9 Primate Chromosome Evolution

Stefan Müller, PhD

Contents

BACKGROUND

During the last two decades, comparative cytogenetics and genomics has evolved from a specialized discipline to a highly dynamic field of research. This development was driven by major technological advancements as well as emergence of the deeper insight that many aspects of human genome function can be better understood when information about its evolutionary changes is taken into account. Whole-genome sequencing projects of biomedical model species and domesticated animals provided important clues to the molecular mechanisms that shaped the human genome. These strategies were complemented by the launch of the chimpanzee genome project, leading to the recent publication of the first chimpanzee draft sequence and its alignment with the human reference sequence.

The objective of this chapter is to (1) review recent technical developments in the field of comparative cytogenetics and genomics provide knowledge about ancestral primate chromosomal traits and on evolutionary landmark rearrangements; (2) recapitulate the molecular cytogenetic evidence for the evolutionary history of human chromosomes with special emphasis on great apes; and (3) summarize the present data about the genomic environment of evolutionary chromosomal breakpoints in human and great apes.

Rates and the direction of evolutionary rearrangements are discussed in the context of the emerging patterns of evolutionary genomic changes and future perspectives including whole genome sequencing and microarray based approaches are addressed.

From: *Genomic Disorders: The Genomic Basis of Disease*
Edited by: J. R. Lupski and P. Stankiewicz © Humana Press, Totowa, NJ

INTRODUCTION

The introduction of fluorescence *in situ* hybridization (FISH) to comparative karyotype analysis *(1)* marked a paradigm shift from the analysis of chromosome morphology toward chromosomal DNA content. The use of human chromosome-specific probes in cross-species chromosome painting experiments for the first time allowed the secure identification of chromosomal homologies between species at a resolution of 3–5 Mb within primates and 5–10 Mb when comparing human with nonprimate mammals. A second landmark was the establishment of a reproducible Zoo-FISH protocol *(2)* for the analysis of any mammalian species with human probes. In addition, chromosome-specific "painting" probes were established from several nonhuman primates as well as from other mammalian and vertebrate species by fluorescence-activated chromosome sorting and subsequent degenerate oligonucleotide-primed polymerase chain reaction amplification *(3)*, for example, from lemurs *(4)*, mouse *(5)*, and chicken *(6)*. The availability of chromosome paint probes from nonhuman species for Zoo-FISH experiments allowed the assembly of comparative chromosome maps in a reciprocal way *(7)* (Fig. 1). This approach is particularly helpful when analyzing distantly related species in which the hybridization efficiency is low. Moreover, when performing reverse painting to human chromosomes, evolutionary breakpoints can be localized on the human sequence map for a subsequent detailed characterization of evolutionary breakpoints. Further development of this strategy led to the concept of multidirectional chromosome painting *(8)*, where members of a species group of interest are systematically analyzed with human paint probes and in addition with paint probes of a species from the targeted species group.

Cross-species chromosome painting certainly has a number of limitations: apart from the limited resolution, intrachromosomal rearrangements usually escape detection. For the construction of more detailed comparative genome maps, several complementary methods have been established, among others Zoo-FISH employing chromosome bar codes and subregional DNA probes, comparative radiation hybrid mapping and, ultimately, whole genome sequencing.

In order to obtain high-resolution comparative chromosome maps, Zoo-FISH with vector-cloned DNA probes can be performed. Bacterial artificial chromosome (BAC) libraries from numerous species are publicly available (http://bacpac.chori.org), among them human, chicken, mouse, rat, cat, pig, and cow, and several nonhuman primates (chimpanzee, orangutan, gibbon, macaque, squirrel monkey, lemur, and others). Because human, mouse, and rat genome projects are essentially complete, "tile path" BAC probes from these species provide an excellent source for comparative FISH mapping studies with a direct link to the genome sequence (www.ensembl.org). For FISH analysis of distantly related species, human BAC probes can be selected from genomic regions with high evolutionary sequence conservation between human and mouse *(9)*.

Alternatively, somatic cell hybrid panels may serve a template for physical mapping studies in order to assemble interspecies homology maps. High-resolution radiation hybrid panels have been established, for example, for the rhesus macaque *(10)*. For chimpanzee, gorilla, and orangutan, lower resolution panels are available *(11)*.

The rapid progress of the human, mouse, and rat genome projects already provides a detailed and comprehensive insight into the ancestral mammalian genome organization. Although the major mammalian evolutionary breakpoints are hidden among the noise that stems from the extreme genome reshuffling in rodents, it is increasingly possible to read the human/mouse sequence alignment like cross-species chromosome painting data. With other more recently

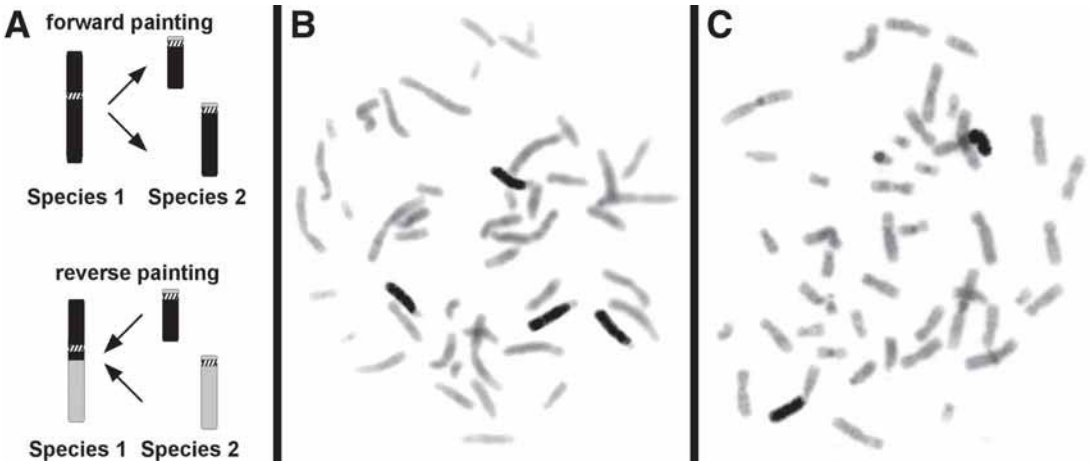

Fig. 1. (A) The principle of reciprocal chromosome painting. (B,C) Reciprocal chromosome painting between human and orangutan: (B) *in situ* hybridization of human chromosome 2 probe to an orangutan metaphase delineates two homologs. (C) Reverse painting of the orangutan 2q homolog to human chromosomes hybridizes to the 2q13-qter segment.

launched genome projects (i.e., chicken, dog, and chimpanzee *[12]*) advancing even faster, the view becomes increasingly clear. For example, the fusion point of human chromosome 2 in 2q13 can readily be identified on the human/chimpanzee comparative sequence map (www.ensembl.org) at the junction of chimpanzee chromosome 12 and 13 homologous sequences.

