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Reiteration frequency mapping: Analysis of repetitive sequence organization within cloned DNA fragments containing the human initiator methionine tRNA gene

(repetitive elements/human genome/tRNA gene/contact hybridization)

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ABSTRACT **The organization of repetitive sequences within three cloned chromosomal segments from human fetal liver DNA containing the initiator methionine tRNA gene was studied. The procedure developed for this study involves the contact hybridization of an electrophoretically separated 5′-^{32}P-labeled restriction endonuclease digest of the cloned segment with total human genomic DNA covalently bound to aminobenzyloxymethyl-paper. The extent of hybridization of each labeled fragment to the area of paper with which it is in contact is proportional to the representation of the sequence within the human genome. We show that sequences with a wide range of genomic repetition are present in the neighborhoods of the three dispersed initiator tRNA loci, each characterized by a different overall organization pattern.**

The basic organizational pattern of the genome of almost all higher organisms is dominated by the interspersion of short repetitive sequences some 300 nucleotides long between single-copy segments that are several hundred to a few thousand base pairs long (1, 2). The functional significance of repeat sequence interspersion remains a subject of speculation, but the pattern suggests that certain families of repeated sequence may function in the control of single-copy sequence expression (3).

In this report we describe the organization of repeated sequences within three cloned genomic fragments containing genes for the human initiator methionine tRNA. Several features distinguish this gene from the "average" single-copy sequence and interested us in the specific organization of repeated elements in its neighborhood. The initiator methionine tRNA genes, in the human genome, represent a family of at least 10–13 genes, dispersed over distantly spaced chromosomal loci, and, therefore as a group share a formal resemblance to the class of repetitive interspersed sequences. The putative transcription unit is only about 100 nucleotides long (4), considerably shorter than the average interspersed repetitive element. In addition, in contrast to the majority of unique sequences in the genome, which are transcribed by RNA polymerase II (5), the tRNA locus is the substrate of RNA polymerase III (6), an enzyme that also has been implicated in the transcription of a subset of repeated sequences (7).

In this report we describe a hybridization procedure that provides a rapid and quantitative means of determining the organization of repeated sequences in cloned chromosomal segments. The method is applied here to the analysis of repeat sequence interspersion surrounding the initiator methionine tRNA loci.

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MATERIALS AND METHODS

Materials. Restriction endonucleases were from New England BioLabs. Human DNA was isolated from placenta by the procedure of Blin and Stafford (8).

Isolation of Recombinant Clones. The recombinant library of human fetal liver DNA in bacteriophage λ Charon 4A, from which the clones containing initiator methionine tRNA genes were isolated, was a gift of Tom Maniatis (9). The probe used in screening was a 180-base-pair fragment bearing the 73-nucleotide sequence of the initiator methionine tRNA purified from the plasmid Pxt 210, which contains several tRNA genes from *Xenopus laevis* (10) and was a gift of S. G. Clarkson. Details of the screening, propagation, and characterization of the recombinant phage bearing the initiator methionine genes will be described in full detail elsewhere.

Fixation of DNA to Aminobenzyloxymethyl (ABM) Paper. The procedure is based on that described by Alwine *et al.* (11). A 310-cm^2 sheet of ABM-paper (Schleicher and Schuell, Transa-Bind) was soaked for 1 hr at 4°C in 100 ml 1.2 M HCl to which 3 ml of a 10 mg/ml freshly prepared solution of sodium nitrite had been added. The paper was washed twice with 500 ml of ice-cold water, followed by two 500-ml washes in 0.2 M sodium acetate, pH 4.0. The paper was blotted on a sheet of Whatman 3 MM paper and placed onto a glass plate. Ten milliliters of a 1.5-mg/ml solution of DNA in water, denatured at 107°C for 15 min, cooled, and adjusted to 0.2 M sodium acetate, pH 4.0, was applied to the paper, and maintained at 4°C overnight in a covered tray. Under these conditions about 2.5 μg of DNA is bound per cm^2 as determined from the A_{260} of material released from the paper on incubation with bovine pancreatic DNase (Worthington, D, 2800 units/mg) at 20 μg/ml for 1 hr at 37°C in 10 mM Tris·HCl, pH 7.5/5 mM $CaCl_2$. The ABM sheet was drained of excess liquid and sealed in a plastic bag (Kapak pouches, Scientific Products, Bloomington, MN) containing 20 ml of 50% (vol/vol) formamide, 7× standard saline citrate (NaCl/Cit) (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0), 50 mM sodium phosphate at pH 6.8, 0.1% $NaDodSo_4$, 1× Denhardt's solution (12), 200 μg of heat-denatured *Escherichia coli* DNA (Worthington) per ml, 100 μg of poly(rA) (Miles) per ml, and 1% glycine. The sheet was kept for 24 hr at 45°C, after which it was stored at 4°C until used.

