Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Enrico Ginelli, K.W. Jones, Gianmarco Corneo

Chromosomal location of human fast intermediate DNA

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **64** (1978), n.5, p. 499–504.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1978_8_64_5_499_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1978.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — *Chromosomal location of human fast intermediate* DNA (*). Nota di ENRICO GINELLI (**), K. W. JONES (***) e GIANMARCO CORNEO, presentata (****) dal Socio S. RANZI.

RIASSUNTO. — Una frazione di DNA umano, chiamata « Human Fast Intermediate DNA », isolata da DNA umano totale rinaturato a valori intermedi di Cot mediante centrifugazione all'equilibrio in un gradiente di Ag^+—Cs_2SO_4, è stata localizzata sui cromosomi metafasici umani con la tecnica dell'ibridazione *in situ*.

Questa frazione di DNA è prevalentemente localizzata a livello dell'eterocromatina pericentromerica dei cromosomi 1,9 e 16.

La localizzazione è evidente anche in molte altre regioni pericentromeriche, incluse quelle dei cromosomi dei gruppi D e G e nella regione distale fluorescente del cromosoma Y.

Parte dei grani autoradiografici è inoltre distribuita sui bracci cromosomici.

In previous works satellite and other repeated DNAs were localized on different, mainly heterochromatic, sites of human chromosomes by *in situ* hybridization [1-7].

We have now studied the chromosomal location of a human Fast Intermediate DNA (F.I. DNA); this has been isolated in renatured form from human placental total DNA by centrifugation of high molecular weight DNA renatured to an intermediate value of Cot in an equilibrium Ag^+—Cs_2SO_4 density gradient [8].

The results obtained indicate that such F.I. DNA is widely distributed in many pericentromeric and other heterochromatic sites in human chromosomes.

DNA *extraction and density gradient centrifugation.*

Human placental DNA was extracted by Marmur's method [9]. High molecular weight DNA, in native or reannealed form (Cot 50), was centrifuged in analytical and preparative Ag^+—Cs_2SO_4 density gradients as reported elsewhere [8, 10]. Seventy $\mu g\ ml^{-1}$ of DNA and Ag^+/DNA-P, molar ratio of 0.2 and 0.3, and thirty-five $\mu g\ ml^{-1}$ of DNA and a Ag^+/DNA-P, molar ratio of 0.2, were used respectively in native and reannealed DNA experiments.

For all the experiments the initial density of the solution was about 1.48–1.50 g ml^{-1}.

(*) Work carried out at Centro di Studi di Patologia Molecolare applicata alla Clinica, Via Pace 15, 20122 Milano. Director: Prof. Luigi Villa, with the support of C.N.R. grants No. CT. 76.01203.04 and 77.01644.04.

(**) Istituto di Biologia Generale, Facoltà di Medicina, Università di Milano, Via Viotti 5, 20133 Milano, Italy.

(***) Institute of Animal Genetics, University of Edinburg, West Mains Road, Edinburg, EH9 3JN Scotland.

(****) Nella seduta del 13 maggio 1978.

In vitro DNA *transcription.*

F.I. DNA, isolated by preparative Ag^+—Cs_2SO_4 density gradient centrifugation of total human DNA reannealed to Cot 50 [8], was transcribed into *c*-RNA as previously described [11].

The DNA was dialysed against 5 M NaCl and then against 0.01 M Tris Buffer, pH 8 and transcription was then performed using equimolar amounts of ^{3}H-ATP, ^{3}H-UTP, ^{3}H-CTP and ^{3}H-GTP (specific activity 15-20 Ci/mM) or ^{14}C-UTP (specific activity 240 mCi/mM), as labelled precursors, and *E. Coli* RNA polymerase [12]. The estimated specific activities of ^{3}H-*c*-RNA and ^{14}C-*c*-RNA were $1.4\text{-}2\times10^7$ cpm μg^{-1} and 5×10^5 cpm μg^{-1} respectively.

DNA/RNA *filter hybridization.*

Filter hybridization was carried out in DNA excess. Loading of Ag^+—Cs_2SO_4 DNA fractions and hybridization conditions were as previously described [8, 13]. A total of 50 μg from each gradient was hybridized with 4×10^4 cpm of ^{14}C-complementary RNA in $2\times$SSC at 55 °C for 20 h. The filters were then RNAase treated, washed in $2\times$SSC, dried and counted in a scintillation counter.

