rodent somatic cell hybrids is discussed. A general procedure for the assignment and regional mapping of genes is proposed. The procedure is applied in a study on the location of a gene coding for Uridine Monophosphate Kinase (UMPK). The results of this study indicate that UMPK is located in band p32 of chromosome 1 of man.

I. INTRODUCTION

The use of the man-rodent somatic cell hybrid system for assignment of genes to chromosomes of man is based on correlation of the presence and absence of gene products with the presence and absence of chromosomes (Ruddle, 1973; Grzeschik, 1973; Ruddle & Creagan, 1975). This presupposes the feasability of establishing presence and absence of specific gene products and chromosomes of man in populations of hybrid cells.

If a specific assay for a human gene product is available, the product will be detectable in a man-rodent hybrid cell system if the gene is not suppressed, rodent gene products do not hamper detection and the method of detection is sufficiently sensitive. Accomplishment of the former two conditions can be tested by investigating expression of the gene and its eventual rodent counterparts in human and rodent parental cell lines as well as in a panel of hybrid cell lines which together carry a complete haploid set of human chromosomes. If a human gene product can be detected in a hybrid cell system, demonstration of the product in a particular cell line indicates the presence of the particular gene, whereas failure of detection indicates that the gene is either completely absent or present in a quantity which is too low for yielding a detectable amount of product.

Identification of human chromosomes in metaphases of hybrid cells can be achieved satisfactorily by parallel or sequential application of staining techniques which discriminate between human and rodent chromosome material and of classical Q, G or R banding techniques (Bobrow &

Cross, 1974; Kim and Grzeschik, 1974; Kucherlapati et al., 1975; Burgerhout, 1975). On the other hand, no reliable method is yet available to prove that particular human chromosome material is absent in hybrid cells. Since de novo breakage of human chromosomes in hybrid cells is a common phenomenon (Hamerton et al., 1975; Friend et al., 1976; Burgerhout et al., in press), this implicates that apparent absence of a particular chromosome of man as a whole in preparations from a single or relatively few hybrid cell line should not be adduced as sufficient argument in itself for or against assignment of any gene to that chromosome.

For a reliable correlation of data from chromosome analysis and phenotype analysis it is necessary to know which should be the minimal level of occurrence of a particular chromosome of man for yielding detectable expression of a particular gene located on that chromosome. This minimal level of occurrence will be referred to as the "minimal significant chromosome frequency". The "frequency" of a chromosome is defined in this study as the ratio of the number of copies of a particular human chromosome and the number of tetraploid or near-tetraploid sets of Chinese hamster chromosomes observed in a sample of mitoses from a particular hybrid cell line, multiplied by 100.

The minimal significant chromosome frequency can in principle be established by testing the detectability of a particular human gene product in a range of diluted preparations from a hybrid in which the frequency of the chromosome on which the gene is located is known. Such a dilution experiment can only be performed for a gene that has been assigned to a particular chromosome, i.e. it can be used for a justification a posteriori of the

minimal frequency at which any human chromosome has been considered to be present in the hybrid cell lines used in the assignment procedure. A priori estimation of that frequency should be feasible by performing a similar dilution experiment on a preparation from human cells. If the analysis of a panel of hybrid cell lines selected with due observance of the estimated minimal significant chromosome frequency results in an assignment, the a posteriori dilution experiment should be carried out on one or more hybrid cell lines which are representative for the hybrid system used. For justification of the assignment procedure as performed, the minimal significant chromosome frequency calculated from the a posteriori dilution experiment should not be higher than the frequency at which any human chromosome has been observed to be present in any hybrid of the assignment panel.

Observing the considerations stated in the preceding paragraphs, we can state the following operational definition of assignment: a gene A is located on chromosome B if investigation of a panel of hybrid cell lines which together carry a haploid set of human chromosomes has resulted in the detection of each chromosome except B in at least one hybrid in which A was not found to be expressed. Since arguments concerning detection of chromosomes are also valid with respect to detection of parts of chromosomes, the definition as stated also describes minimal conditions for regional assignment of genes if "chromosome" is replaced by "chromosome part".

The principles of assignment as described have been applied in the present study on the localization of a gene coding for uridine monophosphate kinase (UMPK, E.C.