RECONSTRUCTION OF PRIMATE KARYOTYPE EVOLUTION

The Ancestral Primate Karyotype

Because chromosomal homology maps between human and approx 30 nonprimate mammals and more than 50 primates have been established, the data set available provides a firm basis on which proposals for common ancestral mammalian chromosomal traits and shared derived primate-specific chromosome forms can be made. For the sake of clarity and simplicity, in the following sections chromosomes are always numbered according to their human homologous counterparts.

When comparing the karyotypes of species from different placental mammalian orders, a surprisingly high degree of conservation can be observed for the majority of species. The homologs of human autosomes 1, 5, 6, 9, 11, 13, 17, 18, and 20 are found conserved as separate entities in several different orders and are therefore assumed to represent ancestral mammalian chromosome forms. In addition, human chromosome 3, 4, 14, 15, and 21 homologs are entirely conserved in other mammalian orders, however, translocated. Syntenic associations of human homologous chromosomes 3/21, 4/8p, and 14/15 are, therefore, also considered to be ancestral for placental mammals. Human chromosome 2, 7, 8, 10, 16, and 19 homologs are found split in two separate syntenic segments, some of them are associated with further chromosomal material. Human chromosome 2 is a fusion product of two separate ancestral homologs 2pter-q13 (2a) and 2q13-qter (2b). Human chromosome 7 is a complex fusion product of

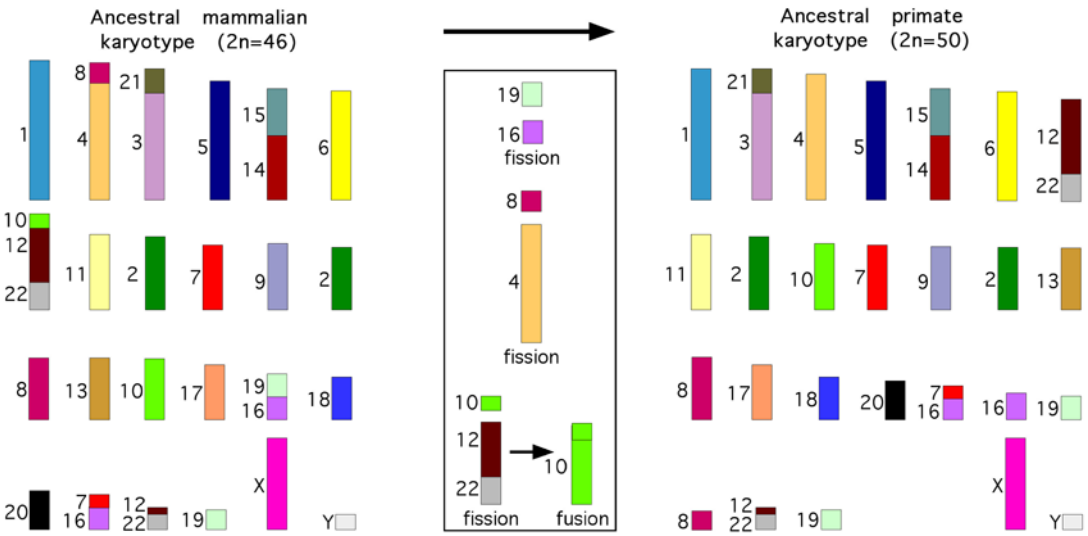

Fig. 2. Inferred ancestral mammalian and ancestral primate karyotypes with the assignment of human homologies to the left, based on multidirectional chromosome painting data. The primate karyotype is derived by fissions of 10p and 12pter-q23.3, 16q and 19q, and 4 and 8p, followed by fusion of 10p/10q.

ancestral chromosome forms (7p21-q11.21, 7q11.23-21.3, 7q22.1-qter) and (7pter-p22, 7q11.21-11.23, 7q21.3-22.1)/16p. Human chromosomes 12 and 22 resulted from a reciprocal translocation t(12;22)(q23.3;q12.3) of the two ancestral homologs 10p/12pter-q23.3/22q12.3-qter and 22q11.2-22q12.3/12q23.3-qter. Chromosome 16q and 19q homologs are associated on a single chromosome, whereas 8q, 10q and 19p are found as separate entities *(13)* (Fig. 2). The ancestral karyotype of placental mammals may therefore have comprised of 2n = 46 chromosomes.

The putative ancestral primate karyotype shows only a very few differences when compared to the ancestral mammalian karyotype. In the inferred primate ancestor presumably fissions of 10p and 12pter-q23.3, 16q and 19q, and 4 and 8p occurred, followed by fusion of 10p/10q, resulting in a karyotype of 2n = 50 chromosomes with conserved human homologous chromosome (segments) 1, 2pter-q13, 2q13-qter, 4, 5, 6, (7p21-q11.21, 7q11.23-21.3, 7q22.1-qter), 8p, 8q, 9, 10, 11, 13, 16q, 17, 18, 19p, 19q, 20, X, Y, and the association of 3/21, (7pter-p22, 7q11.21-11.23, 7q21.3-22.1)/16p, 12pter-q23.3/22q12.3-qter, 22q11.2-22q12.3/12q23.3-qter, 14/15 (Fig. 2).

The following section provides an overview of the current knowledge on karyotype evolution in prosimians and higher primates (anthropoidea), the latter being subdivided in New World monkeys (platyrrhini), Old World monkeys (cercopithecoidea), and apes (hominoidea, gibbons, and great apes).

Prosimians

Prosimians are comprised of lorises, bush-babies, and lemurs. Only five species were fully analyzed using human chromosome painting probes: brown, black, and ring-tailed lemur *(4,9)*, and two bush-babies *(14)*. In addition, black lemur chromosome-specific probes were characterized by reverse painting to human chromosomes *(4)*. The results so far revealed that these prosimians retained ancestral primate chromosome forms 12/22, 3/21, 14/15, and probably

7/16, but showed common derived fissions of chromosome 1 (two fissions), 4, 5, 6, 8, and 15. In addition, the investigated lemurs share four derived fissions and five translocations, the two bush-babies five fissions, and six translocations *(14)*. Numerous further rearrangements were noticed, for example eight fusions only found in the black lemur *(4)*. By conclusion, none of the prosimians analyzed to date has conserved a primitive primate karyotype, instead prosimians have highly derived karyotypes.

New World Monkeys

The 16 genera and over 100 recognized species of New World monkeys (platyrrhini) show a high degree of karyotypic diversity with chromosome numbers ranging from 2n = 16 in *Callicebus lugens* to 2n = 62 in *Lagothrix lagotricha*. With established comparative genome maps between human and more than 20 species by cross-species chromosome painting with human probes, New World monkeys represent the most comprehensively studied group of species among mammals altogether. In addition, several species have been analyzed by multi-directional chromosome painting employing tamarin (*Saguinus oedipus*) *(15)* and woolly monkey (*L. lagothricha*) *(16)* chromosome-specific probes.

