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A compositional map of human chromosome 21

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GC-poor and GC-rich isochores, the long (>300 kb) compositionally homogeneous DNA segments that form the genome of warm-blooded vertebrates, are located in G- and R-bands respectively of metaphase chromosomes. The precise correspondence between GC-rich isochores and R-band structure is still, however, an open problem, because GC-rich isochores are compositionally heterogeneous and only represent one-third of the genome, with the GC-richest family (which is by far the highest in gene concentration) corresponding to <5% of the genome. In order to clarify this issue and, more generally, to correlate DNA composition and chromosomal structure in an unequivocal way, we have developed a new approach, compositional mapping. This consists of assessing the base composition over 0.2–0.3 Mb (megabase) regions surrounding landmarks that were previously localized on the physical map. Compositional mapping was applied here to the long arm of human chromosome 21, using 53 probes that had already been used in physical mapping. The results obtained provide a direct demonstration that the DNA stretches of G-bands essentially correspond to GC-poor isochores, and that R-band DNA is characterized by a compositional heterogeneity that is much more striking than expected, in that it comprises isochores covering the full spectrum of GC levels. GC-poor isochores of R-bands may, however, correspond to 'thin' G-bands, as visualized at high resolution, leaving GC-rich and very GC-rich isochores as the real components of (high-resolution) R-band DNA. Very GC-rich isochores, which are indeed characterized by the highest gene concentration, as expected from previous data, are localized at the telomere. This situation might be common to all telomeres which correspond (like the telomere of the long arm of chromosome 21) to T-bands, namely to the telomeres of many human chromosomes.

Key words: compositional mapping/human chromosome 21/ isochores

Introduction

The human genome, like those of all warm-blooded vertebrates, is made up of isochores, which are long (>300 kb), compositionally homogeneous stretches of DNA that belong to a small number of families characterized by different GC levels (Bernardi *et al.*, 1985; Bernardi, 1989). Isochores show correlations with both genes and chromosomes. On the one hand, the GC levels of genes, introns, exons and of different codon positions show linear correlations with the GC levels of the isochores that harbor them (Bernardi *et al.*, 1985). On the other hand, a number of lines of evidence (Bernardi, 1989) indicate that GC-poor isochores correspond to DNA stretches that are located in G-bands (Giemsa positive, or Giemsa dark bands), whereas GC-rich isochores correspond to DNA stretches that are located in R-bands (reverse bands; these are equivalent to Giemsa negative, or Giemsa light bands). This band–isochore correspondence needs, however, to be clarified further, for the following reasons.

(i) Cytogenetics indicates that the ratio of G- and R-bands is 1:1, whereas the ratio of GC-poor to GC-rich isochores is 2:1. This may mean (a) that standard R-bands comprise more 'thin' G-bands (only detected at high resolution; see below) than standard G-bands comprise 'thin' R-bands, as suggested by recent observations (Ikemura and Aota, 1988); and/or (b) that DNA compaction is higher in G-bands than in R-bands.

(ii) While GC-poor isochores, which are present in G-bands, differ very little from each other in composition, GC-rich isochores, which are located in R-bands, encompass a wide GC range (Bernardi *et al.*, 1985; Bernardi, 1989). This should lead to inter- and/or intra-band compositional heterogeneity in R-bands.

(iii) The interspersion of different GC-rich isochores within individual R-bands (at standard resolution) is indicated by the finding that many genes located in R-bands of a number of chromosomes (Aota and Ikemura, 1986; Ikemura and Aota, 1988) are present in the GC-richest fraction of the human genome, the isochore family H3 (Mouchiroud *et al.*, 1987, 1988; Bernardi *et al.*, 1988, 1989). Since H3 only represents 3–5% of the genome, it cannot account for the totality of DNA of all R-bands in which it is present, and other DNA components, characterized by lower GC levels, must also be present. A corollary of this conclusion is that since gene concentration is highest, by far, in the isochores of the H3 family (Bernardi *et al.*, 1985, 1988; Mouchiroud *et al.*, 1987), regions of low and high gene concentration should exist not only in G- and R-bands respectively, but also within R-bands.