Preparation of ^{32}P-Labeled Restriction Endonuclease Fragments. One microgram of DNA was digested with 6 units of each restriction endonuclease in a volume of 50 μl for 90 min

Abbreviations: ABM, aminobenzyloxymethyl; kb, kilobase(s) or kilobase pair(s); NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.

at 37°C, under conditions specified by the supplier. *E. coli* alkaline phosphatase (Bethesda Research Laboratories, Rockville, MD) was then added to each reaction mixture at 20 units/pmol of 5′ end of restriction fragment, and the mixture was maintained at 37°C for 2 hr. Nitrilotriacetic acid (previously adjusted to pH 7.4 with NaOH) was added to 7 mM with incubation at 65°C for 15 min. DNA was purified by one extraction with 1 vol of phenol/0.5 vol of chloroform/isoamyl alcohol (24:1 vol/vol), followed by three chloroform extractions. The DNA was recovered by ethanol precipitation and phosphorylated in 10 μl containing 2 Weiss units of phage T4 polynucleotide kinase (Bethesda Research Laboratories), [γ-^{32}P]ATP (specific radioactivity, 8500–8900 Ci/mmol, New England Nuclear; 1 Ci = 3.7×10^{10} becquerels) in 10-fold molar excess to 5′ ends, 70 mM Tris·HCl (pH 8.0), 0.1 M NaCl, 10 mM $MgCl_2$, and 3 mM nitrilotriacetic acid for 2 hr at 37°C. Labeled fragments were purified by Sephadex G-100 chromatography and concentrated by ethanol precipitation.

Preparation of the Contact Gel. The 5′-labeled fragments were dissolved in a small volume of Tris/acetate/EDTA buffer and electrophoresed on a 6-mm-thick vertical slab of agarose (LE, Miles) in the Tris/acetate/EDTA system (13) at 1.5 V/cm for 10 hr at 20°C. The slabs were soaked in 0.2 M NaOH/0.6 M NaCl for 4 hr at 20°C, then neutralized in 1 M Tris·HCl (pH 7.0)/0.6 M NaCl for 5 hr at 4°C.

Contact Hybridization. Five hundred milliliters of 2% agarose (LE, Miles) in 3× NaCl/Cit was poured into a polypropylene box (14 cm wide × 7.6 cm high × 18 cm long; Bel Art Products, Pequanock, NJ) equipped with a moisture-tight cover. After solidification of the gel, the box was equilibrated at 68°C in a convection oven for 2 hr. Two sheets of Whatman 3 MM paper cut to cover the agarose bed were saturated with 3× NaCl/Cit and laid over the surface. The gel, containing the electrophoresed restriction fragments, and previously alkali soaked and then neutralized, was placed on the filter paper, taking care that air bubbles were excluded. A strip of ABM-paper to which genomic DNA had been bound, and previously washed in 3× NaCl/Cit at 68°C for 30 min, was cut exactly to the size of the restriction fragment gel and laid onto its surface. Ten strips of Whatman 3 MM paper cut to the same size as the ABM-paper strip, saturated with 3× NaCl/Cit, were placed above the ABM-paper strip, followed by a stack about 5 cm thick of dry strips of Whatman 3 MM paper. The box was covered and sealed with packing tape. The unit was maintained at 68°C for 36 hr. The strip of ABM-paper was removed from the gel surface and washed twice at room temperature in 3× NaCl/Cit/0.1% SDS/0.1% sodium pyrophosphate and twice in the same buffer at 68°C for 30 min each wash, followed by a single 30-min wash in 1× NaCl/Cit/0.1% $NaDodSO_4$/0.1% sodium pyrophosphate at 68°C. The paper was blotted dry and autoradiographed with an intensifying screen at −70°C against a preflashed sheet of Kodak XR x-ray film.