In the inferred ancestral New World monkey karyotype of 2n = 54 chromosomes *(17)*, human chromosomes 4, 6, 9, 11, 12, 13, 17, 19, 20, 22, X, and Y homologs are found entirely conserved as separate chromosomes. Chromosome 5, 14, 18, and 21 homologs show conserved synteny, are however associated with other homologs (5/7a, 14/15a, 8a/18, and 3a/21). The remaining human homologs are fragmented: 1a, 1b, 1c, 2a, 2b/16b, 3b, 3c, 7b, 8b, 10a/16a, 10b, and 15b.

Among the family callitrichidae, two tamarins (*S. oedipus* and *Leontopithecus chrysomelas*), three marmoset species (*Callithrix jacchus*, *Cebuella pygmaea*, and *Callithrix argentata*), and *Callimico goeldii* were analyzed by multidirectional chromosome painting *(17–19)*. The data showed that *C. goeldii* exclusively shares derived syntenic associations 13/9/22 and 13/17/20 with all callithrichids. A study on an interspecies hybrid between a female Common marmoset (*C. jacchus*, 2n = 46) and a male Pygmy marmoset (*C. pygmaea*, 2n = 44) with a diploid chromosome number of 2n = 45 gave further support for the inclusion of *Cebuella* within genus *Callithrix (20)*. Genomic imbalances between this interspecies hybrid and other callithrichidae, visualized by interspecies Comparative genomic hybridization (iCGH), were confined to centromeric and subtelomeric heterochromatin. Cross-species FISH with a micro-dissection derived *C. pygmaea* repetitive probe revealed species-specificity of several 50 Mb and larger blocks of heterochromatin, thus providing a dramatic example for amplification of noncentromeric repetitive sequences within approximately 5 million years of evolution *(20)*.

Zoo-FISH data are also available from the squirrel monkey, capucin monkeys, and three species of titi monkey (family cebidae). The squirrel monkey shares the derived syntenic association 2/15 with callithrichidae *(17)*. Capuchin monkeys have conserved almost completely the ancestral New World monkey karyotype *(21–23)*. Titi monkeys *Callicebus moloch*, *Callicebus donacophilus* (both 2n = 50), and *C. lugens* (2n = 16), are phylogenetically linked by common derived associations 10/11, 22/2/22, and 15/7 *(24–26)*. The low chromosome number of *C. lugens* is the result of at least 22 fusions and 6 fissions.

The diploid chromosome numbers of atelidae (Howler monkeys, genus *Alouatta*, Spider monkeys, genus *Ateles*, Wooly monkeys, genus *Lagothrix,* and Wooly spider monkeys, genus *Brachyteles*) range from 2n = 32 in *Ateles* to 2n = 62 in *Brachyteles* and *Lagothrix*. Species from all four genera have been analyzed by multi-directional chromosome painting *(16,27,28)*. All

atelidae analyzed share the derived fissions of human chromosome 1, 4, and 5 homologs, inversion of the 10/16 and 5/7 homologs, and a translocation 4/15. The ancestral atelidae karyotype is comprised of 2n = 62 chromosomes and is conserved in *Lagothrix* and *Brachyteles*. Howler monkeys represent the genus with the most extensive karyotype diversity within platyrrhini with high levels of intra-specific chromosomal variability *(29,30,27)*. Molecular cytogenetic studies in Spider monkeys (genus *Ateles*) revealed that at least 17 fusions and three fissions are necessary to derive the putative ancestral *Ateles* karyotype conserved in *A. b. marginatus* (2n = 34) *(23,28,31)*.

Old World Monkeys

Macaques and baboons have strongly conserved and uniform karyotypes with 2n = 42 chromosomes. Compared to the human karyotype all chromosomes show conserved synteny, except for two human chromosome 2 (two homologs) *(32)*. The reduced chromosome number is the result of two fusions, leading to syntenic association of chromosome 7/21 and 20/22 homologs. They further conserved the primate ancestral association of 14/15 homologs. Among guenons, fissions are the main mechanism driving the evolutionary trend toward higher chromosome numbers of 2n = 60 in the African green monkey (*Chlorocebus aethiops*) *(33)* and to 2n = 72 in *Cercopithecus wolfi*.

Leaf-eating monkeys have fairly conserved karyotypes, compared with human with chromosome numbers of 2n = 44 in the black and white colobus (*Colobus guereza*) *(34)* and 2n = 48 in the proboscis monkey (*Nasalis larvatus*) *(35)*. These Colobines share the derived association of human chromosomes 21/22. The Asian members further share a reciprocal translocation of the human chromosome 1 and 19 homolog that was followed by a pericentric inversion.

Hominoids

The four gibbon subgenera show distinct karyomorphs with 2n = 38 in *Bunopithecus*, 2n = 44 in *Hylobates*, 2n = 50 in *Symphalangus,* and 2n = 52 in *Nomascus*. During the last decade all four subgenera were studied by Zoo-FISH, which revealed extensive chromosome reshuffling in all gibbons. First studies employed human chromosome paint probes *(36–39)*. More recently, gibbons were reanalyzed by multidirectional painting *(40–42)*.

The inferred ancestral karyotype of all extant gibbons differed from the putative ancestral hominoid karyotype by at least five reciprocal translocations, eight inversions, 10 fissions, and one fusion *(42)*. It included homologs to human chromosomes 7, 9, 13, 14, 15, 20, 21, 22, X, and Y with conserved synteny and homologous segments of human chromosome 1 (three segments), 2, 3, 4, 5, 6 (two segments each), 8, 11, 12, and 17, respectively. Finally, syntenic associations of segments homologous to human chromosomes were included: 3/8, 3/12/19, 5/16, 5/16/5/16, 19/12/19, 10/4 (twice), 12/3/8, 17/2/17/2, and 18/11. The White-cheeked gibbon, Siamang and White-handed gibbon further share at least five common derived chromosome forms: associations 2/7, 8/3/11/18, 4/5, 22/5/16, and an inversion. In the last common ancestor of the White-cheeked gibbon and the Siamang, one fusion and four fissions may have occurred. In addition, each gibbon accumulated a large number of species-specific rearrangements (between 10 in the White-handed gibbon and 28 in the Hoolock).