The facts quoted above indicate that GC-rich isochores (and probably GC-poor isochores as well) correspond to a finer level of chromosomal structure than standard metaphase chromosome bands. Because of the evolutionary and functional significance of isochores (Bernardi *et al.*, 1985, 1988; Mouchiroud *et al.*, 1987, 1988; Bernardi, 1989), it is of interest to identify such a level. Isochores might correspond to the DNA stretches of high-resolution chromosomal bands, and/or of chromomeres and interchromomeres. High-resolution bands have been obtained in mid-prophase mitotic chromosomes (Yunis, 1976, 1981; Viegas-Pequignot and Dutrillaux, 1978). Chromomere–interchromomere patterns are usually obtained in pachytene

bivalents of meoitic chromosomes (Ferguson-Smith and Page, 1973; Okada and Comings, 1974; Luciani *et al.*, 1975), and strikingly resemble high-resolution bands, with chromomeres corresponding to G-bands, and inter-chromomeres to R-bands (Ambros and Sumner, 1987). Isochores might, however, also correspond to a finer structural level than high-resolution bands.

In order to establish this band−isochore correlation and, more generally, to define the compositional pattern of DNA along the chromosomes, we have developed a novel approach to the analysis of chromosomes below the standard band level. We call this approach compositional mapping. Compositional maps are constructed by assessing GC levels around landmarks (localized on physical maps) that can be probed. This simply requires the hybridization of the probes on DNA fractionated according to base composition. For instance, if DNA preparations of ~0.1−0.15 megabase (Mb) in size are used, as done in the present work, compositional mapping can define the base composition of DNA stretches of ~0.2−0.3 Mb, i.e. twice as long, around the sequence probed. (Obviously, using higher mol. wt DNA preparations could push up the size range that is explored from the point of view of composition.) Indeed, the probed sequence may be located at any position on the DNA fragments carrying it, since these fragments derive from the random physical and enzymatic degradation that occurs during preparation. This approach was tried in the present work for a set of 53 unique-sequence probes localized on the long arm of human chromosome 21, which is the best physically mapped extended region of the human genome (Gardiner *et al.*, 1988, 1990).

Results

Figure 1 shows the CsCl profiles of human DNA fractions, as obtained by preparative ultracentrifugation in a Cs_2SO_4/BAMD density gradient, that were used for the hybridization of probes. BAMD is 3,6-bis(acetato-mercurimethyl)dioxane, a sequence-specific DNA ligand (Bünemann and Dattagupta, 1973) that has been widely used in DNA fractionation (Bernardi *et al.*, 1985; Bernardi, 1989).

Figure 2 shows the results of fraction localization for two anonymous probes, 267 and H8, and for the probe of the α-crystallin gene, CRY A. While probe 267 is located in the GC-poor fractions 1−2 (40−41% GC) and probe H8 in fractions 2−3 (41−43% GC), CRY A is centered on the GC-rich fraction 8 (52% GC). With only three exceptions (see Salinas *et al.*, 1986, for a discussion of such cases), each probe tested hybridized most intensely to only one or two fractions. The hybridization results for all probes are presented in Table I, along with the regional localization information.

Currently the physical map of chromosome 21 accounts for essentially the entire long arm, because the identified *Not*I fragments total 40 Mb, the upper estimate for the size of this region. The pulsed-field data then are sufficiently detailed to allow direct correlations of the regions defined by translocation breakpoints with the band pattern (Gardiner *et al.*, 1990). For example, the A regions map to bands q11 and q21; the D regions to q22.1; region B1 to band q22.2; and the remainder to q22.3 (see Table I). This unambiguous placement of translocation breakpoints has allowed the assignment of probes (and pulsed-field fragments) to