2.7.4.4). Giblett et al. (1975) have presented evidence based on family studies for linkage between UMPK and Rh in man. Since Rh in known to be located on chromosome 1 (Ruddle et al., 1972), this linkage implies an indirect assignment of UMPK to that chromosome. The assignment has been confirmed by Satlin et al. (1975) in a study with man-mouse somatic cell hybrids. The present study comprises a further confirmation of this assignment and evidence for regional location of UMPK in band 1p32.

II. MATERIALS AND METHODS

Man-Chinese hamster hybrid cell lines were obtained by fusion of leucocytes or fibroblasts from 12 different human donors with DON-derived a3 or wg3-h Chinese hamster cells. Properties of the cell lines a3 (thymidine kinase deficient) and wg3-h (hypoxanthine phosphoribosyl transferase deficient) as well as procedures of cell culture and isolation of hybrids have been described by Westerveld et al. (1971). The established human cell line T which was used in the a priori dilution experiment has been described by Van Veen et al. (1958).

Cytogenetic descriptions of structurally rearranged chromosomes utilized in the regional assignment procedure have been published elsewhere. The hybrid clones 1/15UT-21, 1/15UT-24, 1/17HE-2 and 1/17HE-36 each carried one of the reciprocal translocation chromosomes which had been detected in the human parental 1/15UT and 1/17HE cells (Van Hemel et al., 1975; De la Chapelle et al., 1975;

Jongsma & Burgerhout, in press). All other clones in the regional assignment panel were carrying radiation-induced or de novo aberration chromosomes described by Burgerhout et al. (1973; 1975; in press). Secondary clones are indicated by a letter behind the name of the parental clone.

Chromosome preparations and cell lysates from a particular cell line were prepared from the same population of cells as described previously (Burgerhout et al., 1973). Chromosome analysis was carried out following parallel application of trypsin-Giemsa (Burgerhout et al., 1973), R banding (Dutrillaux, 1971) and Giemsa-11 (Bobrow et al., 1972) staining techniques. Unless stated otherwise, the frequency of each human chromosome in a particular hybrid cell line was determined by analysis of 20 randomly selected trypsin-Giemsa stained metaphases.

Cell lysates were prepared by repeated freezing and thawing of cells in a lysis buffer described by Meera Khan (1971). Cellogel electrophoresis (Cellogel$^{(R)}$, Chemetron, Milan, Italy) was utilized to characterize the following enzymes: UMPK, 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44), phosphoglucomutase 1 (PGM_1, EC 2.7.5.1) peptidase-C (Pep-C, EC 3.4.3.-), phosphopyruvate hydratase or enolase (PPH_1, EC 4.2.1.11), guanylate kinase (GuK_1, EC 2.7.48), fumurate hydratase (FH, EC 4.2.1.2) and UDPG pyrophosphorylase (UGPP, EC 2.7.7.9).
General procedures of Cellogel electrophoresis and specific methodes for each enzyme except UMPK have been described by Meera Khan (1971) and Van Someren et al. (1974a).

Electrophoresis of UMPK was performed at 4^{o}C for 3 h.

using a 0.02 M Na_2HPO_4/NaH_2PO_4 buffer at pH 7.0. Samples of cell lysate were applied in aliquotes of 5 μl over a length of 10 mm at 5mm away from and parallel to the cathodic shoulder piece. Following electrophoresis the gel was saturated with a staining mixture for UMPK according to Giblett et al. (1974), containing 1,3 mM of NADH and subsequently incubated at 37°C in a moist chamber. The gel was examined visually under 365 nm UV light at intervals of 5 min. to check the appearance of quenched areas indicating UMPK activity. After 20-30 min. of incubation a counterstaining on NADH was applied using a Tris-HCl-EDTA buffered solution of MTT and PMS at pH 8,6 as described by Meera Khan (1971).

Quantitative determination of UMPK activity in cell lysates was performed by measuring oxidation of NADH after electrophoresis. A sheet of Cellogel was marked with lines parallel to the direction of the current, resulting in 9 channels of 1,5 cm width. Five samples of lysate from one particular cell line were applicated as described above in the 1st, 3rd, 5th 7th and 9th channel respectively. After electrophoresis for 3 h under conditions described above, channel 9 was seperated from the rest of the gel. Channels 1-8 and channel 9 were stained with mixtures according to Giblett et al. (1974), containing respectively 2.6 and 0.65 mM NADH. The gels were then incubated at 37°C in a moist chamber. As a consequence of the low NADH concentration, sites of UMPK activity in channel 9 could be visualized under 365 nm UV light at 2 min. after staining. The remaining channels were then separated and a piece of 2 cm length was cut out from each channel at such a distance from the application line that the regions of UMPK activity as visualized in channel 9 would fall within the range.