Film image density was quantitated by scanning with a Joyce–Loebl densitometer. Autoradiographic exposure was adjusted to allow for proportionality of image densities. Densitometric tracings were quantitated by determination of peak areas with an electronic planimeter.

Reiteration Frequency Mapping. The experimental approach described in this report permits the quantitation of the amount of DNA homologous to a specific DNA sequence in a complex DNA population. Restriction fragments of a genomic segment are labeled to high specific radioactivity at their 5′ ends and electrophoresed in agarose. After *in situ* denaturation, the fragments are blotted against a sheet of ABM-paper to which denatured total genomic DNA has been uniformly bound. The transfer from the agarose gel is performed under conditions optimal for nucleic acid hybridization and results in the association of the labeled restriction fragments to complementary sequences bound to the paper matrix in contact with the gel. Because the fragments are terminally labeled, the autoradiographic intensity of each hybridization signal is proportional to the number, rather than the mass, of labeled fragments that hybridize to a given area of the DNA-paper. A blot against genomic DNA reveals the extent to which sequences within a restriction fragment are repeated in the total genome.

In Fig. 1 we demonstrate the assay by analyzing the sequence representation of a reconstructed mixture of DNA fragments contained within λhtm3, consisting of intact Charon 4A, λhtm3, and cloned 3.7- and 2.03-kilobase pair (kb) *Hin*dIII fragments of λhtm3. As seen in Fig. 1, the *Hin*dIII digest of λhtm3 consists of seven fragments. Sequences present in the 1.25- and 2.4-kb *Hin*dIII fragments were each bound to the paper matrix at a concentration of about 2.6×10^{-6} pmol/cm^2, representing the amount of DNA equivalent to a sequence present in 2 copies per haploid genome in 1 μg of total human DNA. The 5.7- and 6.2-kb *Hin*dIII fragments were each represented in a 20-fold molar excess over the 1.25-kb fragment, while the 3.7-kb *Hin*dIII fragment was present in about a 60-fold molar excess, and the 2.03-kb *Hin*dIII fragment was represented some 400-fold more abundantly than the 1.25-kb *Hin*dIII sequence. Contact hybridization of 0.02 μg (1×10^4 dpm, lane b) and 0.2 μg (lane c) of the 5′-labeled *Hin*dIII digest of λhtm3 to the paper-bound DNA mixture was performed as described above, and a 3-day autoradiographic exposure of the blots obtained is shown in Fig. 1. Densitometric measurements of the bands shown in lane c, listed in the figure, demonstrate that the extent of hybridization of each labeled restriction fragment is linearly proportional to the relative molar abundance of its complement in the nucleic acid mixture within the range between 2.6×10^{-6} pmol and 1.1×10^{-3} pmol of se-

