A compositional map of the cen-q21 region of human chromosome 21

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Abstract

A compositional map of the centromere and of the subcentromeric region of the long arm of human chromosome 21 was established by determining the GC levels (GC is the molar fraction of guanine + cytosine in DNA) of 11 YACs (yeast artificial chromosomes) covering this 13–14 Mb region which extends from the α-satellite sequences of the C(terminal) band q11.1, through R(everse) band q11.2, to the proximal part of G(imsa) band q21. The entire region is made up of GC-poor, or L, isochores with only one GC-rich H1 isochore, at least 2 Mb in size, located in band q21. The almost identical GC levels of the centromeric α-satellite repeats (38.5%), of R band q11.2 (39%), and of G bands (38–40%) provide a direct demonstration that base composition cannot be the only cause of the cytogenetic differences between C, G, and the majority of R bands, namely the H3+ R bands (which do not contain the GC-richest H3 isochores). The results obtained also show that isochores may be as long as 6 Mb, at least in the GC-poor regions of the genome, and support previous observations suggesting that YACs from isochore borders are unstable and/or difficult to clone. Genes and CpG islands are very rare in the GC-poor region investigated, as expected from the fact that their concentration is proportional to the GC levels of the isochores in which they are contained. © 1997 Elsevier Science B.V.

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1. Introduction

Compositional mapping (Bernardi, 1989; Gardiner et al., 1990a; Bettecken et al., 1992; Pilia et al., 1993; De Sario et al., 1996) or, more precisely, molecular compositional mapping of human genome regions is of interest in at least three respects. First of all, compositional maps can shed light on the old, yet still controversial, issue of the correlation between base composition and chromosomal banding. Second, compositional maps provide information on such structural features of the genome as isochore sizes and borders. Finally, gene concentration being directly correlated (Mouchiroud et al., 1991; Zoubak et al., 1996) with the GC levels (GC is the molar fraction of guanine plus cytosine in DNA) of the isochores that make up the human genome (see below), compositional maps are equivalent to maps of gene concentration.

Indeed, the human genome is a mosaic of large DNA segments, the isochores (see Bernardi, 1995, for a recent review), which are compositionally uniform, but belong to a small number of families that cover a very wide (30–60%) GC range. Gene concentration is low in GC-poor isochore families L1 and L2 (collectively called L), which represent 62% of the genome, increases in GC-rich isochore families H1 and H2, which correspond to 22% and 9% of the genome, respectively, and reaches the highest value (about 20 times higher than in GC-poor isochores) in isochore family H3, which represents only 3–4% of the genome, the remaining 4% or so being formed by satellite and ribosomal DNAs.

Chromosomal compositional mapping, namely compositional mapping by in situ hybridization of DNA fractions derived from different isochore families, has provided a new approach to the study of genome organization at the chromosomal level. The earliest such
experiments, involving the GC-richest and gene-richest isochores of the H3 family (Saccomone et al., 1992), produced the highest concentration of signals on the thermal-denaturation-resistant T bands (Dutrillaux, 1973), a subset of R bands. These experiments established that T bands comprise the GC-richest, gene-richest, single-copy DNA. Further work, in which the chromosomal location of the other isochore families was studied (Saccomone et al., 1993), showed (i) that T bands contain not only H3 isochores, but also H2, H1 and L isochores; (ii) that R' bands (namely R bands exclusive of T bands) are formed, on average, by H1 and L isochores to almost equal extents, and by scarce amounts of H2 and H3 isochores; and (iii) that G bands essentially consist of L isochores, H1 isochores being present at very low levels.

Very recent work (Saccomone et al., 1996) showed that R bands can be partitioned into three groups on the basis of the in situ hybridization of H3 isochores. Indeed, these are present in 28 T (or H3+) bands, and in 31 T' (or H3*) bands, although at a lower concentration in the latter. The remaining 140 or so R bands (at a 400-band resolution) do not contain H3 isochores and only exceptionally contain H2 isochores, which are normally associated with H3 isochores; these bands were called H3- bands. On the basis of the GC3 levels of genes and of the GC levels of very long sequences localized in H3- bands and in G bands, respectively, it was suggested that H3- bands may have average GC levels as low as G bands.

