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Universal mapping probes and the origin of human chromosome 3

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ABSTRACT **Universal mapping probes (UMPs) are defined as short segments of human DNA that are useful for physical and genetic mapping in a wide variety of mammals. The most useful UMPs contain a conserved DNA sequence immediately adjoined to a highly polymorphic CA repeat. The conserved region determines physical gene location, whereas the CA repeat facilitates genetic mapping. Both the CA repeat and its neighboring sequence are highly conserved in evolution. This permits molecular, cytogenetic, and genetic mapping of UMPs throughout mammalia. UMPs are significant because they make genetic information cumulative among well-studied species and because they transfer such information from "map rich" organisms to those that are "map poor." As a demonstration of the utility of UMPs, comparative maps between human chromosome 3 (HSA3) and the rat genome have been constructed. HSA3 is defined by at least 12 syntenic clusters located on seven different rat chromosomes. These data, together with previous comparative mapping information between human, mouse, and bovine genomes, allow us to propose a distinct evolutionary pathway that connects HSA3 with the chromosomes of rodents, artiodactyls, and primates. The model predicts a parsimonious phylogenetic tree, is readily testable, and will be of considerable use for determining the pathways of mammalian evolution.**

Over the course of evolution, the physical arrangement of genes along a chromosome tends to be conserved among species. When two or more genes are linked on the same chromosome in two or more species, they are said to show conservation of synteny (on the same strand). In fact, the human genome is thought to be a mosaic of about 80 syntenic clusters related to the rodent genome (1). Not only is gene order usually coextensive between syntenic segments, but conservation also extends to the nucleotide level as detected by cross-hybridization among unique DNA sequences. When used as probes, these sequences have the ability to detect homologous regions across species boundaries. If such homologies were properly exploited, a single bank of probes could be assembled to construct both physical and genetic maps of virtually any mammal.

We define universal mapping probes (UMPs) as short [≤15 kilobases (kb)] segments of DNA, derived from the human genome, that possess physical and/or genetic mapping properties among a wide variety of mammals. There are three categories of UMPs. The most elementary is a conserved, but nonpolymorphic, DNA sequence capable of physical but not genetic mapping. A second class of UMPs contains a conserved domain that also exhibits restriction fragment length polymorphism (RFLP), thereby permitting both physical and genetic mapping. cDNAs fall into either of these categories and may serve as a convenient source of UMPs, although identifying a RFLP with sufficient variation in any mammal can be tedious. The third and most useful class of universal mapping probe is one whose conserved sequence is adjoined to a highly polymorphic domain such as a CA repeat. It is this latter category of UMP, which facilitates both physical and genetic mapping across mammalian orders, that we have sought to identify and exploit.

As a demonstration of both the feasibility and utility of our approach, we have identified 14 UMPs derived from human chromosome 3 (*Homo sapiens*; HSA3) that hybridize to a wide variety of mammalian genomes. In addition, the presence of CA repeats in these probes is evolutionarily conserved. These features permit molecular, cytogenetic, and genetic mapping of UMPs throughout mammalia. Our 14 UMPs, together with six cDNAs and one anonymous sequence, have been used to establish comparative synteny of HSA3 with the rat genome. These data, together with previous comparative mapping information between HSA3 and the mouse and bovine genomes, allow us to propose a distinct pathway for the origin and evolution of HSA3 referred to as the FITT (fusion–inversion–translocation–transposition) model.

MATERIALS AND METHODS

Isolation of HSA3 UMPs. The human–CHO hybrid cell line Q314-2 containing HSA3 as its only human chromosome was obtained from the Eleanor Roosevelt Institute (Denver). Rat–mouse hybrid cell lines were prepared as described (2). DNA probes were provided by colleagues cited in the acknowledgments (3, 4). λ phage cloning vector λKT4 was derived from EMBL4N (5). It accepts 12- to 20-kb DNA fragments terminated by *Not* I and *Mbo* I sites and possesses the following landmarks: L arm–T7 promoter–*Not* I–"stuffer"–*Sal* I–*Bam*HI–*Eco*RI–R arm. Genomic rat DNA was cloned into Lambda DASH. *Escherichia coli* LE392 was the host for λ phages. pBluescript (Stratagene) was maintained in *E. coli* strain DH5α.