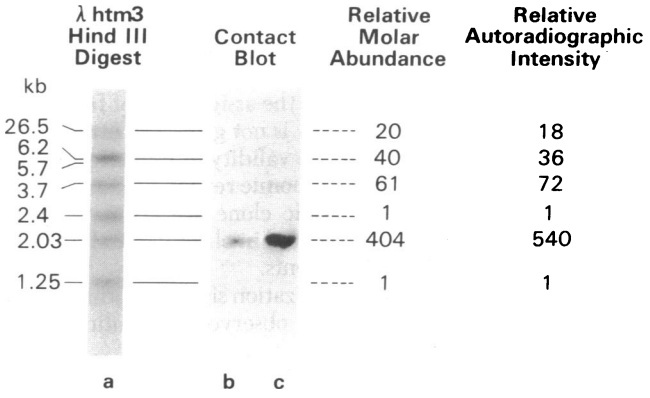

FIG. 1. Reiteration frequency assay. Lane a, autoradiographic image of a 5′-^{32}P-labeled *Hin*dIII digest of λhtm3, prepared and electrophoresed in 1% agarose. The 26.5- and 6.2-kb fragments contain sequences of left and right arms of Charon 4A DNA, respectively, as well as adjacent genomic sequences (see Fig. 2). The 5.7-kb fragment is from within the right arm of Charon 4A DNA. A contact blot was prepared against a strip of ABM-paper to which the following amounts of DNA had been fixed (per 150-cm^2 sheet): 15 ng of λhtm3, 250 ng of Charon 4A, 55 ng of the 2.5-kb *Hin*dIII fragment, and 120 ng of the 2.03-kb *Hin*dIII fragment, each cloned separately in plasmid pBR322. The relative molar abundance of sequences in this mixture contained within each of the *Hin*dIII fragments of λhtm3 are listed, with the unresolved 5.7- and 6.2-kb fragments summed as one. Lanes b and c, a 3-day autoradiographic exposure of the contact blot against the DNA mixture with 0.02 μg (1×10^4 dpm) and 0.2 μg, respectively, of the labeled digest. The numbers listed in the rightmost column are the relative autoradiographic intensities of the corresponding bands in lane c, normalized to the intensity of 1.25- and 2.4-kb fragments, just barely visible in the original autoradiograph.

quence nominally bound, per cm^2, to the paper matrix. An increase in the amount of each labeled fragment in the contact gel from 10^{-3} pmol (lane b) to 10^{-2} pmol (lane c), results in a proportional increase in the extent of hybridization of each labeled fragment.

RESULTS

Initiator Methionine tRNA Clones. The human haploid genome contains between 11 and 13 copies of the initiator methionine tRNA gene, of which 3 representations are shown in Fig. 2. A full description of the characterization of these cloned fragments as well as the organization of the human initiator methionine tRNA genes will be presented elsewhere. λhtm1 and λhtm3 each contain a genomic segment about 17 kb in length and a single methionine tRNA gene, which itself is the sole tRNA locus detected in both segments. The organizations of restriction sites both over the full extent of the two regions and within the *Hin*dIII fragments bearing each tRNA gene are different and suggest that the two fragments represent different chromosomal loci. λhtm4, in contrast, shares a curious homology with λhtm3, being identical in its restriction map up to the *Eco*RI site at about 3 kb 5′ from the tRNA gene. Distal to this point, however, the fragment shows no evidence of map identity.

Reiteration Mapping of the Initiator Methionine tRNA Genomic Fragments. In Figs. 3–5 are shown the reiteration maps of λhtm1, λhtm3, and λhtm4 generated by contact hybridization of 5′-^{32}P-labeled restriction fragments with filter-bound total human placental DNA. The relative autoradiographic intensity of each hybridization signal within a map is listed normalized to a unit value assigned to the intensity detected for the methionine tRNA-bearing fragment in that contact blot. Thus, a signal of unit intensity corresponds to a minimal copy number within the genome of at least 13 members, the representation of the initiator methionine tRNA structural locus. From the similarity in the signal intensity detected on the hybridization of the methionine tRNA-bearing restriction fragments to total genomic DNA with that expected for a 10-copy sequence from the reconstruction experiment shown in Fig. 1, we believe that the assignment of film image density to reiteration frequency is not grossly in error. In addition, as a further check on the validity of the assignment of unit film density as about 13 genomic representations, several fragments within each genomic clone are estimated to be unique copy, as would be expected in chromosomal segments of the size of our cloned fragments.

The reliability of each hybridization signal is within a factor ≤2, the level of reproducibility observed in identical digests run as separate experiments.