In the present work, we have used a new approach (De Sario et al., 1995) in order to map compositionally a 13–14 Mb centromeric–subcentromeric region on the long arm of human chromosome 21, a region known to lack H2 and H3 isochores (Saccomone et al., 1992, 1993, 1996). This allowed us to investigate the base composition of an H3- R band, q11.2, as well as of the C band q11.1, which comprises a long α-satellite segment, and of the proximal part of G band q21.1. This work has provided a direct demonstration that the H3- R band q11.2 is as GC-poor as any G band and as the centromeric α-satellite, thus stressing the fact that the different cytogenetic behaviour of H3- R bands, G bands and C bands cannot be simply due to differences in base composition. Moreover, it has shown that at least GC-poor isochores may be as long as 6 Mb and has confirmed previous observations (De Sario et al., 1996; Gardiner et al., 1995) that YACs from isochore borders are unstable, and/or difficult to clone.

2. Materials and methods

2.1. YACs

Ten clones from a chromosome-21-specific YAC collection (Chumakov et al., 1992) were provided by the Centre d'Etude du Polymorphisme Humain (CEPH). One clone, YR17, was obtained from a partial chromosome 21 YAC library (Bellis et al., 1991). YAC-containing yeast clones were grown in AHC (10 g/l acid-hydrolysed casein, 20 mg/l adenine hemisulfate, 5 g/l (NH4)2SO4, 2% glucose) plates. Three colonies per clone were isolated and grown independently in 5 ml of AHC medium, and then in 200 ml of YPD (1% yeast extract, 2% bactopeptone and 2% glucose). The same cultures were used to prepare plugs for electrophoresis as well as DNA for buoyant density measurements and for PCR (polymerase chain reaction) controls of STSs (sequence-tagged sites).

2.2. YAC sizing

Agarose plugs were prepared according to Bellis et al. (1987). YAC 831B6 was digested to completion with one of the following enzymes: NotI, NruI, MluI, BssHII, SfiI and XhoI (New England Biolabs, Beverly, MA, USA). DNA was fractionated on a 1% agarose gel with 0.3 × TBE (Tris borate EDTA) using a PFGE (pulsed field gel electrophoresis) apparatus. Chromosomes from S. cerevisiae strain AB 1380 (200–2200 kb) and lambda DNA ligated to form <50 to 600 kb concatemers (New England Biolabs) were used as molecular weight markers.

After treatment with 0.25 M HCl and with 1.5 M NaCl, 0.4 M NaOH for 45 min, DNA was transferred overnight from the gels to a positively charged nylon membrane (Hybond N+, Amersham). Blots were kept at 80°C for 2 h, hybridized overnight at 65°C in 0.5 M Na2HPO4 (pH 7.2) and 7% SDS and washed in 0.1 × SSPE (NaCl, NaH2PO4, EDTA, pH 7.4) 0.1% SDS at 65°C (Church and Gilbert, 1984). Human DNA inserts were probed with human Cot1 DNA (GIBCO-BRL). The 2.6 kb amp and the 1.8 kb ura fragments, obtained after double digestion of plasmid pBR322 with BamHI and PvuI, were used to determine YAC orientation. The α-satellite and satellite 1 sequences were probed with αRI(680) (Jorgensen et al., 1987) and pTR1-6 (Kalitsis et al., 1993), respectively. Probes were labelled by incorporation of [α-32P]dCTP with a random oligo primer (Amersham).

2.3. Buoyant densities and GC levels

Buoyant densities of YACs were measured according to De Sario et al. (1995). CorI DNA was used as a probe to detect human DNA in the fractions obtained from preparative, shallow CsCl gradients. GC levels of YACs were calculated from the buoyant densities according to Schildkraut et al. (1962).
3. Results

3.1. YACs

The 11 YACs investigated almost completely cover the 13-14 Mb centromeric-subcentromeric region of chromosome arm 21q (Lawrence et al., 1993). The CEPH YACs were ordered in contigs on the basis of their STSs (Chumakov et al., 1992). Seven of them were shown to be non-chimeric by FISH (Korenberg et al., 1996). YAC YR17, previously localized to band q11 by in situ hybridization (Charliu et al., 1993), was shown in this work to contain the D21S236 and 23CB10L loci, and thus to be located in band q11.2. YAC sizes ranged from 280 to 1700 kb (see Table 1).