Comparative high resolution G-banding analysis of human and great apes indicated, that the only interchromosomal changes are species-specific: a fusion of chromosome 2 in the human lineage, a reciprocal translocation t(5;17) in the gorilla and a band insertion ins(8;20) in the orangutan *(44)*. Chromosome painting of great ape genomes with human probes confirmed the

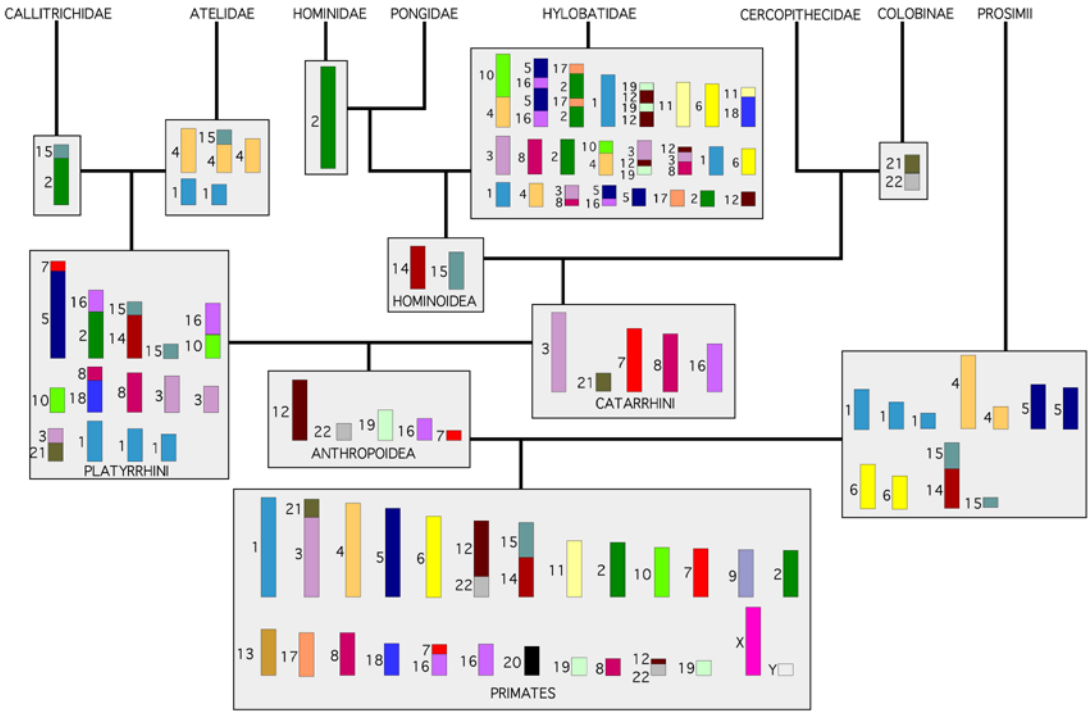

Fig. 3. Superimposition of primate evolutionary landmark rearrangements onto the consensus phylogenetic tree.

fusion of human chromosome 2 and the translocation t(5;17) in the gorilla, but not the insertion ins(8;20) in the orangutan *(1,36,43)*. Further, G-banding indicated that human and great ape genomes would differ by the presence and chromosomal location of constitutive heterochromatin, in the number of nucleolar organizer region-bearing chromosomes, and by several inversions *(44)*. For example, chimpanzee and human chromosome 1, 4, 5, 9, 12, 15, 16, 17, and 18 homologs would differ by pericentric inversions and chromosome 7 homologs of chimpanzee and gorilla by a paracentric inversion. Human and gorilla chromosome 16 homologs as well as human and orangutan chromosome 3 and 17 homologs would have diverged by both peri- and paracentric inversions.

Primate Evolutionary Landmark Rearrangements

On the basis of molecular cytogenetic evidence it is possible to draw increasingly accurate conclusions on landmark rearrangements that occurred during primate evolution (Fig. 3). These landmarks are shared by defined subgroups of primates and thus provide fundamental phylogenetic links between members of these species groups. They can further be superimposed onto the consensus primate phylogenetic tree, which permits an estimate of the timing of these events. The putative ancestor of all primates most probably acquired separate chromosomes 4 and 10 homologs by fission/fusion events and lost the association of 16/19 homologs by fission about 60 million years ago (Fig. 2). The inferred ancestor of higher primates only retained ancestral primate syntenic associations 14/15 and 3/21, but acquired separate chromosome 12 and 22 homologs by reciprocal translocation, a single chromosome 19 homolog by

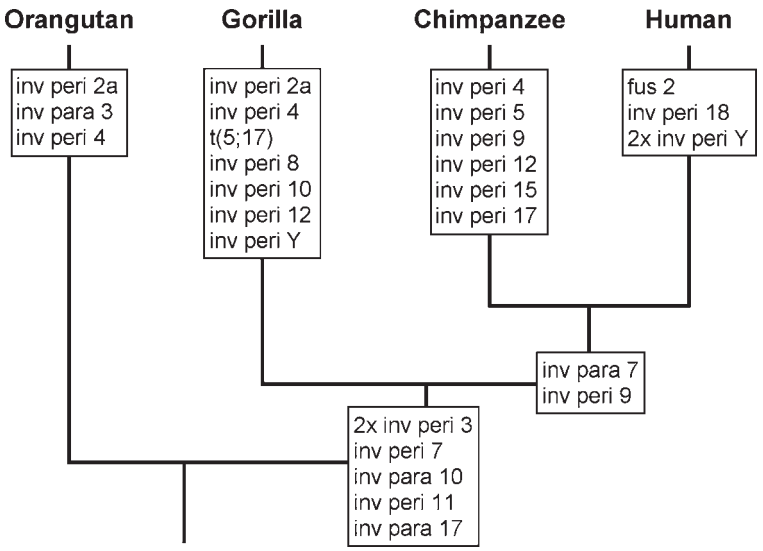

Fig. 4. Overview on chromosome rearrangements that occurred in orangutan, gorilla, chimpanzee and human (*see* The Evolutionary History of Human Chromosomes for details).

a fusion, and lost the association of human 7/16 homologs by fission. These events can be dated before the emergence of New and Old World monkeys (approx 30–40 million years ago). After the New/Old World monkey split, platyrrhini acquired common derived associations 5/7, 2/16, 10/16, 8/18, and several fissions, whereas in the catarrhini ancestor (about 25 million years ago) a fission led to separate chromosomes 3 and 21, whereas fusions resulted in single chromosome 7, 8, and 16 homologs. Approximately 18 million years ago, in the common ancestor of hominoids fission of the association 14/15 took place, leading to separate chromosomes 14 and 15. Finally, less than 6 million years ago and after the divergence of human and chimpanzee, the fusion of human chromosome 2 occurred (Fig. 3).

THE EVOLUTIONARY HISTORY OF HUMAN CHROMOSOMES

This section recapitulates the evolutionary history of selected chromosomes, which are rearranged in human and great apes and for which molecular cytogenetic evidence is available. Figure 4 summarizes all hominoid evolutionary rearrangements.

Chromosome 2

The ancestral condition for all mammals is two separate chromosome 2 homologs. One is homologous to human 2pter-q13, the other to the remaining 2q13-qter segment. Comparative studies with a human chromosome 2q arm-specific paint probe, cosmid clones derived from the human V κ gene cluster, and yeast artificial chromosomes (YACs) indicated that the chimpanzee and the macaque 2pter-q13 homologs may be ancestral, whereas in gorilla and orangutan, independent and probably convergent inversions involving the pericentromeric region of the 2p homolog may have occurred *(45–47)*. More recently, a detailed FISH study with 2q12-14 cosmids revealed the same clone order in human and chimpanzee, which differed from that in the macaque *(48)*.

