A FISH study of chromosome fusion in the ICF syndrome: involvement of paracentric heterochromatin but not of the centromeres themselves

A T Sumner, A R Mitchell, P M Ellis

Abstract

We have used double fluorescence in situ hybridisation to study the involvement of centromeres and paracentromeric heterochromatin in the chromosome abnormalities seen in the ICF syndrome. To detect centromeres, we used a probe which labelled alphoid satellite DNA, and for the paracentromeric heterochromatin a probe for classical satellite II. Our results show that it is always the paracentromeric heterochromatin of the relevant chromosomes that becomes decondensed in this syndrome and which fuses to produce multiradial configurations. However, the centromeric regions, identified by their content of alphoid satellite DNA, appear never to become decondensed and always remain outside the regions of chromosome fusion in the multiradials.

(J Med Genet 1998;35:833-835)

Keywords: ICF syndrome; heterochromatin; centromeres; FISH

The ICF syndrome is a rare human genetic disorder, the symptoms of which include Immunodeficiency, Centromeric instability, and Facial anomalies. The centromeric instability includes decondensation of the heterochromatin of chromosomes 1, 9, and 16, and, very occasionally, of one or two other chromosomes, and the fusion of the heterochromatic regions of these chromosomes to form multiradial configurations, comprising arms from several of these chromosomes. For recent reports on the chromosome abnormalities found in this syndrome, see Smeets *et al.*¹ Brown *et al.*² and Schuffenhauer *et al.*³; the first two of these also review all the cases reported up to their dates of publication.

Although the large blocks of heterochromatin on chromosomes 1, 9, and 16 can become decondensed and may fuse to form multiradial configurations in patients with this syndrome, it has not been clear whether the centromeres themselves are involved in these processes. Using C₄ staining, Gimelli *et al.*⁴ postulated that the centromeres themselves were not directly involved in the formation of multiradials, but that the paracentromeric heterochromatin, which was identified by *in situ* hybridisation, was involved. However, although C₄ staining is generally regarded as a marker for centromeres, the basis of its specificity is not known.⁵

Miniou *et al.*⁶ and Schuffenhauer *et al.*³ also used FISH to show the involvement of classical satellite II in the decondensation and fusion of chromosomes in the ICF syndrome, but did not show images of FISH studies of the centromeres themselves. We have therefore used two colour fluorescence PRINS *in situ* hybridisation (FISH) to study the centromeres and paracentromeric heterochromatin simultaneously in chromosomes from a patient with the ICF syndrome.

Materials and methods

The patient from whom chromosomes were obtained for this study has already been described in detail,² and chromosome preparations were obtained from the patient as described in that paper. Fluorescence *in situ* hybridisation was carried out using a double PRINS technique,⁷ described in detail by Mitchell *et al.*⁸ Two oligonucleotides were used. Oligonucleotide 405 detects alphoid repetitive DNA sequences,⁹ while B18 hybridises to the human satellite II simple sequence family. The sequence of B18 is 5'-CGTTGATTCCATTTGATGTTGATTCCA TTC-3'.¹⁰ Oligonucleotide 405 incorporated digoxigenin-11-dUTP during the PRINS reaction, and the digoxigenin was detected using anti-digoxigenin-FITC FAB fragments (Boehringer Mannheim). The oligonucleotide B18 incorporated bio-11-dUTP (Boehringer Mannheim) during the PRINS reaction, and detection of the biotin was achieved using avidin-Texas red (Vector Laboratories). Chromosomes were labelled with DAPI to produce blue fluorescence. The images were captured using a Zeiss Axioplan fluorescence microscope equipped with a 100 W mercury lamp and a photometric CCD camera. Images were captured and processed using software from Digital Scientific (Cambridge, England). Exact registration of images was achieved using a Chroma P1 filter set mounted on a rotating wheel. False colour images were photographed from the computer screen; the colours were chosen to correspond as closely as possible to the natural colours of the fluorescence, so that DAPI appears blue on the photographs, sites of alphoid satellite DNA green, and satellite II pink.

Results

The probe for alphoid satellite DNA, oligonucleotide 405, labelled all or most of the centromeres in every metaphase examined, whereas

MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, UK

A T Sumner*
A R Mitchell

Cytogenetics Laboratory, Royal Hospital for Sick Children, Edinburgh EH9 1LF, UK
P M Ellis

Correspondence to:
Dr Ellis.

*Present address: 7 Smileyknowes Court, North Berwick, East Lothian EH39 4RG, UK.

Received 13 October 1997
Revised version accepted for publication 23 March 1998

Figure 1 Metaphase spreads from the patient with ICF syndrome showing results of double in situ hybridisation with the probe for alphoid satellite DNA (green) and the probe for satellite II (pink). Chromosomes are counterstained with DAPI (blue). (A) Extended heterochromatin is labelled pink for satellite II (arrow), while the green centromeric signal remains compact. (B) Multiradial configuration (arrowed) showing a single fused block of heterochromatin labelled pink (satellite II), surrounded by separate green signals for the centromeres (alphoid satellite). Note that the very pale pink fluorescence in the multiradial configuration is the result of overexposure of the photograph in this region of highly concentrated satellite DNA.

the satellite II probe, B18, labelled only the paracentromeric heterochromatin of chromosomes 1, 9, and 16 (fig 1). However, when the heterochromatin was decondensed, the thin thread produced label throughout its length with the satellite II probe, while the region labelled with the alphoid satellite probe remained condensed, and showed the same appearance as in chromosomes that did not show any decondensation of the heterochromatin (fig 1A).