3.2. Compositional map

Table 1 presents the list of the YACs analysed, their sizes and the buoyant densities with the corresponding GC levels. Fig. 1 shows the compositional map of the region under investigation. Physical distances were calculated according to the integrated map of Lawrence et al. (1993). Isochore sizes were obtained by calculating the distance between the most proximal and the most distal STSs.

The six most proximal YACs correspond to a 6 Mb GC-poor L isochore. The average GC level is 39%. In fact, the centromeric YAC YAC 831B6 has a buoyant density of 1.704 g/cm³, which would correspond to 44% GC according to Schildkraut et al. (1962). However, two-thirds of this YAC are formed (see below) by α-satellite (38.5% GC), and by satellite 1 (33% GC), which is also present as a small block (50 kb), close to the centromere of chromosome 21 (Kalitsis et al., 1993). In order to account for the calculated 44% GC level of the YAC, its remaining third should be 55% GC, buoyant density being an additive property. This situation is, however, ruled out by the demonstrated absence of H3 and H2 isochores in the region (Sacccone et al., 1992, 1996). The actual GC level of this DNA stretch should, therefore, be at most that of H1 isochores, 44%, which would bring the GC level of the whole YAC down to 40%. It should be noted that the discrepancy between the analytical GC level and that estimated from buoyant density is common for the tandemly repeated sequences of satellite DNAs (Corneo et al., 1968). In agreement with the presence of a border between satellite and non-satellite sequences, possibly coinciding with the heterochromatin/euchromatin border in the region encompassed by YAC 831B6, all the sites observed for the six restriction enzymes used (see Materials and Methods) are localized, unclustered, within a 500 kb region on the centromere-distal (ura) side, whereas the amp probe hybridized to a >1 Mb DNA fragment that contains α-satellite and satellite 1 sequences.

In the proximal part of G band q21, the GC level increases from 39% to 42-43%. This corresponds to a transition from the L isochore to an isochore, at least 2 Mb in size, of the H1 family. Distal to this region, the GC level drops from 42 to 40%, corresponding again to an L isochore.

Four gaps are present in the map. The most proximal one is located between YAC 831B6 and 770B3. An STS localized in this gap, E341, is derived from a multisequence family called chAB4 (Assum et al., 1991). Its structure consists of two >90 kb inverted repeated sequences oriented head to head, and separated by a 60-80 kb long core sequence (Wohr et al., 1996). Since its GC level, as calculated on a 3 kb DNA fragment, is 33%, the genomic region included in the gap might correspond to an L isochore and be compositionally

<table>
<thead>
<tr>
<th>YAC</th>
<th>Size (kb)</th>
<th>Buoyant density* (g/cm³)</th>
<th>GC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>This work</td>
<td>CEPH</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>831B6</td>
<td>1700</td>
<td>1700</td>
</tr>
<tr>
<td>2</td>
<td>770B3</td>
<td>1300</td>
<td>1300</td>
</tr>
<tr>
<td>3</td>
<td>124A7</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>4</td>
<td>759D3</td>
<td>900</td>
<td>1700, 850</td>
</tr>
<tr>
<td>5</td>
<td>YR17</td>
<td>280</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>881D2</td>
<td>1550</td>
<td>1500</td>
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<tr>
<td>7</td>
<td>772A12</td>
<td>840</td>
<td>850</td>
</tr>
<tr>
<td>8</td>
<td>746B10</td>
<td>1160</td>
<td>1300</td>
</tr>
<tr>
<td>9</td>
<td>1851H3</td>
<td>460</td>
<td>—</td>
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<tr>
<td>10</td>
<td>264F9</td>
<td>510</td>
<td>500</td>
</tr>
<tr>
<td>11</td>
<td>78A11</td>
<td>550</td>
<td>—</td>
</tr>
</tbody>
</table>