Chromosome 3

According to one study, the putative ancestral primate chromosome 3 homolog is conserved in the Brown lemur, from which a pericentric inversion led to the ancestral Old World primate homolog conserved in the Bornean orangutan *(49)*. Human/African ape homologs would differ from this chromosome form by two inversions. An even more complex scenario involving several recurrent sites of new centromere seeding was proposed by Ventura et al. *(50)*, according to which human and Bornean orangutan would differ by three inversions. A third hypothesis suggested that from the ancestral simian homolog a common derived and two independent inversions would lead to Bornean orangutan and human chromosome 3 homologs *(51)*. In conclusion, the evolutionary history of human chromosome 3 is probably the most dynamic and complex of all human chromosomes studied in detail so far.

Chromosome 4

Detailed comparative studies on the evolution of human chromosome 4 homologs of great apes and the macaque *(52)* indicated that the human homolog would represent the ancestral hominoid chromosome form. A minimum of seven different breakpoints were observed, some of them were located in the 4p pericentromeric region *(52)*. For three of these inversions breakpoint spanning YAC clones were identified, of which one was confirmative for a previous analysis *(53)*. Interestingly, one clone showed a split signal in chimpanzee and macaque, indicating two independent evolutionary breakpoints in close proximity to each other.

Chromosome 5

Both chromosome bar codes and detailed Zoo-FISH studies with YACs and subregional paint probes identified the chromosome forms shared by macaque, orangutan, and human to be ancestral for hominoids *(54,55)*. From this, the homolog of the chimpanzee is directly derived by a pericentric inversion, those of the gorilla by a reciprocal translocation t(5;17) *(1)*.

Chromosome 6

In a recent FISH study *(56)*, it was shown that the remarkable conservation of chromosome 6 is also present at the subchromosomal level. Despite this, evolutionary centromere relocation events were observed. One of these events may have occurred in the ancestor of great apes, where the centromere moved from 6p22.1 to the present day location. This hypothesis gained support from the observation that in the assumed ancestral location in a cluster of intrachromosomal segmental duplications was found, which the authors explained as remnants of duplicons that flanked the ancestral inactivated centromere *(56)*.

Chromosome 7

A FISH study on evolutionary changes of human chromosome 7 revealed that the ancestral mammalian homologs were comprised of two chromosomes (7a and 7b/16p) as observed in carnivores *(57)*. The ancestral primate segment 7a shared by a lemur and higher Old World monkeys is the result of a paracentric inversion. The ancestral higher primate chromosome form was derived by a fission of 7b and 16p, followed by a centric fusion of 7a/7b in higher Old World primates as observed in the orangutan. In hominoids two further inversions with four distinct breakpoints occurred: the pericentric inversion in the human/African ape ancestor and the paracentric inversion in the common ancestor of human and chimpanzees (Fig. 5) *(57)*.

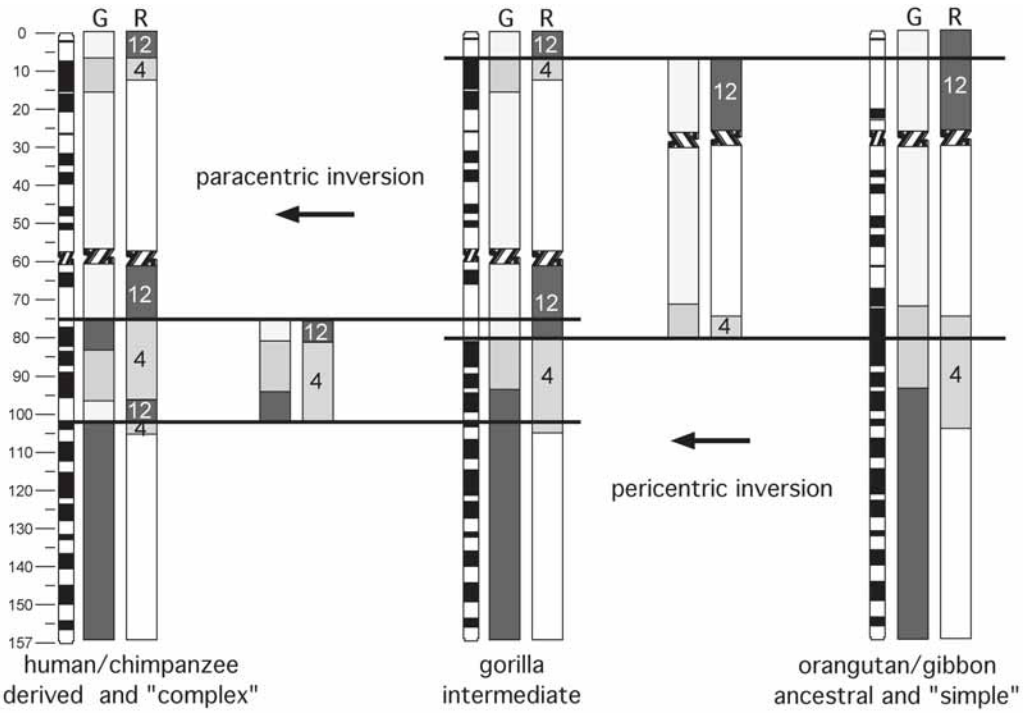

Fig. 5. Hominoid chromosome 7 evolutionary rearrangements delineated by comparative sequence maps of human and rat (R) and cross-species fluorescence *in situ* hybridization with gibbon painting probes (G). Both data sets visualize the inversion breakpoints and their evolutionary direction by an increasingly simple pattern when tracking the rearrangements in the evolutionary reverse direction. Horizontal bars indicate inversion breakpoints.

Chromosome 9

Zoo-FISH with 12 evenly spaced YAC clones *(58)* revealed an identical marker order in Old and New World monkeys, which may therefore represent the ancestral chromosome form for higher primates. A paracentric inversion would derive the ancestral hominoid chromosome form, which was conserved in orangutan and gorilla. A further pericentric inversion would lead to the chromosome form of the last common ancestor of human and chimpanzee. Human conserved this evolutionary intermediate chromosome form, from which the homolog of chimpanzees differs by another pericentric inversion. One of the inversion breakpoints in the chimpanzee homolog was previously identified *(53)*. As for the evolution of human chromosomes 3 and 6, this reconstruction takes into account the evolutionary emergence of neocentromeres.

Chromosome 10

The evolutionary history of human chromosome 10 was tracked by Zoo-FISH with a panel of partial chromosome paint probes, YACs, and BACs *(59)*. These results suggest that in the inferred primate ancestor chromosome 10 homologs were organized as two separate syntenic units, whereas the observation of a single chromosome 10 homolog in two galago species (prosimians) *(14)* would argue for a single chromosome 10 in the ancestral primate. Additional

data on other prosimian species may be required to clarify this issue. Among hominoid primates, the ancestral chromosome form is conserved by orangutan, from which the ancestor of human and African apes is derived by a paracentric inversion. A further species-specific pericentric inversion occurred in the gorilla homolog *(55,59)*.

Y Chromosome

The mammalian Y chromosome shows a broad spectrum of species-specific rearrangements *(60)*. To explain the morphology of the human Y chromosome in comparison to those of the great apes, at least a pericentric and a paracentric inversion specific for the human lineage have to be assumed. Another pericentric inversion was observed in the gorilla homolog. Further, a translocation from chromosome 1 to the Y chromosome took place in a common ancestor of humans and chimpanzees. In addition, submicroscopic deletions and duplications occurred in human, chimpanzee, and orangutan *(60)*.