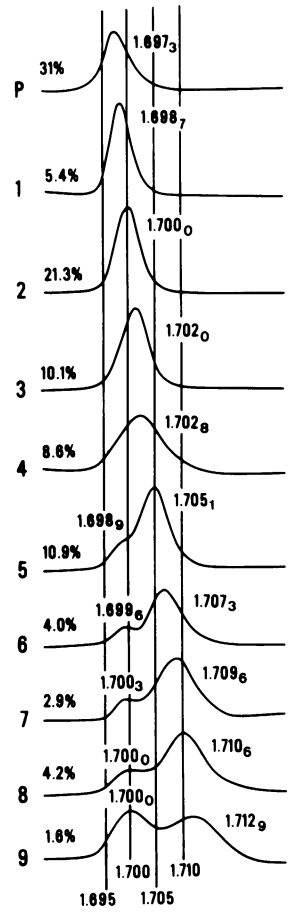

Fig. 1. Analytical CsCl profiles of human DNA fractions, as obtained after preparative ultracentrifugation in Cs_2SO_4/BAMD at a ligand/nucleotide molar ratio R_f = 0.14. Relative amounts and modal buoyant densities are indicated (see also Materials and methods).

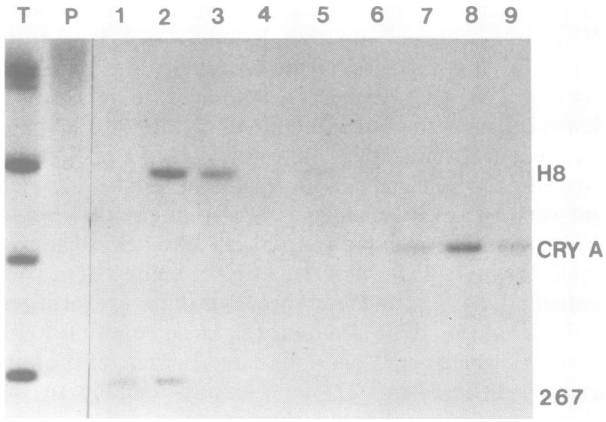

Fig. 2. Hybridization of probes 267, H8 and CRY A on the DNA fractions from Figure 1 (see also Materials and methods).

particular G- or R-bands. In turn, it is now possible to assign the isochores harboring these probes to the same G- or R-bands.

Figure 3 shows the compositional map obtained when the data from Table I are superimposed on the physical map. Fractions are displayed, left to right, in order of increasing GC content. Hybridization is practically absent from fractions 4 and 9. In the case of fraction 9, this is due to the fact that this is essentially formed by satellite IV and by

ribosomal DNA. In the case of fraction 4, the reasons may be the presence of a 1.703 g/cm^3 satellite that is localized in the centromeres of a number of chromosomes (Saunders *et al.*, 1972), and the fact that the GC content of the fraction corresponds to the trough that exists in the compositional distribution of coding sequences between GC-poor and GC-rich sequences (Mouchiroud *et al.*, 1987, 1988; Bernardi *et al.*, 1988). It is not surprising that this compositional distribution is also found in the coding sequences of chromosome 21.

It should be pointed out here that, in many cases, even where there is significant physical linkage data, the order of probes within any region is not completely known. In region C1, however, a strong tendency to increasing GC content is shown in the telomere-proximal area, and there is additional evidence favoring this representation. There are three physical linkage groups in this region, the 231 group containing four probes, the 512 group containing five, and the 520 group containing three. These linkages limit the possible arrangements considerably. Furthermore, there is genetic linkage data showing that the order is CEN-231-E8-520, and preliminary pulsed-field and genetic-linkage data suggesting that the 512 group is directly linked to the 231 group. Together, these groups account for most of the localizations to the 46% GC isochores of fraction 5. This suggests, then, that the remainder of the probes from this region, and therefore the highest GC-content isochores of fractions 7–9, are in the distal part of C1, in the telomeric region of the chromosome.