*Buoyant density values separated by a semicolon were obtained in independent experiments. Three independent colonies were isolated from each YAC clone grown on an AHC plate (see Materials and Methods). In order to detect and discard grossly rearranged clones, the YAC size from each colony was measured by PFGE after hybridization with C1 DNA. As YACs 185H3 and 78A11 had not been previously sized, we selected the colony associated with the highest molecular weight and confirmed the presence of STSs.
similar to its two flanking regions. Three additional gaps are located between YACs 881D2 and 772A12, 185H3 and 264F9, 264F9 and 78A11, respectively.

3.3. Genes

Two genes were mapped to the region covered by our analysis and both are in chromosome band q11.2: R1P140 (Cavaillès et al., 1995) and STCH (Brodsky et al., 1995) have a GC3 level (i.e., the average GC level of third codon positions) of 42.0% and 39.2%, respectively, corresponding to very GC-poor isochores (Clay et al., 1996) consistent with the base composition measured in this chromosome band.

4. Discussion

4.1. The compositional map of the centromeric−subcentromeric region of 21q

A map outlining the main compositional features of the long arm of chromosome 21 was established 6 years ago (Gardiner et al., 1990a) by hybridizing 53 probes for single-copy sequences on compositional DNA fractions. This allowed assessment of the GC levels over the 100−200 kb regions surrounding the corresponding landmarks, which had been previously localized on the physical map. Nine of the landmarks used in Gardiner et al. (1990a) are contained within the region analysed here (see Fig. 1). The present compositional map is consistent with the previous one, except for the region surrounding the D21S110 and D21S59 loci, whose GC level was estimated as 38−39% in Gardiner et al. (1990a) and as 42% here. This discrepancy may be due to the fact that the previous estimate corresponded to a local (0.1−0.2 Mb) GC level, whereas the present one represents the average base composition over a >1 Mb region.

As already mentioned, the GC level of YAC 831B6, as calculated on the basis of its buoyant density, is overestimated. Indeed, since α-satellite and satellite 1 sequences cover 2/3 of this 1.7 Mb YAC, its GC level should be 40% or less, and one-third of YAC 831B6 could be made up by single-copy DNA sequences with
a GC level different from that of the very GC-poor flanking regions. We were unable to measure the base composition of this region because, among the YAC clones distal to 831B6 and included in the map published in Chumakov et al. (1992), some, such as 781G5, carry rearranged sequences (Korenberg et al., 1996), and others, like 857B10, might be derived from chromosome 22 (Wöhr et al., 1996), as none of the markers proximal to D21S215 are specific for chromosome 21. Finally, some other YACs (52H5; Wöhr et al., 1996) contain satellite 3 DNA which is known to be on the p arm and not on the q arm in chromosome 21.

R band q11.2 is made up of GC-poor DNA only. This was already suggested by previous results (Gardiner et al., 1990a), although no definitive conclusion could be drawn at that time, since the three probes mapped to this region provided information on only 25% of the band DNA. Moreover, the most centromeric-distal of the three probes used in Gardiner et al. (1990a) has now been shown to be located in band q21.

The centromeric L and the middle H1 isochores measure 6 and 2 Mb, respectively. These sizes are larger than those observed in chromosome band Xq28, where isochores ranged from 0.2 to 1.3 Mb, being on average 0.6–0.8 Mb (De Sario et al., 1996), and are in agreement with the suggestion (Bettecken et al., 1992; Pillai et al., 1993; De Sario et al., 1996) that GC-poor isochores are larger than GC-rich isochores. The possibility that the segments for YACs 770B3 and 881D2 which are not overlapped by other YACs may hide short GC-richer isochores is unlikely, because, if present, such hypothetical isochores would raise the GC levels of the YACs under discussion from their extremely low values.