EVOLUTIONARY BREAKPOINT ANALYSIS

This section provides an overview on the current knowledge about the genomic context of evolutionary chromosomal breakpoints in human and great apes. By analogy to the functional importance of genomic alterations related with acquired chromosomal aberrations in cancers, it was anticipated that changes of the genomic environment caused by evolutionary chromosome rearrangements might hold the key for a better understanding of the origins of our own species.

The Fusion of Human Chromosome 2

Two head-to-head arrays of degenerate telomere repeats are found directly at the fusion site in 2q13 *(61)*. Their inverted orientation indicates a telomeric fusion. Subsequently, the inactivation of one of the two ancestral centromeres must have occurred. Indeed, close to the fusion point in band 2q21 a degenerated alphoid domain was found by low stringency FISH of a satellite DNA probe *(62)*.

Like in other subtelomeric regions, large blocks surrounding the fusion point are comprised of duplicated sequences *(63)*. Chromosome 2q13 paralogs of 96–99% identity were identified on chromosome 9pter, 9p11.2, 9q13, 22qter, and 2q11.2. The emergence of some of these segmental duplications could be dated back prior to hominid divergence, whereas others appeared to be of more recent origin. It can be speculated that these duplicons may have been the cause for the fusion and that human chromosome 2 is the product of paralogous recombination between two different chromosomes using a duplicated segment as recombination substrate *(63)*.

The fusion point is located in a gene-rich region, with at least 24 potentially functional genes and 16 pseudogenes located within less than 1 Mb distance or in paralogous regions elsewhere in the genome *(64)*. At least 18 of these genes are transcriptionally active, for example members of the cobalamin synthetase W domain (*CBWD*) and forkhead domain *FOXD4* gene families, thus providing an example of genomic innovation connected with duplication and evolutionary rearrangement of subtelomeric and pericentromeric regions.

Inversions of Chromosome 3 in Hominoids

A detailed molecular cytogenetic characterization of evolutionary inversion breakpoints in hominoid chromosome 3 homologs revealed that the ancestral pericentromeric region is

associated with both large-scale and micro-rearrangements *(51)*. Small segments homologous to human 3q11.2 and 3q21.2 were repositioned intrachromosomally in the orangutan lineage. The breakpoint in the human 3p12.3 homologous region of the orangutan is associated with extensive transchromosomal duplications observed in multiple subtelomeric regions. A second breakpoint in the same region, but with a distinctly different location, is flanked by sequences present in all subtelomeric regions of the Siamang (gibbon) genome.

Reciprocal Translocation t(4;19) in the Gorilla

The breakpoints of the reciprocal translocation t(4;19) in the gorilla are located in regions syntenic to human 5q14.1 between *HMGCR* and *RASA1* genes, and in 17p12 with an approx 383-kb region-specific low-copy repeat (LCR)17pA *(65,66)*. The 17p12 region is also susceptible to constitutional rearrangements in human. The authors proposed a series of consecutive evoloutionary segmental duplications involving LCR17pA and approx 191-kb LCR17pB copies that resulted in complex genome architecture in the rearrangements. Detailed comparative analysis of the corresponding region identified remnants of DNA-transposable element MER1-Charlie3 and retroviral ERVL elements at the translocation breakpoint in a pre-gorilla individual *(66)*. In addition, genomic rearrangements involving LCR17pA and LCR17pB resulted in the creation of novel genes at the breakpoint junctions *(66)*.

Inversions of Chromosome 7 in Great Apes

Comparative FISH analysis with BAC clones that were derived from the Williams-Beuren syndrome region in 7q11.23 and which contained LCRs including *NCF1* (p47-phox) sequences revealed duplicated segments in the 7q11.23 homologous region of chimpanzee, gorilla, orangutan, and a gibbon *(67)*. As in human, cross-hybridization was observed in the inversion breakpoint regions at 7q22 and 7p22 in African apes, but not in the homologous chromosome regions in orangutan and gibbon.

Zoo-FISH analysis employing BAC probes confined the 7p22.1 breakpoint of the pericentric inversion in the human/African ape ancestor to 6,8 Mb on the human reference sequence map and the 7q22.1 breakpoint to 97,1 Mb *(57)* in regions with predominantly inter-chromosomal duplications with paralogs on human chromosomes 2–4 and 8–15. These duplicons were already present in the orangutan, but spread to a variety of additional chromosomes in the gorilla. The paracentric inversion breakpoints in the common ancestor of human and chimpanzees were found in 7q11.23 between 76,1 Mb and 76,3 Mb and in 7q22.1 at 101,9 Mb, respectively. Intrachromosomal duplicons mark an at least 110-kb stretch of nearly identical DNA sequence, which is most probably directly flanking both breakpoints. The 7q11.23 breakpoint is further located in close proximity to a 200-kb or larger insertion of chromosome 1 material, which could be dated back to the African ape ancestor *(57)*. Considering that the duplicated sequences flanking the four inversion breakpoints as well as the chromosome 1 transposition were already present in the evolutionary ancestral state prior to the inversions, segmental duplications may have been the cause rather than the result of both rearrangements.

Inversions of Chromosome 12 in Chimpanzee and Gorilla

Both chimpanzee and gorilla show derived pericentric inversions of the chromosome 12 homologs. In a first comparative FISH study, the 12p12 breakpoints in both species were mapped to the same YAC clone, whereas the 12q15 breakpoints were shown to be located in distinctly different regions *(53)*. In addition, a chimpanzee BAC clone was identified, which

also spanned the 12p12 breakpoint *(68)*. Recently it could be demonstrated that this clone did not span the 12p12 breakpoint in the gorilla, thus, demonstrating that both the 12q and 12p inversion breakpoints differ in chimpanzee and gorilla *(69)*. Sequence analysis of the breakpoints in the chimpanzee genome revealed two large duplications in both breakpoint regions, which probably emerged in concert with the inversion because they were shown to be chimpanzee-specific *(69)*.

Fission of the Ancestral Chromosome 14/15 Synteny in the Great Ape Ancestor

The fission that separated human chromosome 14 and 15 homologs led to the inactivation of the ancestral centromere in 15q25 and to the formation of two new centromeres in the locations where they can be found in human. Detailed comparative molecular cytogenetic analysis of the region 15q24-26 revealed 500 kb of duplicons, which flank both sides of the single ancestral centromere in Old World monkeys *(70)*. Notably, the same duplicons are associated with neocentromeres in 15q24-26 in two clinical cases. This suggests that neocentromere formation in human pathology in a region of an evolutionary inactivated centromere may be triggered by the persistence of recombinogenic pericentromeric duplications.

