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Correlation of Physical and Genetic Maps
of Human Chromosome 16

Progress Report
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Abstract

This project is now progressing strongly. Thirteen somatic cell hybrids containing rearranged #16 chromosomes have been constructed, bringing the total number of hybrids constructed by the group to 27 which divides chromosome 16 into 29 regions. 170 probes have been mapped into these regions, most by Southern analysis of the hybrid panel but some by PCR analysis.

Five polymorphisms have been determined on the CEPH panel of families, bringing the total for the project to 10 markers, and a multipoint linkage map correlated with the physical map of the region 16q12.1-q22.1 has been constructed.

Introduction

Although this is the second progress report for this contract it essentially contains all the work carried out since the first progress report covered a period of less than three months during which little had been done other than setting up. The project has been progressing very well and has led to numerous collaborations with other groups involved in mapping this chromosome or studying genes on it.

Scope

This project aims to construct a detailed physical map of human chromosome 16, which will be correlated with the linkage map of this chromosome. The methods to be used to achieve this include the development of a panel of mouse/human hybrid cell lines which contain only parts of chromosome 16. This will be achieved by fusing human cells which have rearrangements of chromosome 16 with mouse A9 cells and selecting for the human APRT gene on the end of the long arm of chromosome 16. The cytogenetic and molecular characterisation of the panel will allow detailed physical mapping of cloned DNA sequences and genes which are expressed in the hybrid cells. The molecular characterisation will be by Southern analysis and *in situ* hybridisation. The cell panel produced should divide this chromosome which contains approximately 3.3% of the human genome into about 50 intervals of average size 2 mb and thus provide a means of mapping any cloned DNA sequence from chromosome 16 into these relatively small regions. Sequences which map into such a region should then be able to be used to generate restriction maps using pulse field gel electrophoresis. This project should lay the foundation for the construction of a restriction map of chromosome 16.

Anonymous cloned DNA fragments of chromosome 16 will be selected from various chromosome 16-specific libraries and mapped using the hybrid cell panel. In selected intervals these will be used to identify RFLPs for which the CEPH panel of families will be typed to correlate the physical and linkage maps. Probes to cloned genes which have been mapped to chromosome 16 will be obtained and these genes will be physically mapped, and, where these probes detect RFLPs, their linkage relationships with other cloned segments will be determined using the CEPH families. Probes on chromosome 16 which have been put through the CEPH families and used to generate linkage maps will be obtained from others on a collaborative basis and physically mapped to further define the correlation of the physical and genetic maps of this chromosome.

Senior Staff Commitments

<i>Investigator</i>	<i>Period Covered by this Report</i>	<i>Next Year of Project</i>
Dr. G.R. Sutherland	40%	40%
Dr. D.F. Callen	50%	50%
Dr. J.C. Mulley	30%	30%
Dr. R.I. Richards	30%	30%

The staff who are funded by the contract spend all of their time working on the project. At the end of 1989 Dr. V.J. Hyland, who was funded by the contract resigned and was replaced by Dr. Eric Kremer early in 1990.

Hybrid Cell Construction

A further thirteen mouse/human hybrids with breakpoints on chromosome 16 have been constructed. These are listed in Table 1. Of particular interest are CY125 and CY130 which are two overlapping interstitial deletions in the 16q21-16q22.1 region. During the process of this work a cell line from the NIGMS cell repository was found to be a balanced translocation rather than a deletion as previously described (Callen et al.b).

Table 1 New mouse/human hybrids constructed.

<i>Hybrid</i>	<i>Chromosome 16 derivative</i>	<i>Source of human parental line</i>
CY196	der(16)t(1;16)(q12;p13.3)	D.M. Danks, Australia
CY197	der(16)t(13;16)(q14;p13.3)	R.J. Desnick, USA
CY185	der(16)t(6;16)(p21.3;p13.11)	Our laboratory
CY175	der(16)t(16;18)(p13.11;q11.23)	Our laboratory
CY123	der(16)t(5;16)(q35.2;p12.2)	S. Moore, Australia
CY165	der(16)t(11;16)(q23;p11.2)	A. Geurts van Kessel, Netherlands
CY150	der(9)t(9;16)(q12;q11.2)	Our laboratory
CY135	der(18)t(14;16)(16;18)(p12;p11q12;p1)	NIGMS, USA
CY130	del(16)(q21q21)	Witt, USA
CY125	del(16)(q21q22.1)	Ferguson-Smith, UK
CY4	der(13)t(13;16)(q34;q22.1)	S. Purvis-Smith, Australia
CY170	der(4)t(4;16)(q35;q22)	NIGMS, USA
CY124	der(15)t(15;16)(q26.1;q22.1)	D. Marsden, USA

The collection of patient material containing chromosome 16 rearrangements from cytogenetics laboratories around the world continues. Material from an additional 19 cell lines, many of which are only partly characterised cytogenetically, (Table 2) has been obtained. This material, and that which is still being collected, will form the basis for the construction of additional hybrids to reach the goal of fifty breakpoints on chromosome 16.

Figure 1

Chromosome 16 showing mapping breakpoints (left of chromosome) and mapped probes (right). Locus names have been abbreviated such that D16S85 has become S85 and so on.

See text for further details.

Table 2 Cell lines containing chromosome 16 breakpoints for which hybrids have not been completed.

