THE PROMETAPHASE BANDS OF HUMAN CHROMOSOMES: COMPOSITIONAL FEATURES AND GENE DISTRIBUTION

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INTRODUCTION

The human genome is a mosaic of long, compositionally homogeneous DNA segments, the isochores, that can be partitioned into five families, two GC-poor families (L1 and L2) representing 63% of the genome, and three GC-rich families (H1, H2 and H3) representing 24%, 7.5% and 4.5% of the genome, respectively (1). Gene concentration increases with increasing GC levels, and reaches a 20-fold higher level in H3 compared to L isochores (2). *In situ* hybridization of DNA from different isochore families provides, therefore, information on the chromosomal distribution of genes. Using this approach, three subsets of R(everse) or G(iemsa)-negative bands, H3+, H3* and H3−, containing large, moderate, and no detectable amounts, respectively, of the gene-richest H3 isochores were identified at a resolution of 400 bands (3). H3+ bands largely overlap with the most heat-denaturation-resistant bands (4), the chromomycin-A3-positive, DAPI-negative bands (5), the bands with the highest CpG island concentrations (6), and the earliest replicating bands (7). Here we have defined the H3+ bands at a 850-band resolution, and have thus identified the human genome regions, having an average size of 4 Mb, that are endowed with the highest gene density.

RESULTS AND DISCUSSION

DNA from the H3 isochore family was hybridized to human prometaphase chromosomes using the biotin/avidin system under conditions in which repeated sequences were competed out (Materials and Methods were already described in Ref. 8). The distribution of hybridization signals on chromosomes (see Fig. 1 for an example) showed that H3 isochores are only located on a small number of R_{850} bands (Figs. 2-5) and on none of the G_{850} bands (R_{400}, G_{400}, R_{850}, G_{850} indicate the R and G bands at resolutions of 400 and 850 bands, respectively). In fact, H3 hybridization signals covered almost all, many, and only a few of the R_{850} bands derived from R_{400} H3+ bands, H3* bands, and H3− bands, respectively (see Fig. 6 for an example).

Indeed, 23 out of the 28 R_{400} H3+ bands only yielded R_{850} bands containing H3 isochores, whereas only some of the R_{850} bands originating from the other five R_{400} H3+ bands showed H3 hybridization signals. For example (Fig. 6), the R_{400} H3+ band 11q13 is resolved into three R_{850} H3+ bands (q13.1, q13.3, and q13.5), and two G_{850} bands (q13.2, and q13.4), whereas the H3+ band 11p15 was one of the five exceptions, with only one (11p15.5) of the three derived R_{850} bands showing hybridization signals. In no case G_{850} bands derived from the R_{400} H3+ bands showed hybridization signals.

In contrast, only some of the R_{850} bands derived from 23 out of 31 R_{400} H3* bands showed hybridization signals. For example, the H3* band 11q23 (Fig. 6) yielded only one R_{850} H3+ band (11q23.3; in fact, only the distal part of it was H3+), whereas the other R_{850} band (11q23.1) was H3−. The remaining eight H3* bands showed the features observed in the vast majority of H3+ bands, in that all the derived R_{850} bands were H3+.

As far as the R_{400} H3− bands are concerned, the higher resolution allowed the identification of 20 bands containing H3 isochores that had not been detected at the lower resolution (3). The majority of these bands were located close to other H3+ or H3* bands (see bands 5q33. 1, 6p21.1, and 12q24.13) and were very thin (see bands 1p13.3, and 7p13). Only some of the R_{850} bands derived from these 20 R_{400} H3− bands exhibited hybridization signals (see band 11p11.2 in Fig. 6). Moreover, in a number of cases, the signal was thinner than the corresponding R_{850} bands, indicating that only part of the R_{850} band contained H3 isochores.

Incidentally, previous work (3) had shown that H2 and H3 isochores co-localize on metaphase chromosomes, with only four exceptions (the telomeric bands 3q29, 6q27, 13q34, and 20p13) which were H2+ and H3−. Now these bands were shown to be H3+, indicating also in these cases a co-localization of H3 and H2 isochores.

Finally, G bands did not reveal the presence of H3 isochores, the only exceptions being two G_{400} bands, 1p36.2 and 19q13.4, which yielded two R_{850} H3+ bands, 1p36.22, and 19q13.42, respectively.