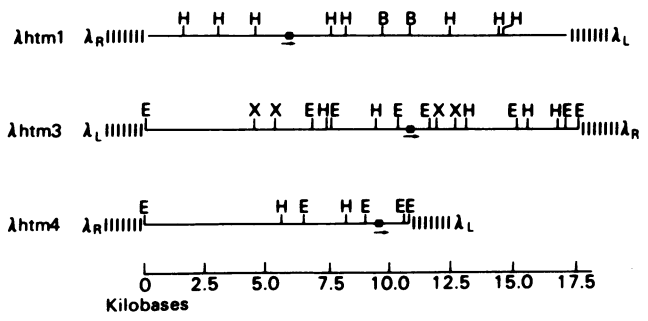

FIG. 2. Restriction maps of cloned chromosomal segments containing the human methionine initiator tRNA gene. Darkened boxes denote the position of the single tRNA gene on the fragment. The arrow points from the 5′ to the 3′ direction of the tRNA gene. Cleavage sites are: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; X, *Xba* I. λ_R and λ_L are right and left arms of Charon 4A DNA, respectively.

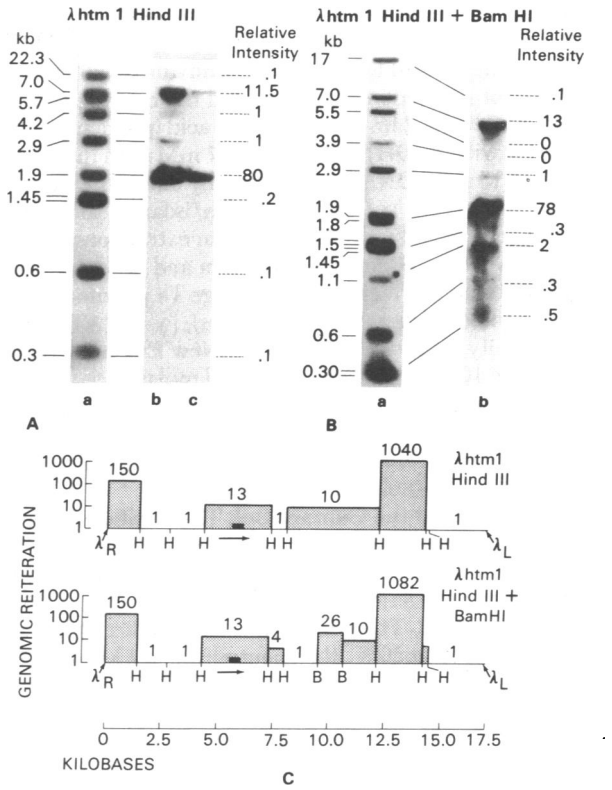

FIG. 3. Reiteration analysis of λhtm1. (*A*) Lane a, *Hin*dIII digest of λhmt1 was labeled and electrophoresed in 1.5% agarose. Lanes b and c are contact blots of 1 μg (6×10^6 dpm) and 0.2 μg, respectively, of λhmt1 *Hin*dIII digest against total human placental DNA fixed to ABM-paper at a concentration of 2.5 μg/cm^2. (*B*) Lane a, *Hin*dIII–*Bam*HI double digest of λhtm1 electrophoresed in 1% agarose for twice the duration of that for the digest used in the blot shown in lane b, obtained on hybridization of 0.2 μg of *Hin*dIII–*Bam*Hi digest (5.6×10^5 dpm) against the same DNA paper used in *A*. Autoradiographic exposure was for 18 hr in *A* and *B*. The relative intensity of each band within a single blot has been normalized to a unit value determined for the intensity of the 2.9-kb *Hin*dIII tRNA-specific fragment in that blot. Bands of relative intensity less than about 2 are barely visible in this autoradiograph, and are quantitated from a 72-hr exposure. The background in *B*, defined by nonspecific hybridization of the 3.9- and 5.5-kb fragments from the right and left phage arms, respectively, was negligible. (*C*) The autoradiographic data in *A* and *B* are summarized. The height of the histogram elements represents the estimated haploid copy number of the sequences within the designated restriction fragment (see text) (symbols as in Fig. 2).