Table I. Localization of DNA probes in chromosomal regions and in compositional fractions

Band[a]	Region[a]	Probe[b]	HGM9[c]	Fraction
R q11.2	(A2)	E9	D21S16	2
	(A3)	M21[d]	D21S13	1,2
	(A3)	233F	D21S4	1,2
G q21	(A4)	511-1H	D21S52	2,3
	(A4)	552-2B	D21S59	1
	(A4)	4U	D21S110	P
	(A5)	236B	D21S11	2
	(A5)	228C	D21S1	P
	(A5)	JG90	D21S95	1
	(A7)	267C	D21S12	1,2
	(A7)	JG108	D21S99	1,2
	(A7)	21pcq	D21S111	1,2
	(A7)	APP		2,3
	(A7)	245D	D21S8	2
R q22.1	(D1)	513-5H	D21S54	1,2
	(D1)	D6*		2
	(D2)	B88*		1,2
	(D2)	JG77	D21S93	1,2
	(D2)	C34*		3
	(D2)	C43*		P
	(D2)	B7*		1,2
	(D2)	C40*		2
	(D2)	B83*		2–5
	(D3)	B18*		2
	(D3)	B15*		5
	(D3)	517-1.7R	D21S63	5,6
	(D3)	SOD1		2,3
	(D4)	524-5P	D21S58	5
	(D4)	525-SH	D21S65	5
G q22.2	(B1)	518-8b	D21S69	5
	(B1)	CP21G1	D21S60	3
	(B1)	H8	D21S17	2,3
	(B1)	ERG		4,5
R q22.3	(B2)	ETS2		5
	(B2)	523-10B	D21S57	5
	(B2)	231C	D21S3	5,6
	(C1)	519-9R	D21S71	5
	(C1)	551-8P	D21S64	5
	(C1)	CBS		5–8
	(C1)	MX1		5,6
	(C1)	MX2		5
(C1)	512-5R	D21S70	5,6	
	(C1)	E8	D21S15	2,3
	(C1)	B3	D21S195	5
	(C1)	BCEI		5,6
	(C1)	520-10R	D21S56	5,8
	(C1)	CRY A		8
	(C1)	E73*		8,9
	(C1)	D22*		5,6
	(C1)	10.2	D21S25	8
	(C2)	LFA		7,8
	(C2)	COL6a1		5,6
	(C2)	COL6a2		5,6

Discussion

The results of Figure 3 indicate that G-bands are compositionally simple, whereas R-bands are complex. Indeed, the large G-band q21 only consists, as far as observed here, of GC-poor isochores. This was shown by the hybridization of eight probes to the pellet fraction and to fractions 1 and 2. The lack of detection of a thin R-band (and of the corresponding interchromomeric region), which is seen at high resolution within q21 (Yunis, 1981; Verma and Babu, 1989) is due, in all likelihood, to the small number of probes, spaced at large intervals, that were tested so far in this region. In the case of the small G-band q22.2, while two probes hybridized to GC-poor fractions 2 and 3, two other probes hybridized to GC-rich fractions 4 and 5. This finding may be correlated with the presence of a thin R-band, which was detected at the 2000 band resolution (Yunis, 1981).

R-bands showed a striking compositional complexity. The whole range of GC levels has been detected in the three R-bands q11.2, 22.1 and 22.3. Indeed, q11.2 is composed of GC-poor isochores, q22.1 of GC-poor isochores in the centromere-proximal part and GC-rich isochores in the centromere-distal part, q22.3 of GC-rich and very GC-rich

[a]Regions are shown in Figure 3. For regional localization of probes, see Gardiner *et al.* (1988, 1990).

[b]Asterisked probes were from U.Tantravahi; the 500 and 200 series from P.Watkins and J.Gusella; JG series from J.Galt; 10.2 from M.Ward; 4U, E9, H8, E8 and B3 from G.Stewart. 21 pcq from C.Wong; CP21G1 from J.Davidson. cDNAs for identified genes were oncogene ETS2, N.Sacchi; β protein precursor from Alzheimer's disease plaques (APP), R.Tanzi and J.Gusella; superoxide dismutase (SOD), Y.Groner; mouse α-crystallin (CRY A), W.Hawkins; lymphocyte function associated antigen (LFA), T.Kishimoto and T.Springer; cystathionine-β-synthetase (CBS), J.Kraus; breast cancer estrogen-induced cDNA, BCEI, ATCC; MX1 and MX2 α-interferon-induced cDNAs, p78 and p78r, M.Horisberger.