Inversion of Chromosome 15 in Chimpanzee

Chimpanzee and bonobo share a derived pericentric inversion of their chromosome 15 homologs. A comparative FISH and *in silico* approach was used to narrow down the breakpoint interval of this pericentric inversion to the 15q11-q13 homologous region *(71)*. The breakpoint mapped to a 600-kb segmental duplication cluster. Sequence analysis indicated that this region comprises a duplication of the *CHRNA7* gene and several Golgin-linked-to-PML duplications. Notably, this evolutionary breakpoint did not colocalize with one of the three major common disease rearrangement breakpoints in 15q11-q13.

Inversion of Chromosome 17 in Chimpanzee

FISH was used to investigate the derived pericentric inversion with breakpoints in 17p13 and 17q21.3 homologous regions, by which chimpanzee chromosome 19 differs from human chromosome 17. Breakpoint-spanning BACs were subsequently used to clone and sequence the junction fragments *(72)*. Both breakpoints were localized in intergenic regions rich in *Alu* and LTR elements, but were not associated with LCRs, duplicated regions, or deletions. The findings suggest that repetitive sequence mediated nonhomologous recombination has facilitated inversion formation. In close proximity of the breakpoints, *NGFR* and *NXPH3* genes are located in 17q21.3 and *GUC2D* and *ALOX15B* in 17p13. Most likely, the genomic structure, the expression level or the replication timing of these genes was not affected by the inversion *(72)*.

Inversion of Chromosome 18 in Human

Human chromosome 18 differs from its great ape homologues by a pericentric inversion, which can be assigned to the human lineage. Recently, chimpanzee BAC clones that span one of the breakpoint regions where were identified by FISH, because in human split signals were observed on 18p11.3 and 18q11 *(73,74)*. Interspecies sequence comparisons indicated that the ancestral break occurred between the *ROCK1* and *USP14* gene. In human, the inversion translocated *ROCK1* near centromeric heterochromatin and *USP14* into proximity of 18p subtelomeric repeats. *USP14* is differentially expressed in human and chimpanzee cortex as well as fibroblast cell lines. Further, a 19-kb segmental duplication with paralogs in pericentromeric

regions of chromosomes 1, 9 and the acrocentric chromosomes is also flanking both 18p11.3 and 18q11 regions in inverted orientation. The authors propose a model, according to which the 19-kb 18q segment containing part of the *ROCK1* gene was first locally duplicated and then transposed to the pericentromeric region of the ancestral 18p. Subsequently, the segmental duplication may have mediated an intrachromosomal crossover, resulting in modern human chromosome 18.

CONCLUSION

Rates and Direction of Evolutionary Chromosomal Changes

Primates, like mammals in general, show strikingly divergent diploid chromosome numbers ranging from 2n = 16 in the New World monkey *C. lugens* to 2n = 72 in the Old World monkey *C. wolfi*. Interestingly, several examples provide evidence against a certain trend toward higher or lower chromosome numbers. In New World monkeys, for example, the ancestor of the monophyletic group atelidae has 2n = 62 chromosomes, compared to the ancestor of all platyrrhini with 2n = 54 chromosomes. More recently in atelidae evolution, the chromosome number in the common ancestor of genus *Ateles* was reduced to 2n = 34.

On average, extant species differ from the inferred ancestral eutherian karyotype by 32 chromosome breaks (*13* and references therein for review). The rate of chromosomal exchange, however, varies greatly. The cat and the mink conserved the ancestral mammalian chromosome forms almost unchanged (11–16 breaks) *(75)*, whereas in the dog genome at least 66 breaks occurred *(76)*. Rodents show even greater differences between evolutionary conserved and extremely reshuffled genomes. A recent summary on the current state of the rat genome project listed 278 disrupted chromosomal syntenies between human and rat and 280 between human and mouse as a consequence of 353 rearrangements *(77)*. The vast majority of these rearrangements can be assigned to the muridae lineage because squirrels, which also belong to the order rodentia, show a highly conserved genome organization (21 breaks) *(78)*. A similar situation is found in primates: human chromosomes differ by only 21 breaks from the eutherian ancestor, whereas gibbon genomes accumulated at least 90 breaks. In summary, a "chromosomal evolutionary clock" does certainly not exist. Instead, the rate of chromosomal evolution may change over time in a phylogenetic lineage and could be interrelated with changing environmental impacts and population dynamics.

Patterns of Evolutionary Genomic Changes

Together with retroposon integrations, indels (insertions and deletions), changes of the mitochondrial gene order, gene duplications, and genetic code changes, evolutionary chromosome rearrangements can be classified as rare genomic changes. A bioinformatics approach to reconstruct the succession human/mouse chromosome rearrangements revealed a surprisingly large number of breakpoint hotspots: 190 of the 245 breaks analyzed reused a genomic region, although the authors emphasized that they were not necessarily located in exactly the same nucleotide position *(79)*. The existence of breakpoint clusters would argue against random breakage *(80)* and in favor of a fragile breakage model. Some other reports support this nonrandom breakage model. On human chromosome 1 and its homologs in placental mammals, evolutionary fissions cluster in two breakpoint hotspots on the long arm *(81)*. Further, at the resolution of chromosome banding, in the macaque 9 out of 17 evolutionary breakpoints correspond to evolutionary conserved fragile sites *(82)*. Because the vast majority of the

rearrangements analyzed *(79)* are confined to the muridae lineage, a careful inspection of breakpoints in other mammals is required in order to determine whether this model applies to mammalian genome evolution in general.

In the majority of mammalian species, Robertsonian (centromeric) and tandem fusions or fissions appear to be the predominant type of evolutionary rearrangements. For example, in all New World primates that were analyzed by Zoo-FISH, 64% of the 149 recorded rearrangements were fissions, 24% fusions, and 10% intra-chromosomal rearrangements. Only two rearrangements were identified as reciprocal translocations. Notably, some of the whole chromosome fusions were not simple centromeric head-to-head fusions, but head-to-tail fusions between centromeric and telomeric regions (*see* ref. *17*). The majority of the evolutionary rearrangements appear to be mediated by highly repetitive regions of the genome and are accompanied by gain or loss of centromeric or telomeric function.

A second group of repetitive sequences, which came into the focus of attention as cause for genomic disorders and evolutionary genome instability, are segmental duplications or LCRs *(83–86)*. So far, they have been found to be associated with almost every evolutionary chromosomal rearrangement in hominoid primates that was characterized in detail. Among these were the fusion of human chromosome 2, the reciprocal translocation t(5;17) in the gorilla, several inversions and the formation of neocentromeres. Within the primate lineage, the explosive expansion of the majority of these elements can be correlated with the burst of primate *Alu* retroposition activity approx 35 million years ago *(87)*. At least for some of the rearrangements in hominoid primates it was demonstrated that the associated segmental duplications were present prior to the rearrangement and may have been a cause for it rather than a consequence. In addition, segmental duplications are statistically significantly enriched in genomic regions, which are differentially expressed in the human and chimpanzee brain *(88)*. It would certainly be interesting to know to which degree the chromosomal distribution of duplicated segments in rat and mouse genomes *(89,90)* corresponds with sites of genome reshuffling in these two species.

