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Absence of Genomic Imprinting at the DiGeorge Locus

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INTRODUCTION

Fluorescence in situ hybridization (FISH) has been used to visualize specific genomic DNA sequences in interphase nuclei. Timing of replication can be measured by FISH to interphase nuclei: nuclei with a sequence that has not replicated reveal two single signals (G_1), whereas those in which the sequence has replicated show two signal doublets (G_2). Asynchronous nuclei show a single signal on one allele and a double hybridization dot on the other homologue. In general, most sequences replicate synchronously on the two homologues, with only 10% of nuclei showing an asynchronous hybridization pattern. However, for the sequences known about to be imprinted, approximately 30% of nuclei reveal asynchronous replication. Little is known whether or not the proximal region of chromosome 22, involved in the DiGeorge syndrome [1], is imprinted. We have, therefore, examined the replication timing pattern of the DiGeorge critical region (DGCR).

MATERIAL AND METHODS

Chromosome Preparation

Chromosome slides were made from 72-hour phytohemagglutin-treated lymphocyte cultures of peripheral blood. The slides were air dried and stored at 20 °C until use.

Probes

Cosmid probes used in this study were as follows: c237, c350 (kindly provided by P. Scambler) and 48F8, cos40 (a gift from G. Thomas), and were previously mapped to the DGCR (Fig. 1). PDN34 mapping at the PW/AS locus was used as the control. Cosmid probes were prepared using an alkali procedure.

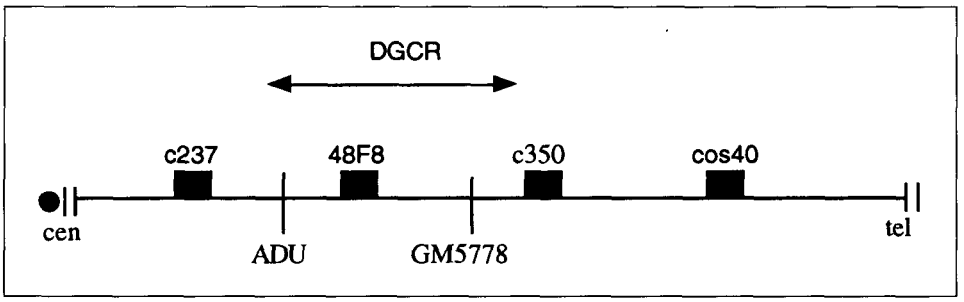

Fig. 1 - Partial physical map of the DGCR.

In situ Hybridization

Probes were labelled by nick translation using biotinylated 14-dATP (BRL). The slides were denatured in 70% formamide/2-SSC for 5 min at 70 °C, and passed through a cold-ethanol series. 60 ng of denatured probes were applied to the slides in a hybridization buffer containing 50% formamide; the slides were incubated overnight at 37 °C. After hybridization, the slides were washed 35 min in 50% formamide/2×SSC and three times in 2SSC at 37 °C. Detection was carried out at 37 °C using a goat antibiotin antibody (Sigma) followed by FITC-conjugated antigoat antibody (Sigma).

Scoring and Analysis

All analyses were performed using a Leitz DM microscope and photographed on Kodak Ektachrome 400. We scored 200 nuclei per slide to determine the percentage of cells with single signals (G_1) double signals (G_2) and single-double signals (G_1/G_2). Each experiment was duplicated for all used probes.

RESULTS

Hybridization analysis of the cosmid probes for the proximal part of chromosome 22 revealed asynchronous replication, with 8-12% of the cells in G_1/G_2. The level of synchronous replication was 50-60% cells in G_1 cells and 30-38% in G_2. In contrast, asyncronous replication was observed in 35% of nuclei with PDN34 (PW/AS region) (Fig. 2-3).

DISCUSSION

FISH is a sensitive method to analyze the timing of replication. It has recently been shown that asynchronous replication occurs in regions known to be subject to parental imprinting [2]. In our study, we found a low level of asynchronous nuclei, suggesting the absence of imprinting at the DiGeorge locus. This approach provides a screening for imprinted genes complementary to other methods, such as phenotypes associated with uniparental disomy (UPD).