DNAs were purified by established procedures (6, 7). Oligodeoxynucleotides were 5′-end-labeled with polynucleotide kinase and [γ-^{32}P]ATP. Genomic and cloned DNAs were ^{32}P-labeled with a random primer kit (Stratagene) and *in situ* hybridization probes were biotin-labeled as described (8). DNA cloned into pBluescript was sequenced by using Sequenase (United States Biochemical).

For Southern blots, DNA from agarose gels was transferred to nylon membranes (Hybond N+; Amersham) and hybridized with 10^7 cpm of the appropriate ^{32}P-labeled DNA probe overnight at 65°C in 10 ml of 10% SDS/7% polyeth-

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Abbreviations: UMP, universal mapping probe; RFLP, restriction fragment length polymorphism; FITT, fusion–inversion–translocation–transposition; FISH, fluorescence *in situ* hybridization.

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ylene glycol (M_r 6000)/200 μg of sonicated salmon sperm DNA per ml. The filters were then washed and processed for autoradiography (7).

HSA3 UMPs were isolated from a library derived from Q314-2 DNA digested to completion with *Not* I and partially with *Mbo* I. This mixture was ligated to *Not* I/*Bam*HI-digested λKT4 DNA and packaged (Gigapack Plus; Stratagene) into mature phage (titer, 2×10^6 phage per ml). HSA3 clones identified by hybridizing plaque lifts from this library to ^{32}P-labeled total human DNA were subsequently digested with *Eco*RI or *Eco*RI/*Not* I, Southern blotted onto nylon membranes, and hybridized with ^{32}P-labeled total human DNA. Only unique sequence (nonhybridizing) *Eco*RI or *Not* I/*Eco*RI fragments were subcloned into pBluescript. Those subclones hybridizing to both human and rodent genomes in a reproducible manner were operationally defined as UMPs.

Fluorescence *in Situ* Hybridization (FISH). Metaphase chromosomes were obtained from human lymphocytes and Fisher rat embryo fibroblast cultures. Cells in exponential growth phase were synchronized by treatment with 5-bromodeoxyuridine and processed for FISH as described (9, 10). When hybridizing UMPs to rat chromosomes, 40 ng of DNA probe per slide was used, the concentration of formamide in the hybridization mixture was lowered to 40%, and the first posthybridization wash was performed at reduced stringency (20 min; 40% formamide; 37°C).

Rat Genetic Mapping. DNA polymorphisms were mapped with a panel of 36 backcross animals obtained by mating F_1 heterozygotes [Long–Evans (LE) × Brown Norway (BN)] to BN individuals. The segregation patterns of parental and recombinant RFLPs for each marker were used to calculate genetic map distance.

Human Genetic Mapping. Linkage analysis of HSA3 was carried out with the CEPH (Centre d'Etude du Polymorphisme Humain) reference pedigree panel (11). RFLP and CA repeat variation studies were performed by modifications of published protocols (12, 13). To date, no CEPH consortium or comprehensive reference map has been constructed for HSA3. Therefore, a HSA3 genetic map was produced by using genotype data from version 4.0 of the CEPH data base. Linkage analysis for constructing this framework map, as well as placement of UMPs therein, was performed as described (14) using the logarithm of odds (lod) score method implemented on the analysis program CRIMAP (Version 2.4; P. Green, personal communication).

RESULTS

HSA3 UMPs. A collection of candidate UMPs was obtained by taking advantage of the fact that in mammals regions enriched in the dinucleotide CpG are preferentially located at the 5′ end of genes (15). The *Not* I restriction site (GCGGC-CGC) contains two CpGs and thus selects for such potentially conserved regions. We identified 612 clones from the Q314 *Not* I/*Mbo* I library as being of HSA3 origin. Of these, 264 (43%) contained a CA repeat. Since the average insert size is ≈15 kb, we estimate there to be about one CA repeat every 35 kb, closely agreeing with previous results (16).

Of the 612 clones, 131 (21%) contained at least one unique *Eco*RI fragment, whereas 88 (14%) possessed a unique *Not* I/*Eco*RI fragment. Ten *Eco*RI or *Not* I/*Eco*RI fragments were tested for their ability to hybridize to Southern blots containing human, mouse, and rat DNA. Three of 10 *Eco*RI fragments hybridized moderately with rodent DNA, whereas 6 of 10 *Not* I/*Eco*RI clones gave moderate to strong signals. We focused our attention on the *Not* I/*Eco*RI clones because of their enhanced cross-species hybridization characteristics.

