Use of chromosomal translocations with in situ DNA hybridisation to confirm localisation of human 5S ribosomal RNA genes

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SUMMARY Two cases of chromosomal translocations involving the long arm of chromosome 1 were investigated for 5S ribosomal gene localisation using in situ hybridisation. In the first family, there was an interstitial translocation of 1q25–32 to chromosome 5; the 5S genes remained on chromosome 1. In the second family, there was a translocation of 1q42–44 to chromosome 21q12; the 5S gene locus in this case was translocated. This shows that the 5S ribosomal genes are at position 1q42–44, confirming a previous assignment based on adenovirus-induced uncoiling and on a partial trisomy (Steffensen et al., 1977).

The accurate assignment of genes to particular loci on human chromosomes allows the construction of linkage maps, and will ultimately assist in antenatal diagnosis and in the understanding of gene function in development and hereditary disease. To date, most gene localisations have been made by studies of the genetic linkage of phenotypic characters, or by the analysis of heterokaryons and cell hybrids, and by the study of gene dosage as expressed in protein products in partial trisomies and monosomies.

In this report we apply hybridisation of 5S ribosomal RNA to the human 5S ribosomal gene locus, making use of two cases where translocations on chromosome 1 have been identified and mapped.

Human cells contain multiple copies of the gene for 5S ribosomal RNA. The 5S ribosomal RNA is a normal constituent of ribosomes, is 120 nucleotides in length, and has a molecular weight of approximately 40 000. There are approximately 600 copies of the 5S gene in human diploid cells per haploid genome (Szabo et al., 1978), though the number of genes in aneuploid HeLa cells may be several times higher (Hatlen and Attardi, 1971). The 5S gene sequence is conserved, at least between mammalian species (Williamson and Brownlee, 1969).

In situ hybridisation of 5S ribosomal RNA to Xenopus laevis chromosomes has located the oocyte 5S genes on the telomeres of the long arms of most of the chromosomes (Pardue et al., 1973). When human chromosomes are fractionated on sucrose gradients and the DNA from each fraction hybridised to 5S RNA, gene sequences appear to be present in several fractions; however, the fractionation allows considerable overlap between size classes (Aloni et al., 1971). In situ hybridisation to normal human metaphase chromosomes shows extensive labelling over the long arm of chromosome 1 at position 1q32–44 (Steffensen et al., 1975).

The assignment of the 5S genes to chromosome 1q was confirmed by Steffensen et al. (1976), using adenovirus 12 to induce uncoiling of chromosome 1 at site 1q42, and identifying the 5S gene locus just distal to this site. In situ hybridisation of a duplication of region 1q31–1q43–44 also confirmed the assignment of the 5S gene locus to position 1q42–43 (Steffensen et al., 1977).

Translocations represent a particularly clear method for showing chromosomal localisation, as the genes will either translocate or not with a given chromosomal fragment. We have studied two translocations involving the long arm of chromosome 1 for the position of the 5S ribosomal genes.
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Patients

FAMILY A
Chromosome analysis of the banded karyotypes (Fig. 1) showed an unusual interstitial translocation. The segment 1q25–32 was inserted below 5q13. Two members of this family were studied; the father with the balanced translocation 46,XY,ins(5;1) (5pter→5q13::1q25→1q32::5q13→5qter;1pter→1q25::1q32→1qter), and the daughter with the partial trisomy for the segment 1q25–32: 46,XX,ins(5;1) (5pter→5q13::1q25→1q32::5q13→5qter)pat.

Meiotic studies showed that this was a non-reciprocal translocation (M. A. Ferguson-Smith, unpublished data).

FAMILY B
Chromosome analysis of the banded karyotype (Fig. 5) showed a balanced translocation between chromosome 1 and chromosome 21: 46,XX,t(1;21) (1pter→1q42::21q12→21qter;21pter→21q12::1q42→1qter).

Methods

Total RNA was prepared from mouse reticulocytes and 5S ribosomal RNA was purified (from 28S, 18S, and 4S RNAs) on a G100 Sephadex column (Reynier et al., 1967; Williamson and Brownlee, 1969). Gel electrophoresis was carried out on 6% polyacrylamide gels to confirm the purity of the RNA, which
Fig. 2  Case A. Autoradiographs developed after exposure for 56 days.

Fig. 3  Case A. Distribution of grains on short (p) and long (q) arms throughout the chromosome groups of (a) the daughter and (b) the father.

No of grains/group
Frequency: \[ \frac{\text{No of grains}}{\text{Total units}} \]
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Chromosome banding studies and in situ hybridisation were carried out on short term peripheral blood cultures (Ferguson-Smith, 1974) and the trypsin-Giemsa banding method (Seabright, 1972) was used for chromosome identification.

Before in situ hybridisation, the chromosome preparations were treated with boiled pancreatic RNAse (100 μg/ml) in 2 × SSC (SSC = 0.15 M NaCl, 0.015 M Na₃ citrate) for one hour at 37°C, thoroughly rinsed in 2 × SSC, and dehydrated in 75% and 95% alcohols. Chromosomes were denatured by heating the slides in 95% formamide,
Fig. 6  Case B. In situ hybridisation of $^{32}$P 5S rRNA to balanced translocation 1q−;21q+. 70 days' exposure.