In situ *hybridization.*

In situ hybridization was performed using ^{3}H-complementary RNA in $3\times$SSC at 55 °C for 20 h on chromosomes which had been prepared from human male blood by the conventional phytohaemagglutinin-air drying procedure.

The chromosomes were heat denatured for 10 seconds, a procedure which has been found to promote more efficent *in situ* hybridization [14].

After hybridization the slides were RNAase treated, washed exhaustively in $2\times$SSC, then dipped in Ilford K 2 liquid emulsion diluted 50 : 50 with water. After drying the slides were exposed for two months.

As shown in Fig. 1, F.I. DNA bands as a separate peak on the light side in a Ag^+—Cs_2SO_4 density gradient when high molecular weight total human DNA reannealed to Cot 50 is centrifuged.

F.I. DNA and the pool of satellite DNAs (corresponding to the minor peak on the right of the gradient) separate in these conditions because, being renatured, they bind fewer silver ions than the main unique DNA.

High molecular weight F.I. DNA has a CsCl density of 1.703 g ml^{-1} in renatured form. Its buoyant density decreases to 1.700 g ml^{-1} when it is renatured at low molecular weight; it accounts for 6 % of total DNA and its approximate reiteration frequency is 10^5 [8].

In a Ag^+—Cs_2SO_4 preparative gradient similar to the analytical gradient of Fig. 1, the DNA fractions corresponding to the F.I. DNA were pooled, dialysed against 5 M NaCl and recentrifuged in a preparative neutral CsCl gradient to allow a greater purification of the DNA template.

In order to verify whether the F.I. DNA shows sequence correlation to the known satellite DNAs its labelled complementary RNA was hybridized to total native human DNA fractionated in Ag^+—Cs_2SO_4 gradients (Figs. 2 A and B) in conditions suitable for the separation of human satellite DNAs [10].

The two experiments reported in Fig. 2 show that the major proportion of the radioactivity, due to F.I. DNA *c*-RNA hybridization, is under the main band DNA and a minor amount of radioactivity is in the regions of the gradients containing satellite DNAs.

On the other hand the hybridization profiles under the main band DNA are in agreement with previous data indicating that this class of sequences is not completely organized in clusters at high molecular weight, but similar nucleotide sequences are spread in DNA molecules having different G-C content and/or different kinetic properties [8].

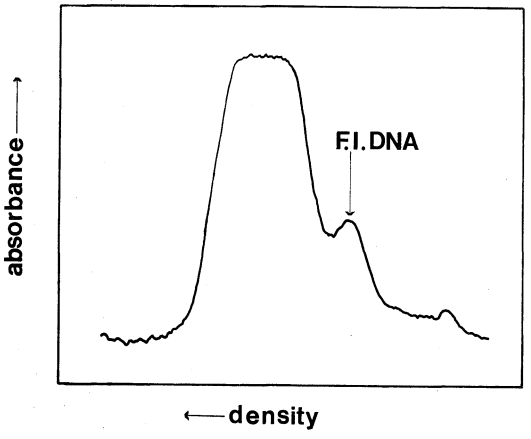


Fig. 1. – Analytical ultracentrifugation in Ag^{+}—Cs_2SO_4 of high molecular weight total human DNA renatured to Cot 50.

The low amount of radioactivity in the regions of the gradients containing the satellite DNAs may indicate a low contamination or some extent of sequence correlation between F.I. DNA and satellite DNAs. These data may also be interpreted as due to the presence of F.I. DNA sequences in the regions of the gradients containing satellite DNAs.

In the gradients shown in Fig. 2 all the highly reiterated sequences corresponding to satellite DNAs I, II, III are almost completely outside the main band DNA as shown by hybridization of *c*-RNAs of the three satellites across the gradients (E. Ginelli, unpublished results). Under these conditions the sequences of satellite DNA IV [15] are located near the DNA peak of satellite III (E. Ginelli, unpublished results).

Therefore F.I. DNA appears to be a class of sequences different from the previously characterized human satellite DNAs.

In order to localize F.I. DNA sequences on human chromosomes, *c*-RNA was *in situ* hybridized to human male blood arrested metaphases prepared by the conventional phytohaemagglutinin-air drying procedure (Plate I, Figs. *a* and *b*).

In both Figs. autoradiographic grains are present in a heavy concentration over the centromeric regions of chromosomes 1, 9 and 16 as shown previously in the case of human satellite DNA II [1].