Of the 88 unique sequence *Not* I/*Eco*RI fragments, 36 (41%) had adjoining CA repeats and 9 of these (25%) also uniquely hybridized to the genomes of a wide spectrum of mammalian orders including primates (human, marmoset), carnivora (dog, cat), artiodactyla (cow, sheep, pig), lagomorpha (rabbit), and rodentia (mouse, rat, hamster). In contrast, only 2 of 52 (3.8%) unique sequence *Not* I/*Eco*RI fragments that lacked a CA repeat hybridized to different mammalian orders. These data indicate that conserved cross-hybridizing sequences are 6.6 (25/3.8) times more frequently associated with stretches of CA repeat than not. In sum, 14 UMPs were obtained (3 *Eco*RI, 11 *Not* I/*Eco*RI fragments; Table 1): 8 contain extensive CA repeat arrays, 3 possess CA repeats in the range of 10–15, and 3, while not adjoined to CA repeats, reveal RFLPs in the human genome.

The HSA3 Cytogenetic UMP Map. The 14 UMPs described above were located on the cytogenetic map of HSA3 by FISH. A mean of 34 metaphase nuclei (range, 22–61) was examined for each probe. In general, the efficiency of *in situ* hybridization was quite high: a mean of 73% of all metaphase spreads (range, 41–100%) possessed at least one chromatid-specific signal and most of the observed signals (mean, 73%; range, 47–95%) reside at specific chromosome bands. These data are summarized in Fig. 1. UMPs are distributed over the length of HSA3, although there appears to be both under- and overrepresentation for some regions. For example, no UMPs were found in 3q25–26, whereas four were located at 3p21 and five were found at 3q22–24. It is of interest to note that these latter two regions are among the richest in CpG island concentration on HSA3, a characteristic that is also correlated with high transcriptional and recombinational activity (19).

FISH of UMPs in Distantly Related Genomes. Fig. 2 illustrates the ability of human-derived UMP probes such as 6-1 and 60-5 to hybridize with the chromosomes of more distant species. Both 6-1 and 60-5 could be unambiguously localized to a specific site in the rat genome (RNO): 6-1 to RNO 8q32 and 60-5 to RNO 4q41. Although the efficiency of hybridization declined (<10% of rat metaphase spreads with a specific signal compared to 73% using human chromosomes), and the amount of background fluorescence increased (<10% of signals at specific bands compared to 73% under standard stringency conditions), symmetrical signal doublets were consistently found at specific locations on chromatid pairs. Such comparative mapping by FISH is rapid and provides

Table 1. Properties of 14 UMPs assigned to HSA3

UMP (restriction site)	Size, kb	HSA3 cytogenetic location	CA repeat length, bp (heterozygosity)	RFLP enzyme (heterozygosity)
5-2 (NE)	4.0	3p21.3–p22	16 (0.76)	None*
5-8 (E)	3.0	3p21	0	*Eco* RI (0.47)
6-1 (NE)	5.0	3p21.1	0	*Hin*cII (0.47)
6-10 (NE)	3.5	3q22–q24	>15	None*
9-2 (NE)	3.5	3q22–q23	12 (0.2)	*Bgl* I (0.2)
12-4 (E)	2.0	3q13.2	10–15	*Bgl* I (0.1)
12-8 (E)	2.4	3p21.1–p21.2	>15	None*
21-3 (NE)	2.5	3q22–q23	>15	None*
21-10 (NE)	1.8	3p24	>15	None*
22-4 (NE)	2.4	3q27	0	*Msp* I (0.45)
33-5 (NE)	5.0	3q22	>15	*Pst* I (0.45)
42-3 (NE)	3.0	3q23	>15	None*
50-2 (NE)	1.8	3p13	10–15	None*
60-5 (NE)	7.0	3p26	16	*Nco* I (0.2)

Restriction sites that delimit each cloned fragment: N, *Not* I; E, *Eco*RI. CA repeat lengths of UMPs 5-2, 9-2, and 60-5 were determined by direct sequencing. All others were estimated by hybridization with ^{32}P-labeled $(CA)_{25}$.