After a total incubation time of 10 min. at 37°C, each 1,75 x 2 cm piece of gel was soaked into 0.5 ml of a 0.1 Tris-HCl buffer of pH 7.8 at 20°C and shaken vigorously on a vortex for 10 sec. Five minutes after soaking the NADH fluorescence of each sample of eluate was measured using a Perkin-Elmer Fluorescence Spectrophotometer MPF-2A, excitation wavelength 360 nm, emission wavelength 460 nm. The difference in fluorescence between the mean values of the runs (channel 1, 3, 5 and 7) and the controls (channel 2, 4, 6 and 8) was taken as a measure for UMPK activity.

The protein content of lysates was measured according to Lowry (1951).

Dilution experiments were carried out by application of the standard electrophoretic procedure on series of lysates obtained by mixing lysate from human or hybrid cells with progressive amounts of DON lysate. Starting from 1:1 mixtures of lysates, the series were prepared such that the concentration of human or hybrid lysate in any sample of the range was twice as high as in the next sample. The appearance of bands of UMPK activity after staining was judged independently by 4 observers.

III. RESULTS

1. Characterization of UMPK

Electrophoretic patterns of UMPK from cells of human, Chinese hamster and man-Chinese hamster hybrid origin are shown in Fig. 1. Human as well as Chinese hamster UMPK

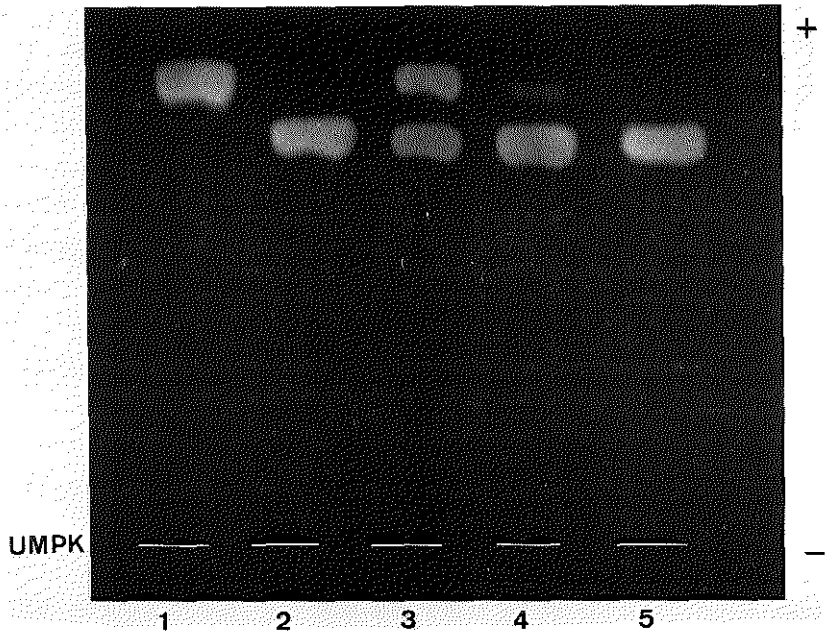

Fig. 1. Zymograms of UMPK on Cellogel Channels: 1, human T cells; 2, Chinese hamster DON cells; 3, mixture of T and DON; 4, man-Chinese hamster hybrid expressing human UMPK; 5, man-Chinese hamster hybrid not expressing human UMPK.

activity is present in a single band. The centre of the human band is about 1 cm anodal to the centre of the Chinese hamster band. Human erythrocytes and leucocytes from four different donors yielded UMPK of identical electrophoretical mobility. Hybrid cell lines yielded either two bands at positions coinciding with the respective ones of human and Chinese hamster UMPK, or one band which was always at the Chinese hamster position.