[c]The HGM9 column provides the Human Genome Mapping 9 nomenclature.

[d]Underlined probes correspond to transcribed sequences (see Neve, 1986; Stefani *et al.*, 1988; R.Neve, personal communication).

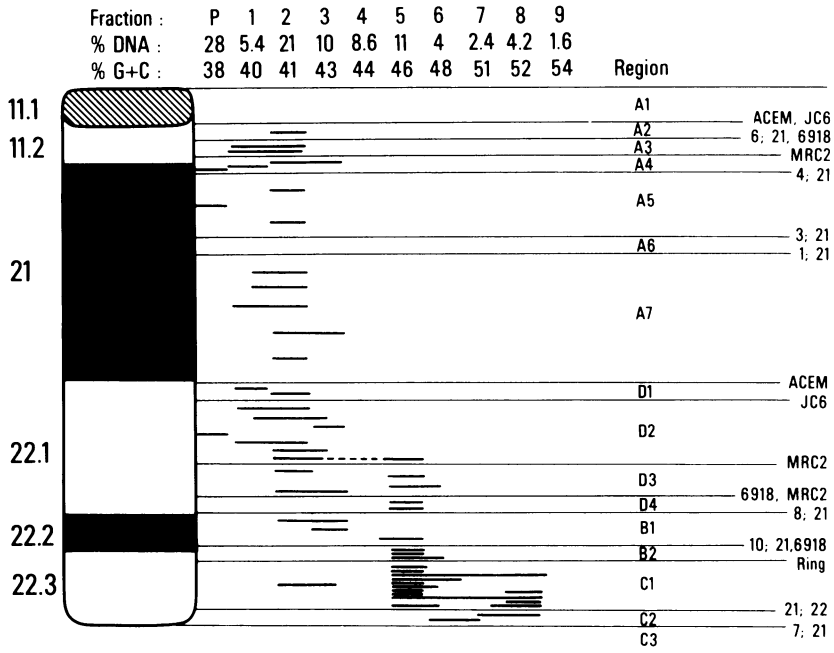

Fig. 3. Compositional map of the long arm of human chromosome 21 obtained when data from Table I are superimposed on the physical map (Gardiner *et al.*, 1988, 1990). Long horizontal lines indicate positions of the breakpoints associated with the rearranged chromosomes listed at the right of the figure (for details of the map and descriptions of the chromosome see Gardiner *et al.*, 1988, 1990).

isochores in the telomere-distal and telomere-proximal parts. This suggests a lack of correlation between GC levels and R-bands. Although this is definitely the case at standard resolution, it may not be so at high resolution for the reasons given below.

(i) In the case of R-band q11.2, only three probes were hybridized, all of them to GC-poor fragments from fractions 1 and 2. The physical map indicates that q11.2 must contain at least 3.0 Mb of DNA, as measured in *Not*I fragments, and therefore these three probes can evaluate the composition of at most 25% of the DNA in the band. It is possible, therefore, that R-band q11.2 is heterogeneous in composition, but that only GC-poor segments have been detected so far because of the particular probes used. This interpretation is supported by the finding of a thin G-band in q11.2 (Yunis, 1981).

(ii) In the case of R-band q22.1, probes from the centromere-proximal half hybridized to the pellet and fractions 1–3, whereas probes from the centromere-distal half hybridized predominantly (but not exclusively) to fraction 5. It should be noted that a thin G-band was detected within q22.1 (Yunis, 1981; Verma and Babu, 1989). Analogous to q11.2, the probes in the proximal and distal halves of q22.1 probably evaluate only 23 and 38% respectively of the total DNA in the band, and therefore some details may be missing.