*No RFLP was found after examining 22 different restriction enzymes.

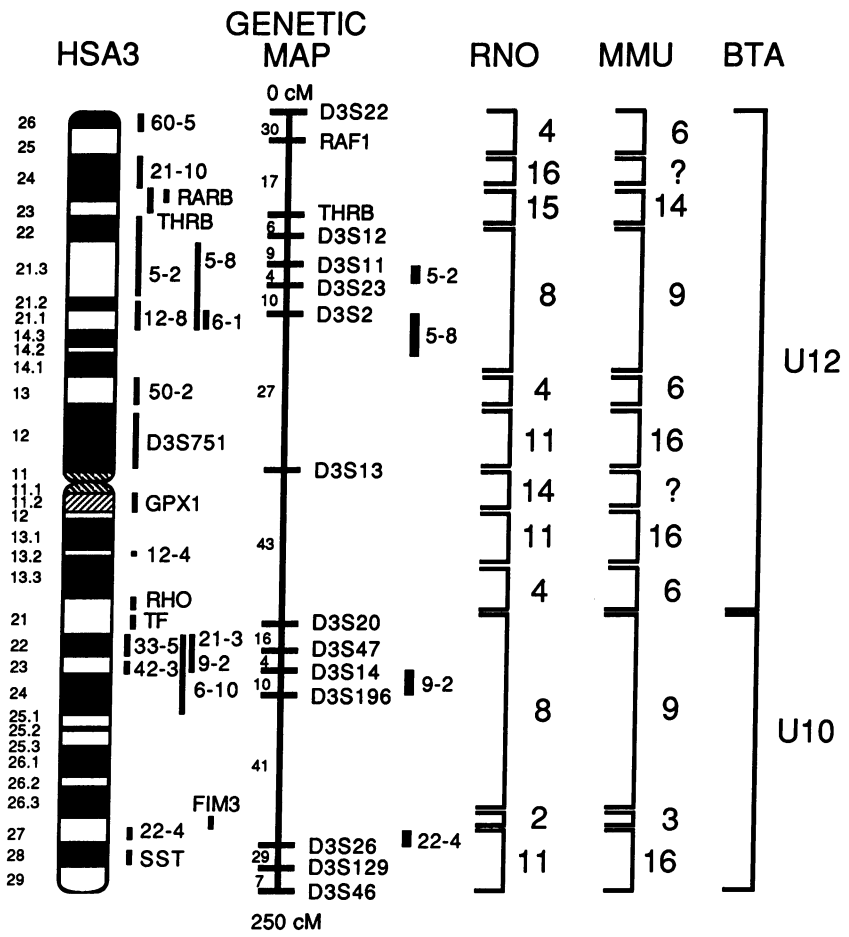

FIG. 1. Comparative syntenic relationships of rat (*Rattus norvegicus*; RNO), mouse (*Mus musculus*; MMU), and cow (*Bos taurus*; BTA) genomes to HSA3. An ideogram of HSA3 appears on the left. UMP probes used in this study to comparatively map to the rat genome are located on the right of HSA3. Transferrin (TF) is included for reference only. The genetic map of HSA3 together with relevant framework markers and the positions of four UMPs (5-2, 5-8, 9-2, and 22-4) are illustrated. The distance between 5-2 and 5-8 is 13.9 centimorgans (cM). The length of this sex-averaged map (250 cM) differs in males (215 cM) and females (335 cM). Portions of HSA3 corresponding to various rat, mouse, and cow chromosomes are indicated on the right. Mouse chromosome assignments were inferred from rat–mouse comparative maps (17); HSA3–bovine comparative maps have been described elsewhere (18). Except for the syntenic boundary between RHO and TF, the limits between adjoining syntenic clusters can only be estimated.

detailed physical information. Although our experience is limited to only two UMPs in this regard, it seems likely that this method will be quite useful for direct chromosome mapping across mammalia.