With exception of 1/15 UT leucocytes, all human fibroblast lines or leucocytes which were used as parental cells were tested for UMPK polymorphism. All sources yielded one

Table 1.

cell line	protein content (mg/ml)	UMPK activity (U/μl lysate)	Maximal dilution at which human UMPK observed	
			Exp. 1	Exp. 2
a3	27	46	-	-
T	22	78	1:256	1:256
A19B1	15	120	1: 16	1: 16
R9A3/12R	29	205	1: 32	1: 16

Note: A unit (U) of UMPK activity is defined as equivalent to the disappearance of 1.10^{-9} Mol NADH during the total incubation time of 10 min. in gel at 37°C plus 5 min. in solution at 20°C.

single band of activity which was situated at the position shown in channel 1 or Fig. 1. The same picture was shown by the permanent human cell line T. Possible polymorphism of UMPK in 1/15 UT parental cells was investigated after completion of the assignment procedure by testing two independent 1/15 UT hybrid clones carrying an intact chromosome 1 as well as two others carrying a 15/1 translocation chromosome (Jongsma & Burgerhout, in press) in which this was the only detectable chromosome 1 material. All four clones yielded a band of UMPK activity at the site shown in channel 1 or Fig. 1, which makes UMPK polymorphism in 1/15 UT very unlikely.

2. Dilution experiments

The a priori dilution experiment was carried out on lysate from the T cell line. After completion of the assignment procedure, the hybrid clones A19B1 and R9A3/12R were selected to be used in the a posteriori dilution experiment. The clones had been derived from fusions of diploid cells from different human donors with Chinese hamster a3 cells. A19B1 carried an intact human chromosome 1 in a frequency

of 56, whereas R9A3/12R carried a rearranged chromosome including the segment 1p36 ⟶ q12 in a frequency of 90. The chromosome frequencies were established by investigation of 50 R-stained metaphases from each cell line. The R-staining technique was chosen because of its reliability for identification of the p arm of chromosome 1 in hybrid cells. Protein contents and total UMPK activities in 1 μl of lysate from the four cell lines are listed in Table 1. The same table shows maximal dilutions at which human UMPK activity was observed in two separate experiments performed on samples from the same batches of lysate. In the a priori dilution experiment, human UMPK is still detectable if one part of a lysate from human cells having a UMPK activity of 78 U/μl is mixed with 256 parts of Chinese hamster lysate with a UMPK activity of 46 U/μl. The a posteriori dilution experiment shows detectability of human UMPK in two hybrid cell lines at respective rations of 1:16 and of 1:16 to 1:32 when lysates with total UMPK activities of 120 and 205 U/μl are mixed with Chinese hamster lysate with a 46 U/μl activity.

3. Chromosomal localization of UMPK

A panel of hybrid clones was selected in order to verify the assignment of UMPK to chromosome 1. With exception of A3MS2I1D and A3MS2I1F, all clones were derived from different fusions. The results of chromosome analysis and UMPK electrophoresis are shown in Table 2. All clones are negative for human UMPK. Each human chromosome except 2 and 10 is present in a frequency of 30 or higher in at least two clones of independent origin; chromosomes 2 and 10 have each been detected in one clone only with respective frequencies of 15 and 70. Each chromosome except 2 is present in at least one clone in a frequency of 60 or

Table 2. Expression of human UMPK and human chromosome frequencies in seven man-Chinese hamster hybrid cell lines

		chromosome frequency												
cell line	UMPK	1	2	3	4	5	6	7	8	9	10	11	12	13
1/22 AM-43	-	.	.	90	80	90	85	80	.	50	.	85	85	85
17 N1b	-	.	.	95	85	85	85	.	35	.	.	75	40	85
A3MS2I1F	-	.	.	65	60	.	35	.	50	.	.	90	40	.
A3MS2I1D	-	.	15	70	50	10	85	30	60	.	.	80	70	.
HRB2	-	.	.	.		.	.	.	.	.	.	90	.	.
A19A1	-	.	.	60	.	75	.	70	20	.	70,	85	.	.
1/17HE-26	-	.	.	55	55	55	90	.	.	65	.	95	.	30

		chromosome frequency										
cell line	UMPK	14	15	16	17	18	19	20	21	22	X	Y
1/22AM-43	-	115	95	75	85	65	75	90	105	90	.	40
17N1b	-	70	25	60	.	85	.	30	100	85	.	.
A3MS2I1F	-	40	30	70	.	.	.	45	145	20	45	25
A3MS2I1D	-	.	.	80	.	.	30	.	.	30	.	.
HRB2	-	.	.	.	.	.	40	.	110	45	60	75
A19A1	-	75	30	30	85	35	30	.	80	.	.	.
1/17HE-26	-	40	.	75	55	30	40	30	95	60	.	.

higher. In addition to the panel, eight independent hybrid clones which were found to carry an intact human chromosome 1 in frequencies of >50 were tested for human UMPK activity. All eight clones were found to be positive.