Fig. 7  Case B. Distribution of grains per length unit of chromosomes 1, 1q−, 21q+, 21, and 22. Arrows indicate position of centromere. Dotted lines around units 16 and 17 in chromosome 21q+ indicate position of translocated portion of chromosome 1.
0·5% SSC at 65°C for 2½ hours, rinsing briefly in 2× SSC, and dehydrating in 50%, 75%, 95%, and 100% alcohols. A 50-fold excess of cold 28S and 18S mouse ribosomal RNA was added to the hybridisation mixture to compete with any possible contaminating 28S or 18S fragments (Prenske et al., 1973). The hybridisation solution contained 4 ng 131I 5S RNA (4·4 - 5·2 × 10⁵ dpm) in 4 μl of 50% formamide, buffered with 0·5 M NaCl, 0·025 M Hepes, 0·01 M EDTA, pH 7·0, per slide. Samples were covered with an acid-washed 22 × 22 mm coverslip, sealed with Cow Gum, and incubated for 16 hours at 43°C.

Unhybridised RNA was removed by treatment with boiled pancreatic RNase (100 μg/ml) for 30 minutes at 37°C. The slides were washed three times in 2× SSC at 4°C, washed three times in 2× SSC at room temperature, and dehydrated through 50%, 75%, 95%, and 100% alcohols before air-drying. Slides were dipped in Ilford Nuclear Emulsion (K2 particle size), diluted 1:1 with distilled water, stored in sealed, light-tight boxes containing anhydrous silica gel, and exposed for 56 to 70 days. The slides were developed at room temperature in Kodak D19, diluted 1:1 with water for 5 minutes, fixed in Ilford Hypam for 4 minutes, washed in tap water for one hour, and stained for 40 minutes in 5% Giemsa in phosphate buffer, pH 6·8.

Results

Family A

Eleven metaphases were analysed from both patients and the grains scored on a karyotype diagram divided into 400 length units, each comprising 0·25% of the total genome length. The normal chromosome 1 and the deleted chromosome 1 showed C-banding after hybridisation (Pardue and Gall, 1970), which enabled the long and short arms to be identified unambiguously. The abnormal chromosome 5q+ was clearly recognisable in the B group chromosomes. The 5S genes remained on the terminal portion of chromosome 1 (Fig. 2). The region 1q25–32 translocated onto chromosome 5 did not contain hybridising sequences.

To allow for chromosome length, the numbers of grains per short and long arms were divided by the total number of length units for each group (Fig. 3). Chromosome 1 is labelled in both cases. The number of grains per length unit of chromosomes 1, 4, 5, and 5q+ of the daughter are shown in Fig. 4. Segments 14 to 17 of chromosome 1 are heavily labelled and correspond to 1q25–4qter. Segments 14 to 15, corresponding to 1q25–32, were translocated to 5q13 and the absence of grains on the insertion chromosome 5 shows that the portion 1q25–32 does not contain the 5S genes. This translocation showed that the 5S genes are located distal to 1q32, from 1q41–4qter.

Family B

Analysis of 18 metaphases showed that the distal part of the long arm of the normal chromosome 1 and the long arm of the G21q+ were heavily labelled (Fig. 6), which shows that the 5S genes are in the translocated portion 1q42–4qter. No hybridising 5S gene sequences were seen on the deleted chromosome 1q−. Fig. 7 shows the distribution of grains over chromosomes 1, 1q−, 21q−, 21, and 22. The only other regions showing significant labelling levels were the short arms of the D group chromosomes, which were labelled to only one third of the extent of the 5S locus (Fig. 8).

The total grains for each 5S locus were 43 and 38 in case A, and 46 and 39 grains in case B. A χ² analysis shows that the differences between the grain counts were not significant.
Discussion

These results confirm, using two translocations, the assignment of the major human 5S ribosomal RNA gene locus to position 1q42-44 (Steifsen et al., 1975, 1976, 1977). The results of Aloni et al. (1971) with HeLa cells may be the result of either the aneuploid karyotype of the cell line, or of incomplete resolution of chromosomes on sucrose-glycerol gradients, prepared from cells treated with mitotic blocking agents. Rearrangements of chromosome 1 are known to have occurred in HeLa cell lines (Miller et al., 1971; Czaker, 1973; Nelson-Rees et al., 1974). There is also conservation of the chromosome 1 localisation of 5S ribosomal RNA gene sequences between man and other primates (Henderson et al., 1978).

It is known that the gene loci for 28S and 18S ribosomal RNAs, on the nucleolar regions at the short arms of the acrocentric chromosomes, show considerable variation in gene number and morphology (Evans et al., 1974; Henderson and Atwood, 1976). No such polymorphisms visible in the light microscope are evident for regions 1q42-43, and in these two cases there is no significant difference in grain count between the normal chromosome 1 and the chromosome 1 involved in the translocation (not transferred in case A, transferred in case B). Therefore, in these two cases, the number as well as the position of the 5S ribosomal RNA genes is approximately conserved.

While the major gene locus for 5S ribosomal RNA has been unequivocally identified previously, and confirmed above, it is not possible to exclude the possibility that small numbers of 5S genes occur at other chromosomal loci. Well spread chromosome preparations gave lower backgrounds than those for which the chromosomes were clustered, presumably because cytoplasmic contamination was less. In general, the background over other chromosomes was random; the only position where hybridisation over background was significant, apart from the localisation 1q42-43, was on the short arms of the D group chromosomes. It is not clear whether this represents a second 5S gene locus, or hybridisation of small amounts of labelled 18S and 28S sequence in spite of the addition of cold 18S + 28S RNA competitor, or an artefact resulting from proteins or RNA molecules binding to the 5S RNA at the site of cellular ribosome assembly. We believe the last explanation is most likely to be correct. Henderson et al. (1978) also found a high background over the nucleolus organiser regions in their attempts to localise mouse globin genes.

Because of their unequivocal nature, translocations represent a useful tool in attempts to locate chromosomal loci for specific gene sequences where DNA or RNA probes are available.

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