In multiradial configurations, the region labelled with the satellite II probe always formed a single block (fig 1B), while the alphoid satellite probe labelled discrete blocks on individual chromosome arms surrounding the block of satellite II. The multiradials are, therefore, formed by fusion of the paracentromeric heterochromatin, while the centromeres themselves remain separate.

Discussion

In this study, we have used oligonucleotide probes which recognise alphoid satellite DNA and classical satellite II DNA to label the centromeres and the paracentromeric heterochromatin, respectively, in chromosomes from a patient with the ICF syndrome. As shown by Mitchell *et al*⁹ for alphoid and satellite III DNA in man, the two families of repetitive DNA studied here also occupy distinct domains. Our results confirm that it is the regions of paracentromeric heterochromatin, containing satellite II, which can become decondensed in this syndrome and can fuse to form multiradials. This is in agreement with morphological studies that pointed to the decondensation and fusion of heterochromatin (see, in particular, the scanning electron microscopy studies reported by Brown *et al*²), and confirms more recent FISH studies.^{3 4 6 11} It is equally clear that the centromeres, as identified by hybridisation of the alphoid satellite probe, do not appear to decondense and are excluded from

the fusions by which the multiradials are formed. This is, of course, consistent with the observation that normally only those chromosomes which carry substantial blocks of paracentromeric heterochromatin are involved in the chromosome abnormalities characteristic of the ICF syndrome.

It is not, at present, possible to explain fully the connection between the various features of the ICF syndrome, but it seems plausible that the same process could give rise to the immunodeficiency and the formation of multiradial chromosomes. Both immunodeficiency and the production of multiradials could be the result of a failed recombination process. It has been shown that recombination can occur between experimentally decondensed segments of heterochromatin.¹² The process involved in the ICF syndrome would presumably be failure to resolve recombination intermediates rather than failure to initiate recombination, otherwise no multiradials would be formed.

- 1 Smeets DFCM, Moog U, Weemaes CMR, *et al*. ICF syndrome: a new case and review of the literature. *Hum Genet* 1994;94:240-6.
- 2 Brown DC, Grace E, Sumner AT, Edmunds AT, Ellis PM. ICF syndrome (immunodeficiency, centromeric instability and facial anomalies): investigation of heterochromatin abnormalities and review of clinical outcome. *Hum Genet* 1995;96:411-16.
- 3 Schuffenhauer S, Bartsch O, Stumm M, *et al*. DNA, FISH and complementation studies in ICF syndrome: DNA hypomethylation of repetitive and single copy loci and evidence for a trans acting factor. *Hum Genet* 1995;96:562-76.
- 4 Gimelli G, Varone P, Pezzolo A, Lerone M, Pistoia V. ICF syndrome with variable expression in sibs. *J Med Genet* 1993;30:429-32.
- 5 Sumner AT. *Chromosome banding*. London: Unwin Hyman, 1990.
- 6 Miniou P, Jeanpierre M, Blanquet V, *et al*. Abnormal methylation pattern in constitutive and facultative (X inactive chromosome) heterochromatin of ICF patients. *Hum Mol Genet* 1994;3:2093-102.
- 7 Koch JE, Kølvræ S, Petersen KB, Gregersen N, Bolund L. Oligonucleotide priming methods for the chromosome-specific labelling of alpha satellite DNA in situ. *Chromosoma* 1989;98:259-65.
- 8 Mitchell AR, Nicol L, Malloy P, Kipling D. Novel structural organisation of a *Mus musculus* DBA/2 chromosome shows a fixed position for the centromere. *J Cell Sci* 1993;106:79-85.

- 9 Mitchell A, Jeppesen P, Hanratty D, Gosden J. The organization of repetitive DNA sequences on human chromosomes with respect to the kinetochore analyzed using a combination of oligonucleotide primers and CREST anti-centromere serum. *Chromosoma* 1992;101:333-41.
- 10 Hollis M, Hindley J. Satellite II DNA of human lymphocytes: tandem repeats of a simple sequence element. *Nucleic Acids Res* 1988;116:363.
- 11 Stacey M, Bennett MS, Hulten M. FISH analysis on spontaneously arising micronuclei in the ICF syndrome. *J Med Genet* 1995;32:502-8.
- 12 Kokalj-Vokac N, Almeida A, Viegas-Péguignot E, Jeanpierre M, Malfroy B, Dutrillaux B. Specific induction of uncoiling and recombination by azacytidine in classical satellite-containing constitutive heterochromatin. *Cytogenet Cell Genet* 1993;63:11-15.