<u>Karyotype</u>	<u>Source</u>
t(1;16)(?;?p13)	Peters, Australia
del(16)(p11.2p11.1)	NIGMS
t(3;16)(q27;q12.1)	Palmer, USA
t(12;16)(q13;q22)	Vivigen Inc., USA
t(11;16)(p11.2;p13.3)	Vivigen, Inc., USA
t(9;16)(?;p13)	Breuning, Netherlands
t(4;16)(q25;p13)	Mikkelsen, Denmark
t(11;16)(q11.2;p11.1)	NIGMS, USA
t(5;15;16)(?;?;p11)	Fraccaro, Italy
t(16;17)(q13;q25.3)	Mikkelsen, Denmark
t(1;16)(q43;q21)	Noel, France
t(5;16)(q22.1;q22.1)	Purvis-Smith, Australia
t(5;16)(q14.2;q22.1)	Our laboratory
t(2;16)(p15;q22.3)	Our laboratory
t(9;16)(p22;q23.1)	NIGMS, USA
t(3;16)(p26;q23)	Brothman, USA
t(16;19)(q23;q13.4)	Mikkelsen, Denmark
t(3;16)(q23;q24)	Palmer, USA
t(6;16)(q15;q24)	NIGMS, USA

Mapping of probes

A total of 170 probes have now been mapped to the cell panel. This is shown in Fig.1 where breakpoints of hybrids (CY numbers) and other chromosome rearrangements are shown on the left and the probes on the right. Probe names are given in brackets preceded by the D16S number. Probes have not always been mapped as yet against all the hybrids. Those probes with the most detailed mapping are generally on the left hand column while those on the middle and right hand columns are mapped to less precise regions.

We have now developed a series of two multiplex PCR reactions based on primers derived from gene sequences (Richards et al, submitted, the genes involved are indicated by the "\$" symbol in Fig.1). This set of STS's have provided a rapid and efficient method for evaluating and mapping the breakpoints of new hybrids.

A refined physical map of the long arm of #16 has been prepared for publication (Chen et al.).

In situ hybridisation

Mapping of the probes in the vicinity of the four fragile sites on chromosome 16 is by *in situ* hybridisation. We now have successfully developed the use of biotinylated probes using a fluorescent detection system. This has provided a rapid procedure to map probes in the vicinity of fragile sites.

CEPH family studies

Since the last report, DNA typing has been finalised from the CEPH families for the markers D16S8 (PvuII), D16S96 (TaqI), D16S79 (TaqI) and D16S131 (TaqI) in chromosomal bands 16p13.13, 16p13.12, 16p13.11 and 16p12.3. One additional marker, D16S36 (TaqI) needs to be typed before a multipoint linkage map is constructed in this region.

Also, since the last report, DNA typing has been finalised from the CEPH families for the marker D16S10 in chromosomal band 16q21 near the fragile site FRA16B. A multipoint linkage map incorporating 10 marker loci between 16q12.1 and 16q22.1 has been constructed in the region near FRA16B and prepared for publication (Kozman et al.). The order of loci was : cen-D16S39-MT-D16S59-D16S65-D16S10-FRA16B-D16S91,D16S4-D16S69-D16S47-HP-pter.

The development of multilocus maps of specific regions of chromosome 16 extends the linkage map of the chromosome.

Collaborations arising from this project

1. *Mapping of cosmid contigs (Drs. Hildebrand and Stallings, Los Alamos).*
Physical mapping of cosmids from the larger contigs is in progress. Twenty such cosmids have been mapped and demonstrate that the contigs are distributed over the whole of chromosome 16.
2. *Mapping of anonymous DNA probes known to be on chromosome 16 (Dr. Lerner, USA; Dr. Harris).*
Six cosmid probes from Dr. Lerner containing NotI restriction sites and twenty-four plasmids from Dr. Harris containing unique chromosome 16 sequences have been mapped to the hybrid panel (Lerner et al.). This has enabled the ordering of breakpoints, especially in the vicinity of 16q22.1.
3. *Correlation of genetic and physical maps (Dr. Julier, France and Dr. Keith, USA).*
The polymorphic probes used by these two groups to construct genetic maps of chromosome 16 are currently being physically mapped. To this time 21 probes have been mapped. This will allow the correlation between the genetic and physical maps.
4. *Mapping the gene for Battens disease (Dr. Gardiner, UK).*
The gene for Battens disease has been mapped by linkage to chromosome 16. The hybrid panel has been used to physically map those probes which show close genetic linkage. This locates the disease gene to 16p11.2.
5. *Mapping regions of the genome involved in oncogenesis (Dr. Breuning, The Netherlands; Drs. Tsuda and Hirohashi, Japan).*
Regions of chromosome 16 involved in oncogenesis involve p13 (the inv(16) of ANLL, M4EO and t(8;16) in ANLL, M5) and q23 (loss of heterozygosity of this region in hepatocellular carcinomas). Collaboration with these groups involves provision of detailed physical maps of the appropriate regions with updates as more information becomes available, and supply of requested hybrid cell lines and probes.
6. *Mapping microsatellite repeats (Weber, USA).*
Microsatellite (AC/GT) repeats isolated by Webber are being physically mapped to chromosome 16.
7. *Mapping of genes (various collaborators).*
Probes and gene sequences for genes known to be on chromosome 16 have been obtained. These have been physically mapped to the hybrid panel either by PCR (when appropriate gene sequence is available) or by probing Southern blots.
8. *Use of the hybrid panel for other genome research (various collaborators).*
There have been numerous requests for hybrid cell lines which may be of use for the mapping of other chromosomes. For example CY2 and CY3 has been supplied to numerous groups involved with the long arm of the X-chromosome. Similarly, Dr. Goodfellow (Canada) has used CY5, CY6 and CY7 for mapping probes on chromosome 10.

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