(iii) Finally, in the case of R-band q22.3, except for one probe hybridizing to fractions 2 and 3, hybridization took place to fractions 5 and 7–8. A strong tendency to an increasing GC content of isochores was seen in the telomere-proximal region (see legend to Figure 3). Because of the large number of probes available in this region, fully 65% of the DNA could be examined. The compositional picture of band q22.3 is therefore probably one of reasonable detail.

These observations are in agreement with the pulsed-field data of the physical map, in that the general finding there had been that probes in R-bands were found on relatively small *Not*I restriction fragments, reflecting the higher level of unmethylated CpG islands (Bird, 1986) of these regions, while the opposite was true for probes in the G-bands. Again, however, the exceptions were probes in the centromere-proximal half of q22.1.

It should be stressed that the assignment of specific isochores to particular G- and R-bands relies on the physical map of the chromosome. Obviously, any errors or misinterpretations in the physical map construction will result in corresponding errors in isochore mapping. The assumptions used in the physical map were: (i) measurements of pulsed-field fragment sizes are accurate; (ii) the long arm of the chromosome does not contain appreciably more than 40 million base pairs of DNA; and (iii) DNA is uniformly distributed along the metaphase chromosome. Verification of (i) and (ii) requires independent pulsed-field maps, and these are under construction (Burmeister *et al.*, 1989; Creau-Goldberg *et al.*, 1989; Owen *et al.*, 1990). Where direct comparisons are possible, the data do not indicate any systematic error. Also, the physical map correlates well with the cytogenetic map (Gardiner *et al.*, 1990). Clearly, however, this does not prohibit some probes from being misplaced relative to G- and R-bands. This is possible in particular for probes mapped near the boundaries of bands, and may account for the presence of two probes in GC-rich isochores in G-band 21q22.2. This is quite unlikely, however, to account for the extent of the GC-poor isochores in the proximal part of R-band 21q22.1. (This DNA spans several millions of base pairs.) If DNA is not uniformly packaged in the metaphase chromosome, but rather is more compacted within G-bands, as suggested by the large difference in relative amounts of GC-rich and GC-poor isochores (Bernardi *et al.*, 1985) and by the data of Ambros and Sumner (1987), then many of these probes, and the isochores containing them, may belong in the G-band 21q21.

While this aids the correspondence of GC-poor isochores and G-bands, it raises problems for the physical and cytogenetic maps. In particular, it leaves rather too little DNA to be spread throughout the distal bands, and distorts the correlation of the pulsed-field map with the cytogenetic map. Clearly, resolving these questions requires more details in both the physical and the compositional maps.

The distribution of identified genes found so far on the long arm of chromosome 21 fits with previous indications of gene distribution in the human genome (Bernardi *et al.*, 1985; Mouchiroud *et al.*, 1987, 1988; Bernardi, 1989). Indeed, while one gene has been localized to G-band q21 (APP), one gene to G-band q22.2 (ERG; this is probably located in a thin R-band within q22.2) and one (SOD) to the telomere-proximal part of R-band q22.1, the other ten genes are in R-band q22.3 (one in region B2, six in region C1 and three in region C2). It should be mentioned here that two more genes, the GART and the α,β-interferon receptor genes are also located in R-band q22.1, while the S100 protein gene is located in region C2 of R-band q22.3. Moreover, the number of anonymous transcribed sequences is strikingly higher in R-band q22.3 compared to other bands (see Table I).

To sum up, the present results have shown a featureless, GC-poor pattern in the large G-band q21 (which might, however, show some change when more extensive data become available), and a more complex situation in the small G-band q22.2. Data have been much more striking for R-bands. Indeed, the GC-poor segments hybridizing to the probes from q11.2 and from the centromere-proximal part of q22.1 may correspond to thin G-bands already detected by high-resolution banding. On the other hand, the centromere-distal part of q22.1 is compositionally complex as shown by the presence of both GC-poor and GC-rich isochores. The most interesting results concern, however, R-band q22.3. With a single exception, all the probes were localized on GC-rich segments, some of which belong to the GC-richest isochore family H3 and cluster near the telomere. In this connection, we note not only that almost all human telomeres correspond to R-bands (Dutrillaux and Lejeune, 1971), but also that narrow terminal regions of 20 telomeres (including that of the long arm of chromosome 21) are especially resistant to thermal denaturation and give rise to T-bands (Dutrillaux, 1973) and very GC-rich segments (Ambros and Sumner, 1987).