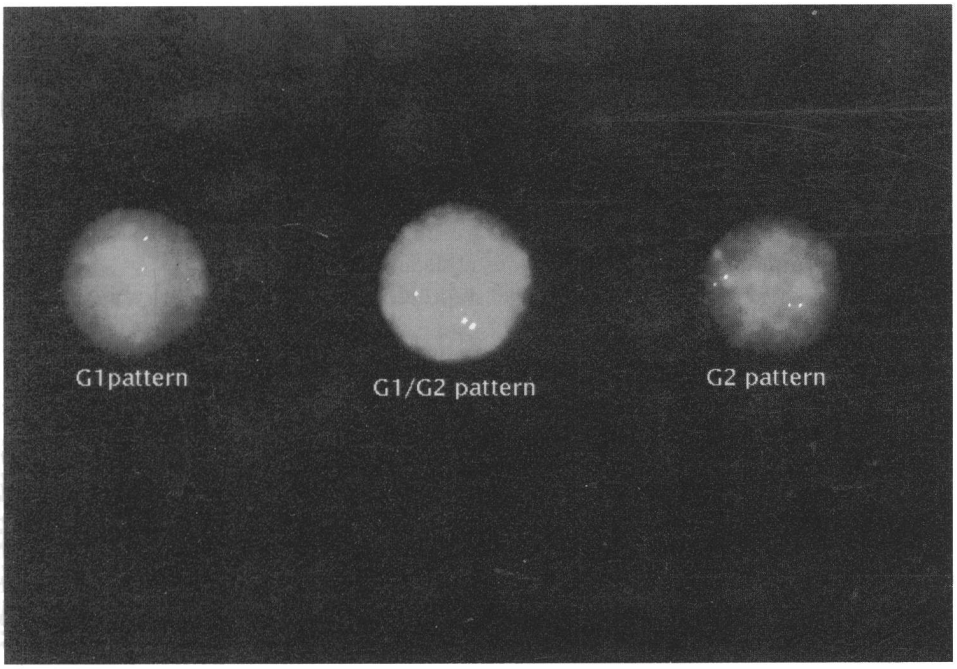

Fig. 2 - Replication pattern with the chromosome 22 sequence. G_1 pattern = the sequence has not yet replicated; G_1/G_2 pattern = only one homologue has replicated; G2 pattern = the sequence has replicated.

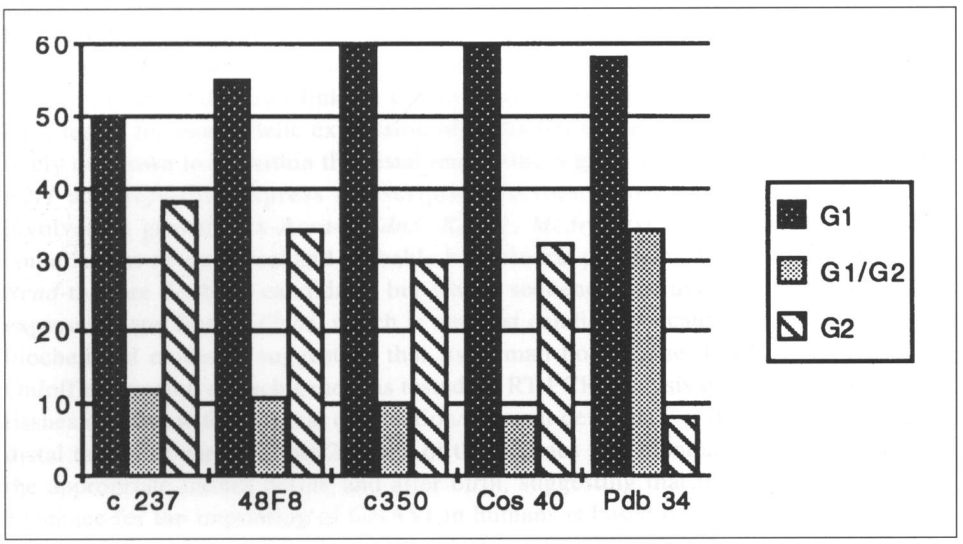

Fig. 3 - Schematic representation of the percentage of different nuclei observed with cosmid probes.

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The UPD appproach can efficiently exclude major imprinting effects over a large region, but minor effects which do not result in clinical abnormalities cannot be excluded.

A possible mechanism to explain UPD is the "correction" of chromosomal trisomy. Therefore, frequencies of chromosomal nondisjunction are different in males and females. It is then difficult to exclude the existence of paternaly imprinting.

The replication-timing method is not absolute. There is no evidence to suggest that all loci showing asynchronous replication are imprinted or that all regions subjects to parental imprinting will show asynchronous replication.

It appears then, that to exclude parental imprinting it is neccesary to combine several approches.

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2. Kitsberg D, Seilig S, Brandels M, Simon I, Koshet I, Driscoll D, Nicholls R, Cedar H: Allele-specific replication timing of imprinted gene regions. Nature 1993; 364: 459-463.

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