In Fig. 3 *A* and *B* are shown the reiteration maps of a *Hin*dIII single and *Hin*dIII–*Bam*HI double digest of λhtm1. A summary of the estimated genomic repetition frequency of the sequences within each fragment is shown in Fig. 3*C*. The strips in Fig. 3*A*, lanes b and c, were produced by contact hybridization against 1 μg and 0.2 μg of labeled digest, demonstrating that, in general, while absolute intensities increase proportionally with the concentration of probe in contact with the filter, there is little difference in the relative intensity of each hybridization signal. Two intensely hybridizing fragments are detected in Fig. 3*A*, the 1.9- and the 7-kb *Hin*dIII fragments. The 1.9-kb fragment is centered at 13 kb (Fig. 3*C*) and contains the most heavily reiterated sequences on the 17-kb segment, estimated to be present in at least 1000 copies per genome. The second most intensely hybridizing sequence is localized between the *Hin*dIII site at about 2 kb and a fragment of the right arm of Charon 4A, contained in the 7-kb *Hin*dIII fragment. It generates a signal about 10% that of the 1.9-kb *Hin*dIII fragment and is estimated to represent a family of about 150 copies.

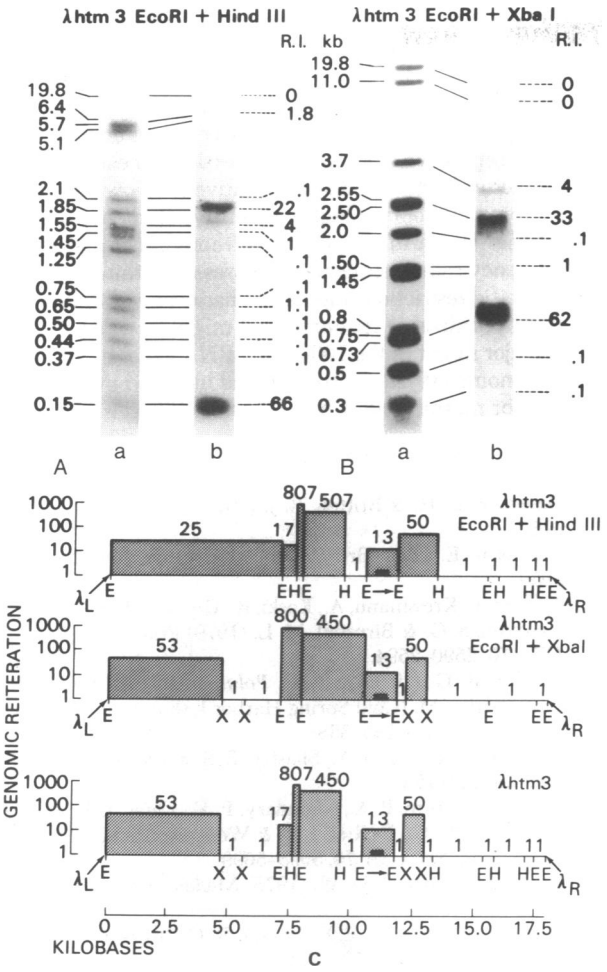

FIG. 4. Reiteration analysis of λhtm3. (*A*) Lane a, *Eco*RI–*Hin*dIII digest in 1.5% agarose. Lane b, Contact blot of 0.5 μg (1×10^6 dpm) of digest against human placental DNA bound to ABM-paper at 2.5 μg/cm². (*B*) Lane a, *Eco*RI–*Xba* I digest in 1% agarose run about twice the distance of the gel used in the blot in lane b, obtained by hybridization of 0.4 μg (4×10^5 dpm) of DNA. Fragment intensities are normalized to the 1.45-kb *Eco*RI tRNA-containing fragment in each blot. Autoradiographic exposure was for 18 hr in *A* and 36 hr in *B*. Nonspecific hybridization of the 20-kb left arm of Charon in *A*, and the same fragment along with the 11-kb piece from the right arm in *B*, is seen to be negligible. (*C*) The data in *A* and *B* are summarized, as described for Fig. 3*C*. The bottom histogram represents a composite of the two upper ones. R.I., relative autoradiographic intensity.