However, many other centromeric sites are labelled. These include prominent label over chromosomes 4, 7, 10, 12, 13, 15, 17, 19, 20, 22, and the distal fluorescent part of the Y chromosome. In this regard it is interesting to note that some chromosomes of groups D and G which are known to exhibit association at metaphase, probably in relation to their participation in nucleous organization [16], show very consistent hybridization grains in the heterochromatic regions near the centromere (arrowed in Plate I, fig. *b*).

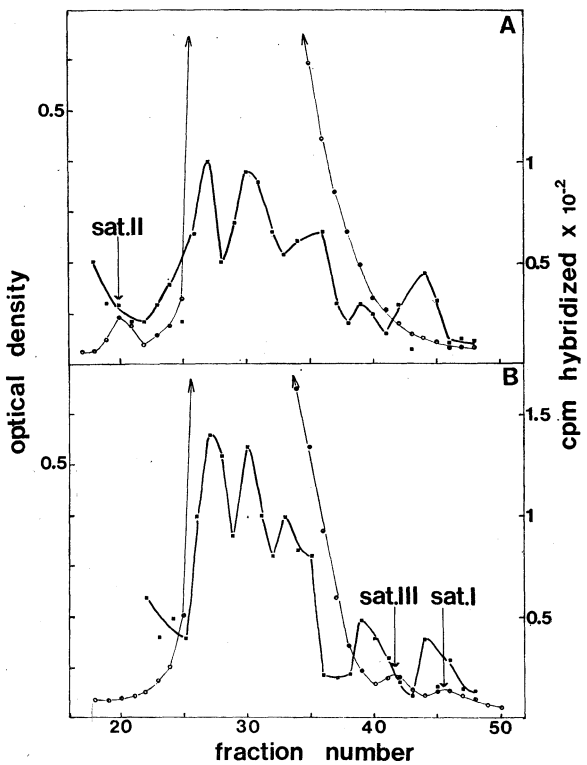


Fig. 2. – Hybridization of Fast Intermediate DNA ^{14}C-labelled *c*-RNA to fractions of human native DNA centrifuged in Ag^+—Cs_2SO_4 density gradient (A): Ag^+/DNA-P = 0.3; (B): Ag^+/DNA-P = 0.2 ■-■ = Radioactivity; o–o = optical density.

Minor autoradiographic grain concentrations are also seen over centromeric regions of chromosomes 3, 5, 6, 8, 11, 14, 18, 21. In agreement with the previous study on sequence organization of F.I. DNA [8] grains are also seen scattered in other regions of many chromosomes, including the X chromosome. These extracentromeric sites are sometimes terminal (telomeric) as well as interstitial and are frequently isochromatid in distribution.

Consistent with the extracentromeric distribution of autoradiographic grains interphase nuclei show hybridization over sites other than heterochromatin.

It therefore seems likely that the F.I. DNA sequences have a wider distribution in the human karyotype than do the satellite DNAs described thus

far. However it must be stated that the heat denaturation method used here appears to give a higher efficiency of hybridization [14] so that comparisons between the present finding and those described previously may not be strictly valid. For example, it appears that mouse satellite DNA, which previously has been described as located exclusively in centromeric heterochromatin [17–18], is also detectable in other regions of the mouse genome after heat denaturation of fixed chromosomes. Re-examination of previous assignment of human satellite DNAs is therefore indicated.

In conclusion, the localization of F.I. DNA in many heterochromatic sites of human chromosomes is in agreement with previous data indicating that the DNA fraction is in part satellite-like. The distribution of previously known human satellite DNAs and human Fast Intermediate DNA appears to cover almost all the pericentromeric heterochromatic sites of human chromosomes.

