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Isolation and Regional Localization by *in situ* Hybridization of a Unique Gene Segment to Chromosome 21

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SUMMARY: A chromosome 21 (ch. 21) flow-sorted library was screened for the presence of unique DNA segments which are specific for the 21st chromosome. By combining the techniques of somatic cell genetics and *in situ* hybridization, we have identified several of these recombinant probes and have regionally mapped one of them to the distal half of the long arm of chromosome 21 (q22.1->qter). This represents the first report of the sublocalization of a unique DNA segment to chromosome 21 by *in situ* hybridization.

Down Syndrome (DS) is a chromosomal disorder caused by the presence of an extra copy of all or part (21q22->qter) of ch. 21 (1,2). To date, accurate diagnosis of DS can only be made by chromosomal analysis, a time consuming and expensive process. Since the incidence of DS in the general population is fairly high, ~ 1 in 1000 live births (1), the availability of a more efficient assay would be both advantageous and economical. Recombinant DNA techniques have allowed the identification and isolation of unique DNA segments which can be mapped to chromosomal regions by Southern blot analysis using somatic cell hybrids and *in situ* hybridization. Several of these unique segments have been used to quantitate chromosome dosage and would be useful in the diagnosis of aneuploid conditions (3,4).

We have identified a series of unique probes which have been tentatively assigned to human ch. 21 by Southern blot analysis of

Abbreviations: DS, Down Syndrome; ch., chromosome.

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DNA from normal diploid fibroblasts, mouse fibroblasts and a mouse-human hybrid whose only human ch. is 21 (WA17d). The assignment of one of these probes (DS21D1) to ch. 21 has been confirmed by *in situ* hybridization and regionally mapped to 21q22.1->qter.

MATERIALS AND METHODS

Cell Lines: Total genomic DNA was prepared as described (5) from normal diploid cell lines, the mouse-human somatic cell hybrid WA17 (6) and from the murine parent of the hybrid cell line, A9.

Isolation of DNA Probes: An EcoRI-generated chromosome 21 flow-sorted library (7) was screened with nick-translated total human DNA (10^8 cpm/ugm) by the Benton-Davis technique (8) to identify ch. 21 specific sequences which lacked repetitive DNA. The sequences were then hybridized to Southern blots (9) containing 20 mcg each of total human, WA17 and A9 DNA to confirm that they contained unique human DNA.

In situ Hybridization and Autoradiography: The hybridization protocol of Harper and Saunders (10) as modified by Zabel et al. (11) was used. Slides were stained immediately after developing using the method of Zabel et al. (11). The chromosome spreads were analyzed microscopically and grain locations were indicated on a chromosome idiogram (12).

RESULTS

Isolation of a Unique DNA Segment: Of the 5,000 recombinant phage examined, approximately 170 (3.4%) showed no hybridization to labelled total human DNA and were selected for a second screen. Several of these were chosen randomly, amplified individually, restricted with EcoRI, blotted and rescreened for repetitive sequences by hybridization against total human DNA. Of these approximately 70% did not show any homology to repetitive human DNA. In the final screen, the recombinant clones were labelled and hybridized to Southern blots containing 20 mcg each of total human, WA17 and A9 DNA. Clones which showed unique bands after hybridization and autoradiography to total human and WA17 DNA were tentatively assigned to ch. 21. The first probe to be identified, DS21D1, carried a 7.0 kb insert and was chosen for further characterization by *in situ* hybridization.

In situ Hybridization: The recombinant clone, DS21D1, was hybridized overnight to metaphase chromosome preparations from a normal 46,XX, individual. The composite hybridization results from 80 metaphase spreads are illustrated in the idiogram in Fig. 1. The major site of hybridization was the long arm of ch. 21 as shown in the metaphase spread in Fig. 2. On the average, 5.9 grains per metaphase spread were observed and of the total amount of label, approximately 7% was localized to the long arm of 21.