Regional assignment of UMPK was performed by use of a panel of 13 hybrid clones carrying various segments of chromosome 1 in frequencies of >50. Table 3 shows the composition of the panel and the results of testing the expression of human UMPK and of seven markers which have been assigned to chromosome 1 of man previously (Ruddle et al., 1972; Van Someren et al., 1974b; Meera Khan et al., 1974).

Table 3. Expression of UMPK and other chromosome 1 markers in hybrid cell lines carrying aberrations involving chromosome 1

cell line	chromosome 1 material detected	UMPK	PGD	PPH_1	PGM_1	UGPP	GuK_1	Pep-C	FH
R9A3/31B	qter→cen	-	-	-	-	+	+	+	+
1/15 UT-24	qter→p22	-	-	-	-	+	+	+	+
HRB 2 LA	qter→p32	-	-	-	-	+	+	+	+
HRB 2	qter→p32	-	-	-	+	+	+	+	+
1/17 HE-36	qter→p32	+	-	-	+	+	+	+	+
R9A3/4A	qter→p34	+	-	-	+	+	+	+	+
R9A3/34H	qter→p36	+	+	+	+	+	+	+	+
1/17 HE-2 and 30	pter→p32	-	+	+	-	-	-	-	-
1/15 UT-21	pter→p22	+	+	+	+	-	-	-	-
R9A3/5A	p36→cen	+	+	+	+	-	-	-	-
R9A3/12R	pter→q12	+	+	+	+	-	-	-	-
HRA11	pter→q21	+	+	+	+	-	-	-	-

IV. DISCUSSION

The identical electrophoretical mobility of human UMPK from red and white blood cells as well as from diploid fibroblasts in our system indicates that the human UMPK studied by us in man-Chinese hamster hybrids is identical with the red cell UMPK studied by Giblett et al. (1974, 1975). This conclusion is in accordance with the results of electrophoretical studies on UMPK from different human tissues performed by Teng et al. (1976).

The results of the *a priori* dilution experiment show that our electrophoretical procedure allows identification of human UMPK if one part of a lysate from human cells having an UMPK activity of 78 U/μl is mixed with 256 parts of Chinese hamster lysate with a UMPK activity of 46 U/μl. In terms of activity, this means a dilution of 1:151. For calculation of the minimal significant chromosome frequency in hybrids, the following assumptions have been made:

(1) Both man and Chinese hamster carry one structural locus for UMPK per haploid genome;
(2) The activities of UMPK from human and Chinese hamster origin in hybrid cells are both proportional to the number of gene copies present;
(3) The ratio between human and Chinese hamster UMPK activity is proportianal to the ratio of human and Chinese hamster gene copies.

Based on these assumptions, one may state that in a hybrid carrying the human chromosome on which UMPK is situated in a frequency of 100, human UMPK activity is 20% of total UMPK activity. This dilution ratio of 1:5 is a factor of 30 higher than the extreme one at which human UMPK is still detectable in the a priori dilution experiment. Thus the minimal significant chromosome frequency for human UMPK should be $\frac{100}{30}$ or 3,3.

The a posteriori dilution experiments yield extreme dilution ratios of 1:16 and of 1:16 to 1:32 for the hybrid cell lines A19B1 and R9A3/12R in which the chromosome which presumably carries UMPK is present in respective frequencies of 56 and 90. The respective UMPK activities of A19B1 and R9A3/12 R are 120 and 205 U/μl lysate. Assuming that the detectability of human UMPK in our procedure is determined by the ratio of human and Chinese hamster UMPK in the lysate, this yields minimal significant chromosome frequencies of $\frac{120}{120+15x46} \times 56 \approx 8$ from A19B1 and of $\frac{205}{205+15x46} \times 90 \approx 21$ to $\frac{205}{205+31x46} \times 90 \approx 11$ from R9A3/12R. These frequencies are a factor 3 to 6 higher than the one of 3,3 which follows from the a priori experiment.