REFERENCES

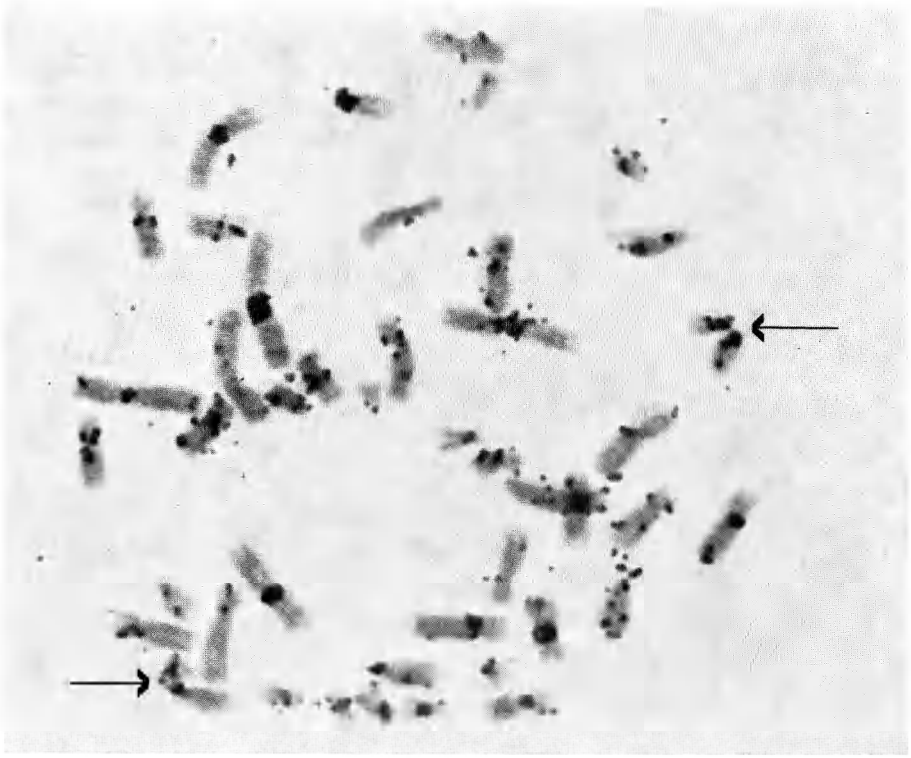
[1] K. W. JONES and G. CORNEO (1971) – « Nature (Lond.) New Biology », *233*, 268–271.
[2] G. F. SAUNDERS, T. C. HSU, M. GETS, E. L. SIMES and F. E. ARRIGHI (1972) – « Nature (Lond.) New Biology », *236*, 244–246.
[3] K. W. JONES, J. PROSSER, G. CORNEO and E. GINELLI (1973) – « Chromosoma (Berl.) », *42*, 445–451.
[4] K. W. JONES, I. F. PURDOM, J. PROSSER and G. CORNEO (1974) – « Chromosoma (Berl.) », *49*, 161–171.
[5] J. R. GODSEN, R. A. BUCKLAND, R. P. CLAYTON and H. J. EVANS (1975 a) – « Expl. cell Res. », *92*, 138–147.
[6] J. R. GODSEN, A. R. MITCHELL, R. A. BUCKLAND, R. P. CLAYTON and H. J. EVANS (1975 b) – « Expl. cell Res. », *92*, 148–158.
[7] K. A. MARX, J. R. ALLEN and J. E. HEARST (1976) – « Chromosoma (Berl.) », *59*, 23–42.
[8] E. GINELLI and G. CORNEO (1976) – « Chromosoma (Berl.) », *56*, 55–68.
[9] J. MARMUR (1961) – « J. molec. Biol. », *3*, 208–218.
[10] G. CORNEO, E. GINELLI and E. POLLI (1971) – « Biochim. biophys. Acta (Amst.) », *247*, 528–534.
[11] K. W. JONES (1973) – In: *New Techniques in biophysics and cell biology* (R. H. Pain and B. J. Smith eds.), vol. 1, pp. 29–66. London, J. Wiley and Sons 1973.
[12] M. CHAMBERLIN and P. BERG (1962) – « Proc. nat. Acad. Sci. (Wash.) », *48*, 81–94.
[13] M. MELLI, E. GINELLI, G. CORNEO and R. DI LERNIA (1975) – « J. molec. Biol. », *93*, 23–38.
[14] L. SINGH, I. F. PURDOM and K. W. JONES (1977) – « Chromosoma », *60*, 337–389.
[15] G. CORNEO, L. ZARDI and E. POLLI (1972) – « Biochim. biophys. Acta (Amst.) », *269*, 201–204.
[16] M. A. FERGUSON-SMITH (1964) – « Cytogenetics », *3*, 124–134.
[17] K. W. JONES (1970) – « Nature (Lond.) », *225*, 912–915.
[18] M. L. PARDUE and J. G. GALL (1970) – « Science », *168*, 1356–1368.

EXPLANATION OF PLATE I

a) Karyotype of human male metaphase chromosomes after hybridization with Fast Intermediate DNA ^{3}H-complementary RNA. Chromosomes are arranged in the conventional manner. This fig. very clearly shows the dense accumulation of silver grains over pericentromeric heterochromatin of chromosomes 1, 9 and 16.

b) Hybridization of Fast Intermediate DNA ^{3}H-labelled *c*-RNA with human male metaphase chromosomes. Arrows indicate chromosomes of groups D and G involved in centric association.

a



b