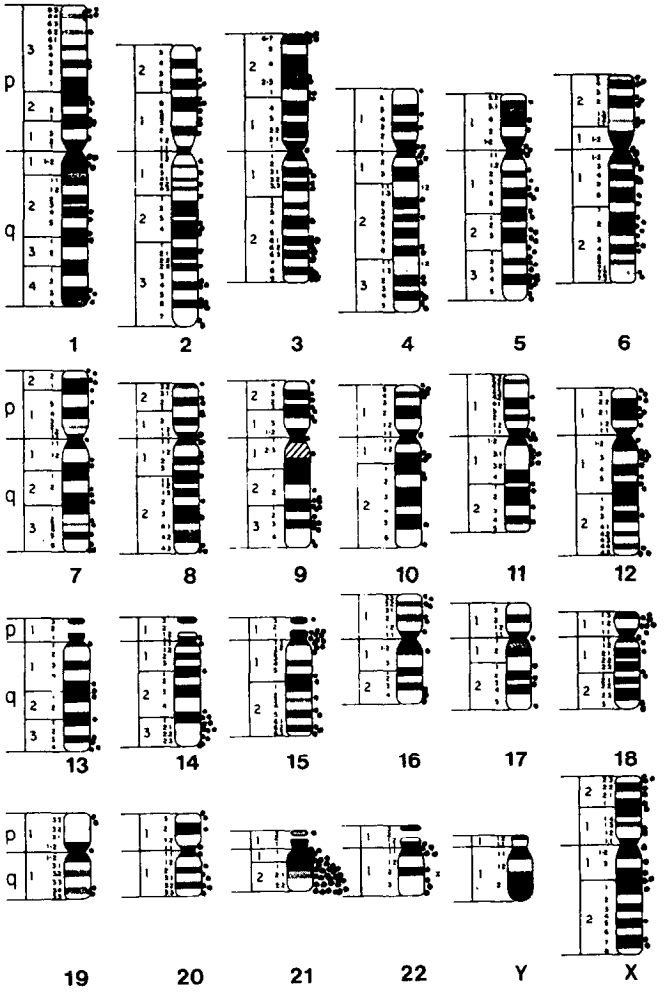

FIGURE 1: Idiogram of high resolution Gbanded mid-metaphase chromosomes showing the composite hybridization data of recombinant probe DS21D1 from 80 metaphase spreads. As is shown, the major area of hybridization is to the long arm of ch.21. Over 53% of the label on this chromosome hybridizes to q22.1->qter.

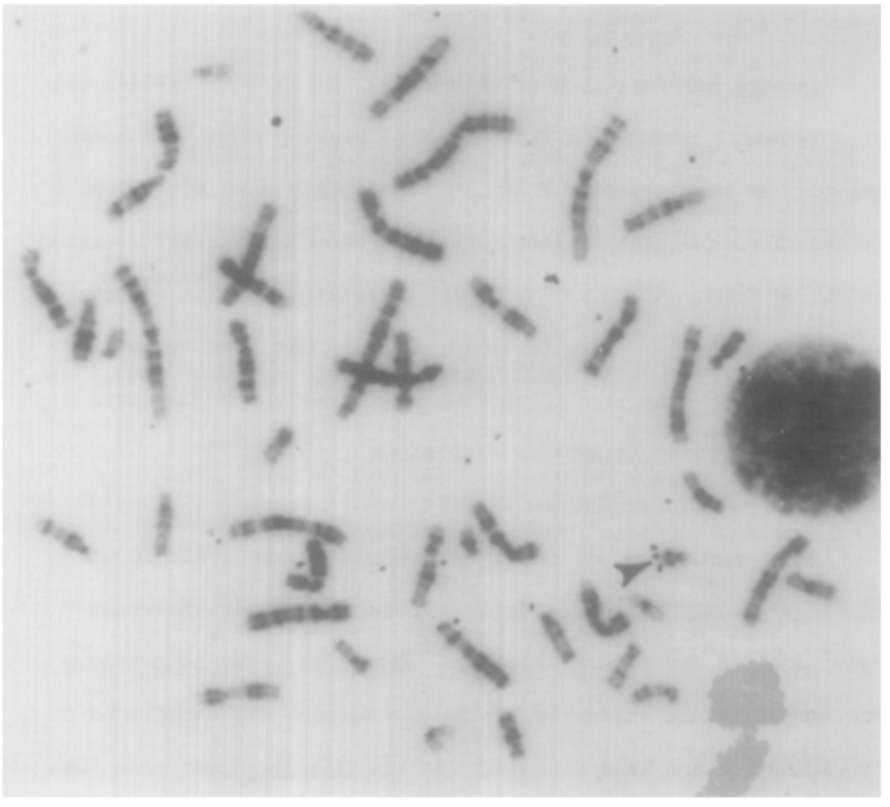

FIGURE 2: In situ hybridization of probe DS21D1 to a G-banded human metaphase spread. The major area of hybridization to the terminus of ch. 21 is indicated by an arrow.

Of the grains visualized on ch. 21, 53% were localized to the 21q22.1->qter region of the chromosome. In addition, 30 more metaphase spreads which contained a labelled ch. 21 were examined in order to obtain more precise mapping data. The majority of these grains were also localized to the region q22.1->qter. Probe DS21D1 also appeared to have some homology to the centromere of ch. 15. However, over 50% of the grains which localized to this region were obtained from one of the three slides used in the analysis; whereas the ch. 21 region q22.1->qter was consistently labelled on all of the slides. These in situ results corroborate the assignment of DS21D1 to chromosome 21 and have delineated the mapping to q22.1->qter.

DISCUSSION

In situ hybridization of unique gene segments has proved to be a powerful method for their precise mapping along the human genome. We have screened a ch. 21 library and have identified a series of unique gene fragments which we have tentatively assigned to ch. 21 on the basis of their hybridization patterns to total human and WA17 DNA. One of these, DS21D1, has been regionally mapped to the long arm of ch. 21, specifically to the area q22.1->qter, using *in situ* hybridization.

The centromere of ch. 15 also showed some evidence of homology to DS21D1. We believe this may represent background hybridization for several reasons. First, in order to preserve the morphological integrity of the chromosome, the conditions of hybridization are less stringent for the *in situ* experiments than for Southern blots, By lowering the stringency conditions used in the Southern blot hybridization, we observed more homology to total genomic DNA with DS21D1, indicating that this unique probe has some affinity for other DNA segments which may or may not be located on ch. 21. Second, many low repetitive DNA families are concentrated in the centromeric regions of DNA (13). If the probe had some homology to a low repetitive DNA element, this could explain the apparent hybridization to the centromere of ch. 15. Alternatively, the probe could encode a gene on ch. 21 which had some degree of homology to a pseudogene on 15.

We believe this to be the first *in situ* localization of a unique 21 DNA segment. Since this is also the area of the chromosome which is present in a trisomic amount in DS, the probe DS21D1 may allow for the diagnosis of DS using recombinant techniques.

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