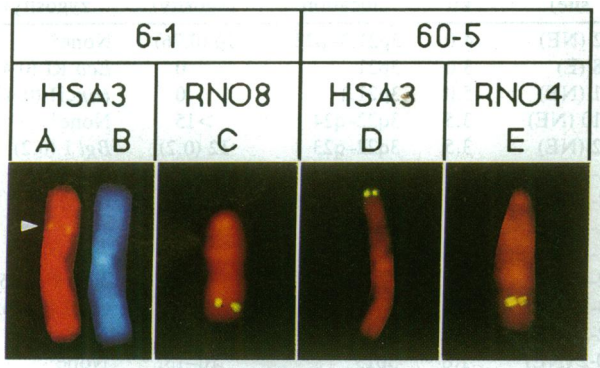

FIG. 2. FISH of UMPs 6-1 and 60-5 to human and rat metaphase chromosomes. (*A* and *B*) Propidium iodide (PI) (red-orange) and 4′,6-diamidino-2-phenylindole (DAPI) (blue) fluorescence images, respectively, of HSA3 hybridized to fluorescein isothiocyanate (FITC) (yellow)-labeled UMP 6-1 (arrowhead). (*C*) Merged DAPI–FITC image of RNO8 hybridized to 6-1. (*D* and *E*) Merged DAPI–FITC images of 60-5 hybridized to HSA3 and RNO4, respectively.

Genetic Mapping of UMPs to HSA3. Table 1 indicates the potential to map genetically all of our UMPs using CA repeat or RFLP analysis. Of the 14 HSA3-specific UMPs, 7 revealed RFLP properties with an average heterozygosity of 0.33 (Table 1; range, 0.10–0.47). Two of these, 5-8 and 22-4, showed the highest heterozygosity and were mapped on HSA3 to the intervals between framework markers D3S2–D3S13 and D3S196–D3S26, respectively (Fig. 1). In addition, 11 UMPs contained, or were estimated to contain, $(CA)_n$ repeat lengths with $n \geq 15$. Heterozygosity at loci of this size is known to be quite high, thereby facilitating meiotic mapping (20). Two $(CA)_n$-containing UMPs, 5-2 and 9-2, were located to intervals between framework markers D3S11–D3S23 and D3S14–D3S196, respectively. UMP 5-2 with n = 16 had a heterozygosity value nearly 4 times greater than UMP 9-2 (n = 12).

Mapping UMPs to Rodent Chromosomes. Each of the 14 UMPs listed in Table 1, together with 5 previously known HSA3 cDNAs and 1 anonymous probe, have been mapped to a specific rat chromosome using a panel of rat–mouse hybrid cells (2). The results are illustrated in Fig. 1. In addition, FIM3 was located on RNO2 by testing the progeny from a backcross mating of BN/LE × BN for linkage between *FIM3* and the prolactin receptor previously mapped to RNO2. Two recombinants, among 36 backcross offspring, demonstrate that *FIM3* is indeed linked to the prolactin receptor. Thus,

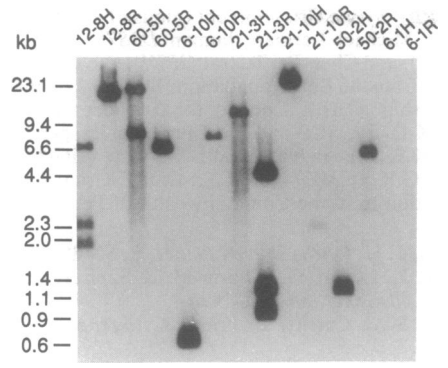

FIG. 3. Preservation of the CA repeat/conserved domain motif from rats to humans. Southern blots of *Eco*RI digests of human and rat cognate UMP clones were hybridized to ^{32}P-labeled $(CA)_{25}$. Sequencing demonstrated 60-5H contained a 16 CA repeat array.

these 21 probes demonstrate that HSA3 is composed of at least 12 syntenic clusters derived from 7 distinct rat chromosomes.