Protein measurements have been carried out in order to check whether mixing of lysates as performed in the dilution experiments might lead to significant changes in protein content, which might influence human enzyme activity in the gel as measured following eletrophoresis.

Since protein contents of a3, T, A19B1 and R9A3/12R are within the range of a factor of 2, we do not believe that this factor has influenced our results.

The data listed in Table 2 show that with exception of chromosome 2, each human chromosome has been detected in one or more hybrids which did not yield a detectable amount of human UMPK in a frequency which is well above the minimal significant frequency as indicated by the a posteriori dilution experiments. It should be noted that chromosome frequencies observed tend to be underestimations since no chromosome is scored as present in a cell if doubt exists with regard to its identity or intactness. The results shown in Table 2 and the observation of human UMPK activity in 8 independent clones carrying a complete chromosome 1 in a frequency higher than 50 provide a confirmation for the assignment of UMPK to chromosome 1, though the possibility that UMPK is located on chromosome 2 cannot be excluded formally. The search for further information clones is in progress.

In the regional assignment panel (Table 3) the complete material of all bands of chromosome 1 except p32 is present in at least two clones which lack human UMPK. The clones excluding the 1qter⟶1p31 segment as a possible location for UMPK are derived from different fusion experiments, whereas the region 1 pter⟶p33 is excluded by two primary clones from one fusion (1/17 HE-2 and 1/17 HE-30). All clones carrying the complete material of 1p32 do express human UMPK. These data indicate that UMPK is located in band 1p32. The assignment of UMPK to 1pter⟶p21 by Shows & Brown (1976) is in agreement with this conclusion.

In a previous paper (Burgerhout et al., in press) we reported the regional assignment of PGM_1 to 1p32. Of the

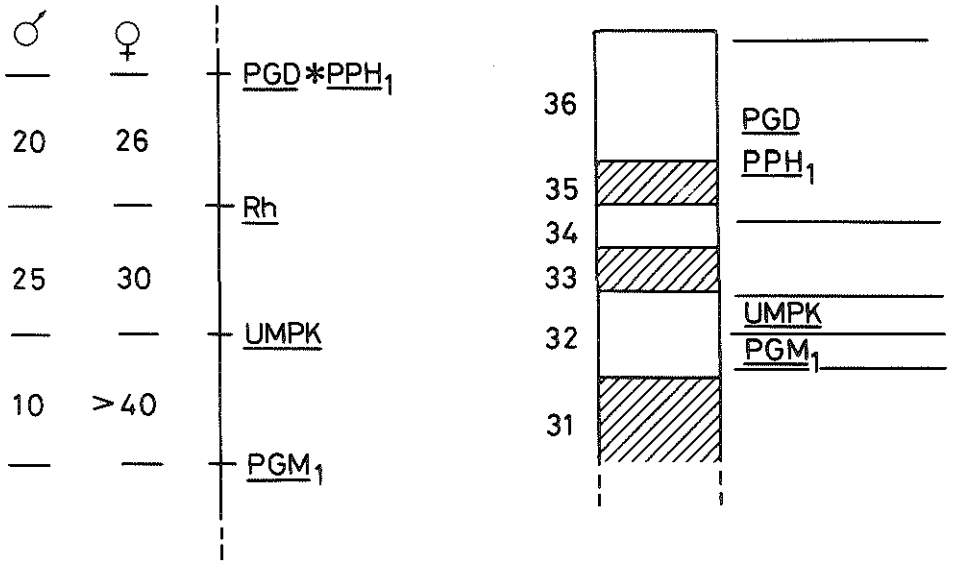

Fig. 2. Comparison of maps obtained by family studies (left) and by somatic cell genetic studies (right). Numbers refer to recombination frequencies (left) and to the Paris Conference (1971) chromosome band nomenclature (right).

other chromosome 1 markers, PGD and PPH_1 are known to be located on 1pter⟶p34, whereas UGPP, GuK_1, Pep-C and FH are situated on the q arm (Burgerhout et al., in press). The segregation pattern of PGM_1 and UMPK relative to the other markers in the clones HRB2, 1/17 HE -36, 1/17 HE -2 and 1/17 HE -30 (Table 3) indicate that UMPK lies distal to PGM_1.