The present results lead to several conclusions. (i) Since the colocalization of H2 and H3 isochores (which represent 12% of the human genome) in R_{850} H3+ bands appears now to be the rule, the fraction of these isochores in those bands (which represent 17% of the total genome) correspond to the majority, 70%, of the DNA contained in them. (ii) In some cases, however, the coverage of R_{850} H3+ bands by hybridization signals is overestimated. For example, the present experiments suggest that almost 50% of band Xq28 is H3+, whereas compositional mapping has shown that only 5% is formed by H3 isochores (9). (iii) In a number of R_{850} H3+ bands, H3 hybridization coverage was limited to a fraction of the band. This indicates that the present results provide information

concerning a resolution higher than 850 bands; thus, they may correspond, in many cases, to the practical highest resolution that can be attained, namely 1250 bands. (iv) 83% of the bands, namely the R_{850} H3− and the G_{850} bands, present low or very low gene concentrations; since genome size is remarkably constant in mammals and since such regions are conserved in syntenic regions of chromosomes from mammalian orders that diverged about 100 millions years ago (10-14), this suggests some functional role for the gene-poor majority of the genome and the corresponding intergenic regions. (v) Finally, the present results are relevant for the choice of the regions of the human genome that deserve sequencing priority.

Interestingly, these regions correspond to gaps in the physical map of the human genome (3,15). The difficulty experienced in cloning these regions into YACs and/or in avoiding high levels of chimerism and deletion in most probably related to their high recombination level, a property which apparently is conserved when these regions are cloned in yeast.

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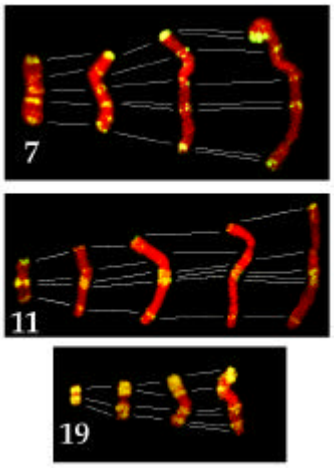
FIGURE LEGENDS

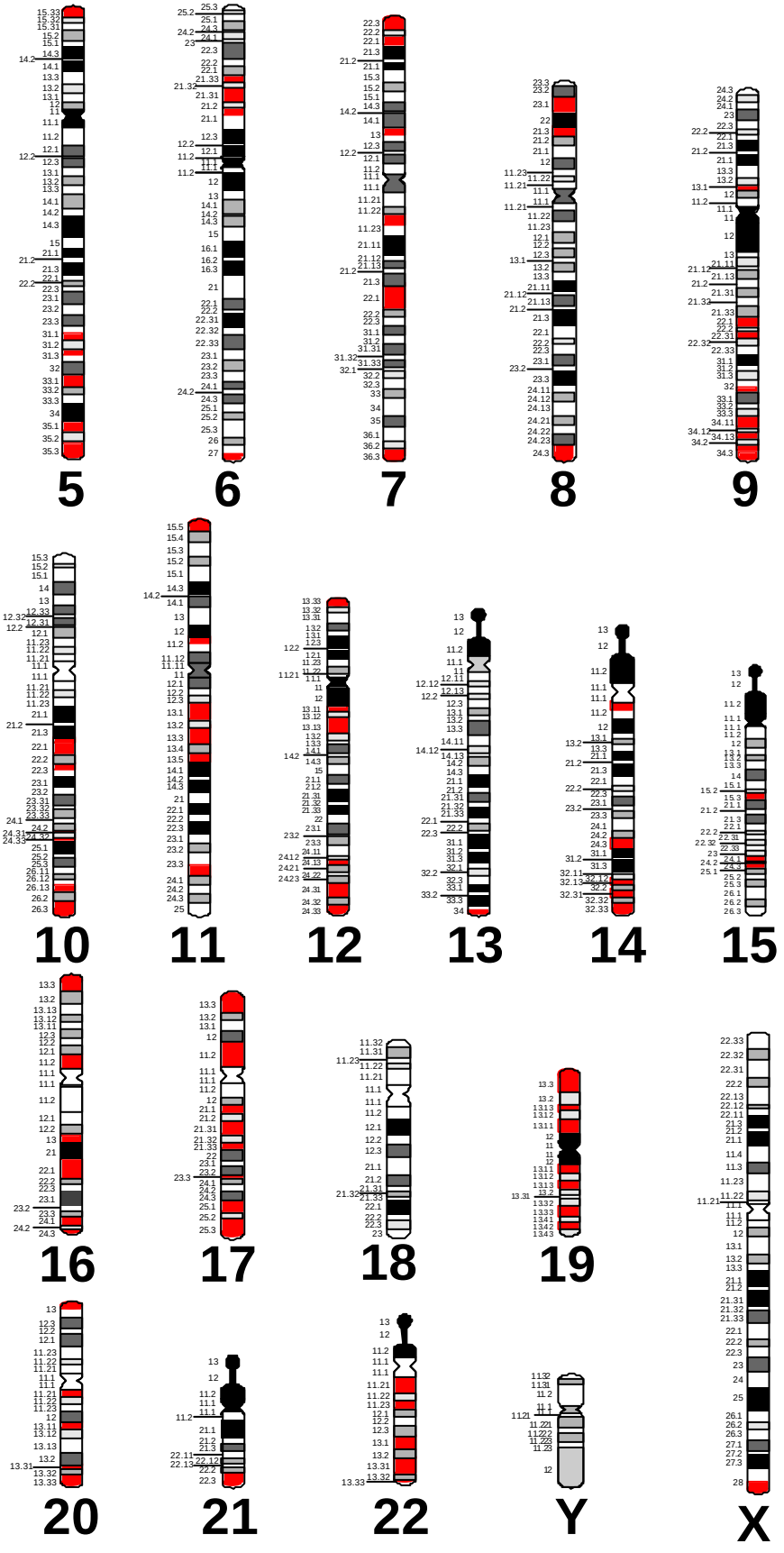
Fig. 1. Human chromosomes 7, 11 and 19 hybridized with the biotin-labeled DNA from the H3 isochore family, at different levels of resolution. The hybridized regions were visualized by fluorescein (yellow signals) and chromosomes were red-stained with propidium iodide. Each panel presents chromosomes with a band resolution ranging from about 300 to about 850.