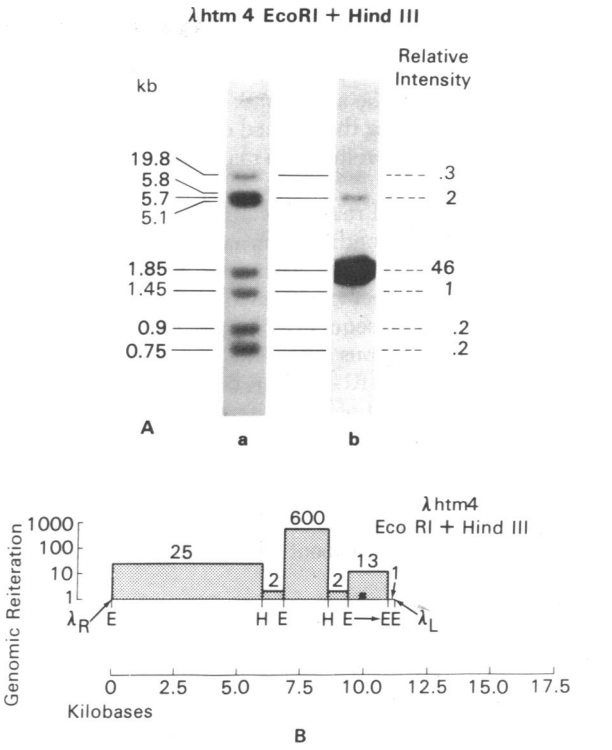

FIG. 5. Reiteration analysis of λhtm4. (*A*) Lane a, *Eco*RI–*Hin*dIII digest of λhtm4 labeled and run in 1.5% agarose. Lane b, Contact blot obtained with 0.4 μg (8×10^5 dpm) of digest. Fragment intensities are normalized to the 1.45-kb *Eco*RI tRNA-specific fragment. Autoradiographic exposure was for 36 hr. Nonspecific hybridization represented by the signal corresponding to the 20-kb left arm of Charon 4A was not subtracted from specific signals. (*B*) The data in *A* are summarized, as described for Fig. 3*C*. The reiteration number of the 0.2-kb *Eco*RI fragment, not resolved on this gel, was deduced from the corresponding segment in λhtm3.

The 2.9-kb *Hin*dIII fragment, which contains the methionine tRNA locus, is seen to hybridize to about the same extent as the 4.2-kb *Hin*dIII fragment centered at about 10 kb, and the latter fragment would thus appear to contain at least a 10- to 15-copy sequence. The remaining *Hin*dIII fragments of 1.45, 0.6, and 0.3 kb are estimated to be uniquely represented as noted in Fig. 3*C*.

The reiteration map of the *Hin*dIII–*Bam*HI double digest of λhtm1 in Fig. 3*B* was derived from a contact hybridization against a 1% agarose separation (in contrast to the 1.5% agarose separation in Fig. 3*A*) and demonstrates the reproducibility of restriction band hybridization intensities seen in separate experiments. The repeated sequence within the 4.2-kb *Hin*dIII fragment is localized between the *Bam*HI sites at 9 kb and the *Hin*dIII site at 12 kb. The 1.45-kb *Hin*dIII–*Bam*HI fragment centered at about 9 kb falls within a triplet with an intensity equal to about 4 copy equivalents, permitting a maximum assignment of no more than 2 genomic repeats to the segment. The 1.9-kb *Bam*HI–*Hin*dIII fragment centered at 11.5 kb is not resolvable from the abundant 1.9-kb *Hin*dIII sequence family, and thus an absolute assignment for this fragment cannot be determined in this digest.

In Fig. 4*A* is shown the reiteration map of an *Eco*RI–*Hin*dIII double digest of λhtm3, separated in 1.5% agarose, and in 4*B* is shown a corresponding analysis of an *Eco*RI–*Xba* I double digest separated in 1% agarose. A summary of the genomic repetition frequency of each fragment is shown in Fig. 4*C*. In Fig. 4*A* the most extensively hybridizing sequence is the 0.15-kb *Hin*dIII–*Eco*RI fragment centered at about 7.5 kb, represented by at least 800 genomic copies. This sequence lies to the left of the second most abundantly represented segment, the 1.85-kb *Eco*RI–*Hin*dIII centered at about 9 kb and represented by about 400–500 genomic copies. The 1.55-kb *Eco*RI-*Hin*dIII fragment centered at about 12 kb has about 50 representations. Within the 5.7-kb *Eco*RI fragment adjacent to the left arm of Charon 4A a sequence with a similar representation is noted. The 0.65-kb *Eco*RI-*Hin*dIII fragment centered at about 7 kb is present in about the same abundance as the methionine tRNA structural locus. The remaining fragments on λhtm3 appear to be unique.