A comparison between a map of the 1p3 region inferred from man-Chinese hamster hybrid studies (Burgerhout et al., in press; this study) and a recent linkage map of Rh, PGD, PPH_1, UMPK and PGM_1 (Hamerton, 1976) is shown in Fig. 2. The relative positions of PGD, PPH_1, UMPK and PGM_1 in both maps are compatible. From the recombination frequencies of UMPK and PGM_1 one may conclude that the meiotical

length of the chromosome material which constitutes band 1p32 is at least 10 cM in males and > 40 cM in females. However, the fact that only five male and three female individuals have contributed to the recombination figures gives rise to reservation over the significance of this conclusion.

REFERENCES

BOBROW, M. and CROSS, J.: Differential staining of human and mouse chromosomes in interspecific cell hybrids, Nature 251: 77-79 (1974)

BOBROW, M., MADAN, K. and PEARSON, P.L.: Staining of some specific regions of human chromosomes particularly the secondary constriction of No. 9., Nature New Biol. 238: 122-124 (1972)

BURGERHOUT, W.G.: Identification of interspecific translocation chromosomes in human-Chinese hamster hybrid cells, Humangenetik 29: 229-231 (1975)

BURGERHOUT, W.G., LEUPE-DE SMIT, S. and JONGSMA, A.: Regional assignment of seven genes on chromosome 1 of man by use of man-Chinese hamster somatic cell hybrids. II. Results obtained after induction of breaks in chromosome 1 by X-irradiation (in press)

BURGERHOUT, W.G., SOMEREN, H. VAN, and BOOTSMA, D.: Cytological mapping of the genes assigned to the human A1 chromosome by use of radiation-induced chromosome breakage in a human-Chinese hamster hybrid cell line. Humangenetik 20:159-162 (1973)

CHAPELLE, A. DE LA, MILLER, R.C., GREENE, A.E. and CORIELL, L.L.: A (1;17) translocation, balanced, plus trisomy 21, 47 chromosomes. Repository identification No. GM-201. Cytogenet.Cellgenet. 14: 82-83 (1975)

DUTRILLAUX, B. and LEJEUNE, J.: Sur une nouvelle technique d'analyse du caryotype humain. C.R. Acad.Sci., Paris: 2638-2640 (1971)

FRIEND, K.K., DORMAN, B.P., KUCHERLAPATI, R.S. and RUDDLE, F.H.: Detection of interspecific translocations in mouse-human hybrids by allealine Giemsa staining. Exptl. Cell Res. 99: 131-136 (1976)

GIBLETT, E.R., ANDERSON, J.E., CHEN, S.-H, TENG, Y.S., and COHEN, F.: Uridine monophosphate kinase: a new genetic polymorphism with possible clinical implications. Am.J.hum.Genet. 26:627-635 (1974)

GIBLETT, E.R., ANDERSON, J.E., LEWIS, M. and KAITA, H.: A new polymorphic enzyme, uridine monophosphate kinase: gene frequencies and a linkage analysis. In: ROTTERDAM CONFERENCE (1974): Second international workshop on human gene mapping. Birth Defects: Original Article Series Vol. 11, no. 3, pp. 159-161 (The National Foundation, New York, 1975)

GRZESCHIK, K.H.: Utilisation of somatic cell hybrids for genetic studies in man. Humangenetik 19: 1-40 (1973)

HAMERTON, J.L.: Report on the committee on the genetic constitution of chromosomes 1 and 2. In: BALTIMORE CONFERENCE (1975): Third international workshop on human gene mapping. Birth Defects: Original Article Series, Vol. XII, 7, 1976, pp. 7-23 (The National Foundation, New York)

HAMERTON, J.L., MOHANDAS , T., McALPINE, P.J. and DOUGLAS, G.R.: Localization of human gene loci using spontaneous chromosome rearrangements in human-Chinese hamster somatic cell hybrids. Am. J. Hum. Genet. 27: 595-608 (1975)

HEMEL, J.O. VAN, BIERVLIET, J.P. VAN, and JAGER-VAN DER GRIFT, P.W. DE: A girl with 46. XX,t (1;15) karyotype. Cytogenetic and clinical observations. Clin. Genet. 8: 213-217 (1975)