Fig. 2.-5 Ideograms of human chromosomes at a 850 band resolution (16) showing the H3+ bands as red bands.

Fig. 6. Ideogram of human chromosome 11 at 400 (left) and 850 (right) band resolution showing the chromosomal regions containing H3 isochores. Red, yellow and white bands on the left chromosome indicate the H3+, H3* and H3− bands. Red bands on the right chromosome indicate the regions hybridizing the H3 isochores.

FIG. 1

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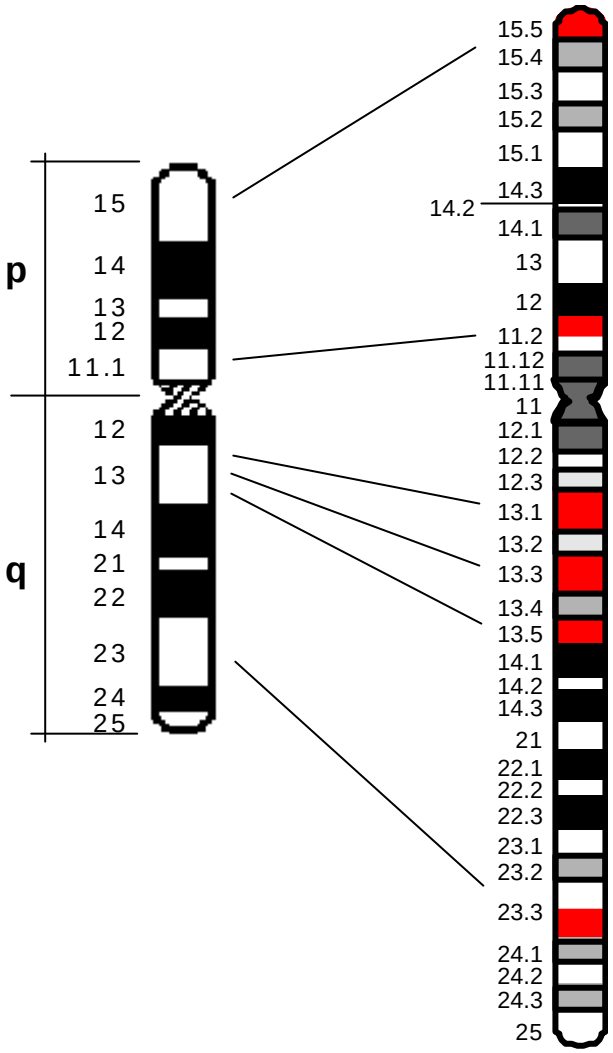
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