Our results confirm and considerably extend previous comparative mapping data between HSA3 and the genomes of the rat (17) and, by inference, the mouse (21). Previously, RNO4, -8, -11, and -15 and MMU3, -6, -9, -14, and -16 have shown homology with HSA3 (22, 23). It is now clear that ≈80% of HSA3 demonstrates comparative synteny with RNO4, -8, and -11. The remaining 20% is accounted for by contributions from RNO2, -14, -15, -16, and possibly other chromosomes. Homology of HSA3 with RNO2, -14, and -16 has not been reported. Indeed, UMP 21-10 is the first DNA probe to identify RNO16. In addition, the segments of RNO4, -8, and -11 (also MMU6, -9, and -16) were thought to be contiguous along HSA3 but are obviously far more interrupted than heretofore recognized. Fig. 1 also identifies portions of the mouse genome where they are known to correspond to the comparable rat chromosome (17), as well as previously published comparative HSA3 bovine data (18). Two BTA linkage groups, U12 and U10, appear to be sufficient to cover all of HSA3.

Conservation of CA Repeat Motifs. There is a striking correlation between the presence or absence of a CA repeat in human-derived UMPs and their rat homologs. Seven human UMPs (12-8, 60-5, 6-10, 21-3, 21-10, 50-2, and 6-1) were used as probes for isolating the corresponding rat sequence. As shown in Fig. 3, both human and rat cognate clones 12-8, 60-5, 6-10, 21-3, 21-10, and 50-2 possess CA repeats. Four (12-8, 60-5, 21-3, and 50-2) of the six contain robust CA repeat lengths and should serve as highly polymorphic probes for genetic mapping of these UMPs in the rat as well. Conversely, UMP 6-1 did not possess a CA repeat in either human or rat genomes. The complete concordance among all seven human–rat pairs for the presence or absence of a CA repeat adjacent to highly conserved sequences is striking and suggests that such dinucleotide reiterations are of considerable selective advantage.

DISCUSSION

The ability to identify and isolate UMPs for construction of comparative syntenic maps between HSA3 and the rat (and, by extension, mouse) genome has been demonstrated. HSA3 is defined by at least 12 syntenic clusters derived from seven different rat chromosomes. Since HSA3 represents ≈7% of the human genome, it should be possible to develop the complete ensemble of 300 UMPs, one from each metaphase band of every human chromosome, and thereby construct a grand unified map of the mammalian genome with a resolving power of ≈10^7 base pairs. Such a map, anchored to the human

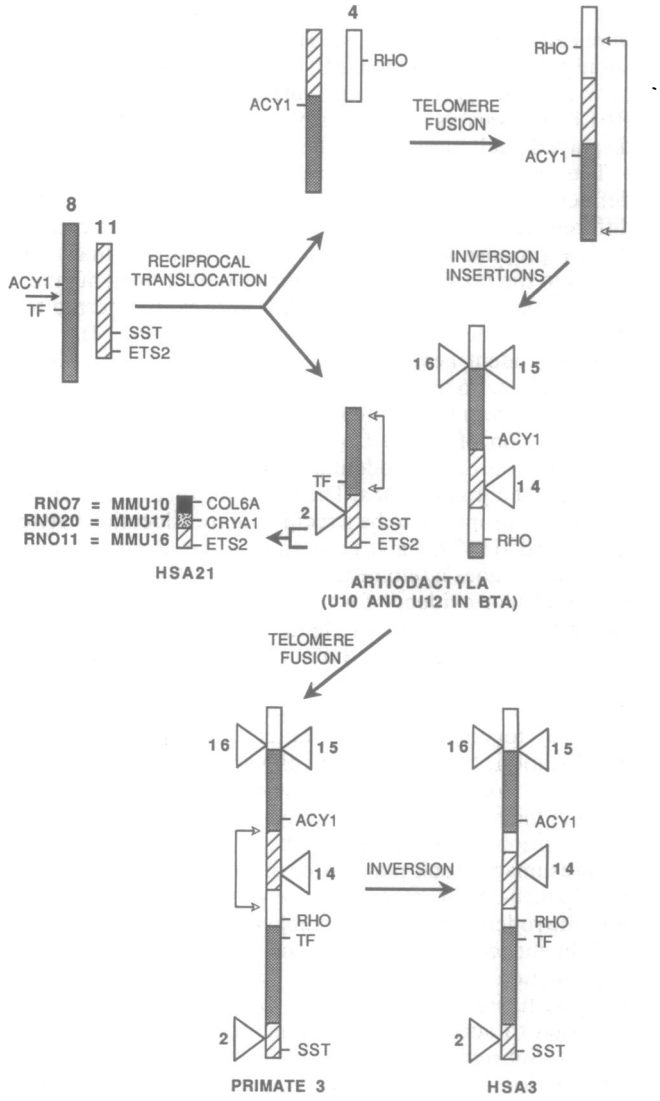

FIG. 4. Proposed major steps in evolution of HSA3 according to the FITT model. Locations of *ACY1* (aminoacylase 1), *TF* (transferrin), *RHO* (rhodopsin), *SST* (somatostatin), *ETS2* (avian erythroblastosis virus oncogene homolog 2), *COL6A* (collagen type VI), and *CRYA1* (crystallin α peptide) serve as landmarks.