### 4.2. Gene concentration

Only one characterized gene has been mapped to the 13–14 Mb region included in our analysis. Of the 131 cDNAs mapped so far to chromosome 21, 45% are located in the 7.6 Mb telomeric band 21q22.3, which accounts for 19% of the DNA of 21q and consists mainly of very GC-rich isochores of the H3 family (Gardiner et al., 1990a). CpG islands are also rare, as indicated by the fact that in chromosome 21 the largest NotI fragments are clustered in the proximal region near the centromere (Gardiner et al., 1990b; Wang and Smith, 1994).

### 4.3. Interspersed repeated sequences

The distribution of these sequences was studied by separately hybridizing two probes, for Alu and L1 repeats, respectively, on NotI restriction fragments from the hybrid cell line WAV 17 (Sainz et al., 1992). As expected, the 7.0 Mb chromosome 21 NotI fragment located in the centromeric region has one of the lowest frequency of Alu repeats, with a 2.8-fold under-representation of Alu, as compared with the average NotI band. In contrast, the frequency of occurrence of L1 repeats in this region is close to the average. This is consistent with Alu sequences being more abundant in GC-rich isochores and L1 being mainly present in GC-poor isochores (Meunier-Rotival et al., 1982; Soriano et al., 1983).

### 4.4. Compositional homogeneity and YAC stability

A comparison of our compositional map with that of Chumakov et al. (1992) indicates a small number of YAC clones in those regions where there is a change in the GC level. A similar phenomenon of correspondence between low frequency (and rearrangements) of YACs and gaps on the physical map was observed in human chromosome bands 21q22.3 (Gardiner et al., 1995) and Xq28 (De Sario et al., 1996). As regards YAC stability, the good reproducibility of the buoyant density measurements (Table 1) suggests that the YACs from the subcentromeric region of chromosome 21 were more stable compared with the YACs mapped to Xq28. One possible explanation is that the subcentromeric region is much more homogeneous compositionally than chromosome band Xq28. The different stability of genomic DNA cloned in YACs could also depend on the different amount of repetitive sequences, as the centromeric–subcentromeric region of chromosome 21 contains fewer Alu sequences than Xq28. (Along the same lines, YAC 831B6, which contains a large block of α-satellite sequences, was rather unstable, as shown by the fact that only one of the three colonies isolated contained a YAC of the predicted size). An additional reason, not exclusive of the previous ones, is that in the present case only yeast colonies containing a YAC having the expected molecular weight were selected.

### 4.5. Base composition and chromosome banding

Three comments are appropriate:

1. The available compositional maps show that isochores can be shorter than band size in Xq28 (Chumakov et al., 1992), but longer than band size in 21q11.2 (present work), and stress the lack of a match between isochose size and band size.

2. The existence of a H3− R band completely deprived of H1 isochores and the well-established presence of H1 isochores in other H3− bands (Saccone et al., 1993) indicates that at least two families of H3− R bands exist, which do or do not comprise H1 isochores. In fact, H3− R bands, like G bands, are likely to cover a spectrum of H1 levels.

3. While previous work (Saccone et al., 1996) provided strong indications for the majority of R bands (namely the H3− R bands) to have average GC
levels as low as G bands, the compositional mapping of band q11.2 provides unequivocal evidence for a specific H3+ R band which is compositionally indistinguishable from the flanking C band and from G bands in general. Under these circumstances, it is difficult to escape the conclusion that base composition alone cannot be responsible for the different cytogenetic properties of C, G and H3− R bands.

This suggests that other explanations like protein–DNA complexes (Comings, 1978) or the different folding of the AT-rich scaffold and packing of chromatin loops (Saitoh and Laemmli, 1994) could account for such differences, but does not imply that base composition plays no role in the cytogenetic properties of T (H3*) and T (H3+) bands, which comprise small or large amounts of H3 isochromosomes (always accompanied by H2 isochromosomes), respectively.

In conclusion, the overall picture which is emerging from this and previous work (Saccone et al., 1992, 1993, 1996) fits with the small overall GC difference between G and R band DNAs (Holmquist et al., 1982; Claiborne et al., 1990) and with the small relative amounts of H1, H2 and H3 isochromosomes, and stresses the shortcomings of the current cliché of “GC-rich R bands and GC-poor G bands”.

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References