JONGSMA, A.P.M., SOMEREN, H. VAN, WESTERVELD, A., HAGEMEIJER, A. and PEARSON, P.L.: Localization of genes on human chromosomes by using human-Chinese hamster somatic cell hybrids: assignment of PGM_1 to chromosome C6 and mapping of the 6PGD, PGM_1 and Pep-C genes on chromosome A1. Humangenetik 20: 195-202 (1973)

KIM, M.A. and GRZESCHIK, K.H.: A method for discriminating murine and human chromosomes in somatic cell hybrids. Exptl. Cell Res. 88: 406-410 (1974)

KUCHERLAPATI, R.S., HILWIG, J., GROPP, A. and RUDDLE, F.H.: Mammalian chromosome identification in interspecific hybrid cells using "Hoechst 33258". Humangenetik 27: 9-14 (1975)

LOWRY, D.H., ROSEBOROUGH, N.J., FARR, A.L. and RANDALL, R.J.: J. Biol. Chem. 193: 265 (1951)

MEERA KHAN, P.: Enzyme electrophoresis on cellulose acetate gel: zymogram patterns in man-mouse and man-Chinese hamster somatic cell hybrids. Arch. Biochem. Biophys. 145: 470-483 (1971)

MEERA KHAN, P., DOPPERT, B.A., HAGEMEIJER, A. and WESTERVELD, A.: The human loci for phosphopyruvate hydratase and guanylate kinase are syntenic with the PGD-PGM_1 linkage group in man-Chinese hamster somatic cell hybrids. In: New Have Conference (1973): First international conference on human gene mapping. Birth Defects: Original Article Series, Vol. 10, no. 3 (The National Foundation, New York, 1974).

RUDDLE, F.H.: Linkage analysis in man by somatic cell genetics Nature 242: 165-168 (1973)

RUDDLE, F.H. and CREAGAN, R.P.: Parasexual approaches to the genetics of man. Ann. Rev. Genet. 9: 407-486 (1975)

RUDDLE, F.H., RICCIUTI, F., McMORRIS, F.A., TISCHFIELD, J., CREAGAN, R., DARLINGTON, G. and CHEN, T.R.: Somatic cell genetic assignment of peptidase C and the Rh linkage group to chromosome A-1 in man. Science 176: 1426-1431 (1972)

SATLIN, A., KUCHERLAPATI, R. and RUDDLE, F.H.: Assignment of the gene for human uridine monophosphate kinase to chromosome 1 using somatic cell hybrid clone panels. Cytogenet. Cell Genet. 15: 146-152 (1975)

SHOWS, T.B. and BROWN, J.A.: Localization of the human uridine monophosphate kinase gene to the p21—— pter region of chromosome 1. In: BALTIMORE CONFERENCE (1975), Third international workshop on human gene mapping. Birth Defects, Original Article Series, XII, 7, 1976, pp. 227-230. The National Foundation, New York.

SOMEREN, H. VAN, BEIJERSBERGEN VAN HENEGOUWEN, H., LOS , W., WURZER FIGURELLI ,E., DOPPERT, B., VERVLOET, M. and MEERA KHAN, P.: Enzyme electrophoresis on cellulose acetate gel. II. Zymogram patterns in man-Chinese hamster somatic cell hybrids. Humangenetik 25: 189-201 (1974[a])

SOMEREN, H. VAN, BEIJERSBERGEN VAN HENEGOUWEN, H., WESTERVELD, A. and BOOTSMA, D.: Synteny of the human loci for fumurate hydratase and UDPG pyrophosphorylase with chromosome 1 markers in somatic cell hybrids. Cytogenet. Cell Genet. 13: 551-557 (1974B)

TENG, Y.-S, CHEN, S.-H. and GIBLETT, E.R.: Red cell uridine monophosphate kinase: effects of red cell aging on the activity of two UMPK gene products. Am. J. Hum. Genet. 28: 138-142 (1976)

VEEN, J. VAN DER, BOTS, L. and MES, A.: Arch. Virusforsch. 8, 230 (1958)

WESTERVELD, A., MEERA KHAN, P., VISSER, R.P.L.S. and BOOTSMA, D.: Loss of human genetic markers in man-Chinese hamster somatic cell hybrids. Nature (Lond.) New Biol. 234: 20-24 (1971)