In the *Eco*RI–*Xba* I digest shown in Fig. 4*B*, similar intensity relationships are observed for corresponding genomic segments. The repetitive elements near the left arm of Charon 4A are localized more precisely between the Charon 4A segment and the *Xba* I site at 3.7 kb. A short, unique, 0.3-kb *Eco*RI–*Xba* I segment is detected 3′ to the 1.5-kb *Eco*RI fragment containing the tRNA structural gene. The assignment of the 2.55-kb *Eco*RI–*Xba* I fragment centered at about 13 kb as unique is

tentative, due to ambiguity in resolving its hybridization signal from the heavily reiterated 2.5-kb *Eco*RI fragment, and presents uncertainty as to whether the fragment shares repeated sequences present in the adjacent 0.73-kb *Xba* I fragment. A composite summarizing the derived organization of repetitive elements from the two reiteration maps is shown in the bottom histogram of Fig. 4*C*.

The *Eco*RI–*Hin*dIII reiteration map of λhtm4 is shown in Fig. 5*A*, and a corresponding summary of segment repetition is shown in Fig. 5*B*. The intensely hybridizing 1.85-kb *Eco*RI–*Hin*dIII fragment, estimated to share homology with at least 600 genomic sequences, appears to be identical (or similar) to the analogous fragment in λhtm3. However, in λhtm4, the 1.85-kb *Eco*RI–*Hin*dIII fragment is bounded on its 5′ border by a segment of low reiteration, the 0.9-kb *Eco*RI–*Hin*dIII segment, present in no greater than about two copies, in contrast to the heavily reiterated segment present in the analogous locus in λhtm3. Within the 5.7-kb fragment extending from the right arm of Charon 4A to the *Hin*dIII site at 5.7 kb a sequence with about 25–50 genomic representations can be detected.

DISCUSSION

By application of the mapping procedure outlined in this study, we have described the pattern of repetitive sequence interspersion within three cloned chromosomal segments containing the human initiator methionine tRNA. Reiteration frequency mapping provides a rapid quantitative approach to the localization of repetitive sequences and can be readily applied to the identification of DNA sequences within a cloned genomic fragment that are represented in RNA populations (unpublished observations).

The estimates of genomic reiteration of the localized repeated sequences must be viewed as a *minimal* family size, because we have not determined the sequence divergence of each repeat family. The extent of hybridization of a particular sequence from a family of highly divergent members would be criterion limited, in that hybridization of the probe sequence to more poorly matched relatives in the genome would increase as the stringency of hybridization conditions was lowered. Although in sea urchin (14) and *Drosophila melanogaster* (15) sequence divergence of repetitive element families is surprisingly limited, in the genomes of other higher organisms (16, 17), such as human (18), divergence may be a more common characteristic, and should thus underscore the possibility of an underestimate of family size in this analysis.

It should be stressed that until a sequence within a given restriction fragment is more precisely resolved, we can not distinguish whether the repeated elements detected correspond to a single unit or to a cluster of several different repeated elements.

Perhaps the most striking feature that characterizes the three cloned chromosomal segments that contain the initiator methionine tRNA loci is the *absence* of an obvious common local pattern of repetitive element interspersion. In each clone the tRNA structural gene lies distant, relative to its own nucleotide length, from neighboring highly repetitive elements, in λhtm1 more so than in λhtm3 or λhtm4. Although it is possible that low-frequency repeat sequences are present within the smallest tRNA-specific restriction fragments characterized in this study, no single hybridizable sequence that might be associated with all or a major subset of the 1000 or so tRNA genes in the haploid human genome (19) lies within several hundred nucleotides of the initiator methionine tRNA genes described in this report.

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