Our finding of the highest GC level in the telomere-proximal region has two important implications. First of all, it establishes a link between high GC and thermal stability of DNA within metaphase chromosomes. It should be stressed that such stability is not due to the telomeric $(TTAGGG)_n$ stretches, since these are very short (4 kb) and are present in all human telomeres (Moyzis *et al.*, 1988). Secondly, it suggests that the highest GC isochores of the H3 family should be localized in the heat-resistant telomeric regions mentioned above, or, in other words, that the situation found in q22.3 should be common to a number of telomeric regions. Since the H3 component of the human genome has the highest gene concentration, another implication of these data is that high gene concentrations should be found in a number of telomeric regions, those producing T-bands, which should, therefore, be primary targets for more detailed mapping and sequencing.

In conclusion, already at this early stage, compositional mapping has provided some novel insights into chromosome organization. It is to be expected, however, that when a sufficient density of information is reached, compositional mapping will shed light on the base compositions and the sizes of the isochores making up the genomic regions investigated, so providing the equivalent of the highest possible resolution banding without the uncertainties of cytogenetics. Needless to say, such information is of special interest because of the link between GC levels of isochores and gene concentration.

Materials and methods

DNA preparation

The DNA sample used was obtained from fresh human placenta as described (Zerial *et al.*, 1986). DNA was in the 0.1–0.15 Mb size range (as determined by electrophoresis on 0.2% agarose gel, using lambda and T4 DNA as size standards).

Preparative centrifugation

Conditions were those described in Figure 1(B) of Zerial *et al.* (1986). Nine DNA fractions were obtained from each of the 12 centrifuge tubes spun in the same rotor. Corresponding fractions (as judged by the absorbance profiles) were pooled, dialyzed overnight against 10 mM Tris, 10 mM EDTA, pH 7.5, and exhaustively at 4°C against 10 mM Tris, 1 mM EDTA, pH 7.5. Fractions were then analyzed in an analytical ultracentrifuge, using conditions previously described (Thiery *et al.*, 1976). At the high R_f value used, the amount of pelleted, AT-rich DNA represented almost one-third of the total. This fractionation condition was chosen because it leads to a better separation of GC-rich fractions, which are characterized by a higher compositional heterogeneity and a higher gene concentration. The shoulders on the GC-poor side of the profile from fraction 5 on appear to correspond to human satellite IV (Macaya *et al.*, 1977). The pooling of corresponding fractions from different tubes (a procedure required to obtain relatively large amounts of DNA fractions) led to some loss of resolution, because of the imperfect correspondence of fractions from different preparative tubes. This phenomenon is likely to be more serious for the GC-rich fractions, which represent relatively small amounts of DNA and differ considerably from each other in modal buoyant densities.

Restriction enzyme digestion and hybridization

DNA fractions were digested with *Eco*RI or *Hin*dIII, loaded on 0.8% agarose gel in Tris–acetate buffer, pH 8.0. The amounts of DNA loaded were equivalent to that from 10 μg of total DNA. Alkali-denatured fractions were transferred by blotting on Genatran (Plasco) filters. A subset of the probes that allowed the construction of the physical map of the long arm of chromosome 21 (Gardiner *et al.*, 1988, 1990) was used in the present work. They are listed in Table I by region. Probes were labeled to specific activities of $>10^8$ c.p.m./μg, by the random oligo primer method (Feinberg and Vogelstein, 1984). Hybridizations were by standard techniques (Maniatis *et al.*, 1982).

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