genome by both physical (FISH) and genetic (CA repeats, RFLPs) markers, allows genetic information to be both transferable and cumulative. The implications of this approach are considerable, especially for improving the genetic accessibility of organisms so long ignored yet exhibiting biologically interesting disease or behavioral phenotypes.

We have also demonstrated the striking concordance of the presence or absence of CA repeats in the vicinity of highly conserved DNA sequences from rodents to humans. However, Stallings *et al.* (16) concluded from a survey of GenBank that there is not extensive conservation of CA repeat positions between humans and rodents, although they do note that 6 of 17 (35%) sites were conserved where homology could be established. One possible explanation for this difference is that our process of UMP selection results in capturing the most highly conserved sequences and, with them, adjacent CA repeats of ancient origin are carried along by a linkage selection effect. Alternatively, CA repeats may exist at similar rather than identical positions in the two species. That is, the presence of a CA repeat may be functionally quite important, but its exact position need not be strictly defined. In either case, strong conservation of a

CA repeat and its neighboring sequence has an important implication for comparative gene mapping in mammals: it should allow the cognate UMP to be genetically mapped in its own genome by CA repeat variation. Mapping UMPs by RFLP analysis is also possible since half of the probes displayed such variation in the human genome (Table 1).

The results presented here demonstrate that HSA3 is defined by at least 12 syntenic clusters located on seven different rat chromosomes. Despite this apparent complexity, we propose a simple evolutionary pathway that relates HSA3 to the rat karyotype, requiring only a small number of chromosome rearrangements. This scheme is referred to as the FITT model of chromosomal evolution (Fig. 4).

While rodent genomes have not remained static we are, nevertheless, concerned about those past events that might connect modern rat and human karyotypes. For this purpose, the evolution of HSA3 begins with the primitive placental mammalian order of rodentia (24) and is described here in terms of rat chromosomes. The initial step in the construction of HSA3 is proposed to be a reciprocal translocation between RNO8 and -11. This gives rise to two chromosomes, one of which undergoes a telomere fusion with RNO4. Cytogenetic and molecular evidence for telomere fusions, specifically as they relate to the origin of HSA2, are well documented (25, 26) and may account for the occurrence of interstitial telomere-related sequences in the human genome (27). A large inversion of the 4-8-11 translocation–fusion product would produce, together with the other derivative of the 8-11 translocation, two linkage groups known as U12 and U10 in *B. taurus*. We propose that these are the major events that connect the rodent and artiodactyla lineages. The insertional transposition of appropriate segments of RNO2, -14, -15, and -16 presumably took place at or during the formation of the 4-8-11 and 8-11 chromosomes, events that evidently occurred after the rodent lineage was established. These transpositions most likely occurred as transposon-mediated events, another phenomenon for which there is ample evidence. A second telomere fusion between the 8-11 and 8-11-4 products is then proposed to account for the formation of primate chromosome 3. Subsequent minor inversions ultimately lead to the construction and contemporary linear order of HSA3. The distal portion of U10 in the BTA genome, together with segments homologous to MMU10 and -17, ultimately form HSA21. We presume that this occurred during, or just after, the rise of artiodactyla.

In a general sense, the FITT model relies on only two kinds of events: those involving nonspecific breakage and rearrangement of syntenically conserved chromosomal segments, and those involving insertional transposition of very small regions. We find no evidence that fragile sites or breakpoints found in the chromosomes of human malignant cells (28) necessarily define syntenic boundaries. Importantly, the FITT hypothesis allows for a coherent and parsimonious phylogenetic tree, is readily testable, and will be of considerable value for defining the pathways of mammalian evolution. A more extensive application of the concepts described here will facilitate the rapid construction of a grand unified map of the mammalian genome.

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