FUTURE PERSPECTIVES

Sequencing whole genomes of a large number of mammalian species would obviously provide the most comprehensive information on the molecular mechanisms of genome organization and evolution. Recently eight nonprimate mammals (among them the African elephant, the European common shrew, the European hedgehog, guinea pig, the nine-banded armadillo, the rabbit, and the domestic cat) and the orangutan were added to the National Human Genome Research Institute sequencing pipeline (www.nhgri.nih.gov). These species will complement sequence data from other species, which are already available (*Drosophila*, *Fugu*, *Caenorhabditis elegans*, mouse, and rat) or rapidly emerging (cow, dog, chicken, zebrafish, chimpanzee, and rhesus macaque). Despite these efforts, whole genome sequencing projects will always be limited to key species from different phylogenetic lineages. For an in depth study of primate genome evolution, genomic sequence from at least two species from each major evolutionary branching point would be desirable to reduce species-specific noise. An alternative scenario could be that comparative molecular cytogenetic- and array-based screening technologies will guide the way to relevant genomic regions, which will then be targeted by genome sequencing projects.

A key technology with great promise for evolutionary studies employs genomic clone-based microarrays. In the past, iCGH to metaphase chromosomes provided only limited infor-

mation about genomic imbalances between species owing to a low resolution of approx 10 Mb *(20,91)*. The introduction of comparative genomic hybridization to microarrays with almost 2500 human BAC clones evenly spaced in approx 1-Mb distance (array CGH) lead to a 10-fold increase in resolution *(92)*. A first evolutionary array CGH study *(93)* identified 63 sites of DNA copy-number variation between human and the great apes. Most of these copy number changes affected interstitial euchromatic chromosome regions suggesting that such large-scale events are not restricted to subtelomeric or pericentromeric regions.

Another recently introduced genomic microarray based technique was termed "array painting" *(94)*. It involves a BAC microarray as the target for reverse painting of aberrant chromosomes for the analysis of chromosomal breakpoints. The complete composition and the breakpoints of aberrant chromosomes can be analyzed at the same resolution as mentioned above for array CGH. Initial experiments with gibbon chromosome paint probes successfully demonstrated that this technology has the potential to significantly speed up the molecular characterization of evolutionary breakpoints *(95)*.

The impact of the higher order nuclear architecture on the complex epigenetic mechanisms responsible for cell type specific gene expression patterns in multi-cellular organisms has become an important field of genome research. Initial studies on the gene-density correlated radial arrangement of chromatin revealed a remarkable evolutionary conservation over a period of at least 30 million years *(96)*. As in certain human cell types, in a variety of higher primate species gene-dense chromatin is preferentially located in the nuclear center, whereas gene-poor chromatin shows an orientation toward the nuclear periphery. This probabilistic but highly nonrandom arrangement is still strictly maintained in species like gibbons with extremely reshuffled genomes. This evolutionary conservation argues for a still unknown functional significance of distinct radial higher-order chromatin arrangements. It may also be hypothesized that evolutionary chromosome rearrangements are triggered by certain nonrandom chromatin arrangements. Alternatively, such events may lead to the dislocation of chromosomal material to a different nuclear environment, which in turn may have consequences for the transcriptional activity of the affected loci.

SUMMARY

The current knowledge about genome organization in a large number of mammalian species, based on molecular cytogenetic and whole genome sequence data, already enables a number of fundamental conclusions about the evolutionary forces that shaped the human genome, whereas some other evolutionary aspects of genome organization still remain speculative. For the future, it may be expected that comparative cytogenetics, gene mapping, and genomics will become even more integrated methodolocical approaches in evolutionary studies, which enhance and complement each other concerning resolution and applicability to a wide range of species.

REFERENCES

1. Wienberg J, Jauch A, Stanyon R, Cremer T. Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics 1990;8:347–350.
2. Scherthan H, Cremer T, Arnason U, Weier HU, Lima-de-Faria A, Froenicke L. Comparative chromosome painting discloses homologous segments in distantly related mammals. Nat Genet 1994;6:342–347.
3. Telenius H, Pelmear AH, Tunnacliffe A, et al. Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow-sorted chromosomes. Genes Chromosomes Cancer 1992;4:257–263.

4. Müller S, O'Brien PC, Ferguson-Smith MA, Wienberg J. Reciprocal chromosome painting between human and prosimians (Eulemur macaco macaco and E. fulvus mayottensis). Cytogenet Cell Genet 1997;78:260–271.
5. Rabbitts P, Impey H, Heppell-Parton A, et al. Chromosome specific paints from a high resolution flow karyotype of the mouse. Nat Genet 1995;9:369–375.
6. Griffin DK, Haberman F, Masabanda J, et al. Micro- and macrochromosome paints generated by flow cytometry and microdissection: tools for mapping the chicken genome. Cytogenet Cell Genet 1999;87:278–281.
7. Arnold N, Stanyon R, Jauch A, O'Brien P, Wienberg J. Identification of complex chromosome rearrangements in the gibbon by fluorescent in situ hybridization (FISH) of a human chromosome 2q specific microlibrary, yeast artificial chromosomes, and reciprocal chromosome painting. Cytogenet Cell Genet 1996;74:80–85.
8. Müller S, Stanyon R, O'Brien PC, Ferguson-Smith MA, Plesker R, Wienberg J. Defining the ancestral karyotype of all primates by multidirectional chromosome painting between tree shrews, lemurs and humans. Chromosoma 1999;108:393–400.
9. Cardone MF, Ventura M, Tempesta S, Rocchi M, Archidiacono N. Analysis of chromosome conservation in Lemur catta studied by chromosome paints and BAC/PAC probes. Chromosoma 2002;111:348–356.
10. Murphy WJ, Page JE, Smith C, Jr., Desrosiers RC, O'Brien SJ. A radiation hybrid mapping panel for the rhesus macaque. J Hered 2001;92:516–519.
11. Marzella R, Carrozzo C, Chiarappa P, Miolla V, Rocchi M. Panels of somatic cell hybrids specific for chimpanzee, gorilla, orangutan, and baboon. Cytogenet Genome Res 2005;108:223–228.
12. Pennisi E. Evolution. Chimp genome draft online. Science 2003;302:1876.
13. Froenicke L. Origins of primate chromsomes-as delineated by Zoo-FISH and alignments of human and mouse draft genome sequences. Cytogenet Genome Res 2005;108:122–138.
14. Stanyon R, Koehler U, Consigliere S. Chromosome painting reveals that galagos have highly derived karyotypes. Am J Phys Anthropol 2002;117:319–326.
15. Müller S, Neusser M, O'Brien PC, Wienberg J. Molecular cytogenetic characterization of the EBV-producing cell line B95-8 (Saguinus oedipus, Platyrrhini) by chromosome sorting and painting. Chromosome Res 2001;9:689–693.
16. Stanyon R, Consigliere S, Bigoni F, Ferguson-Smith M, O'Brien PC, Wienberg J. Reciprocal chromosome painting between a New World primate, the woolly monkey, and humans. Chromosome Res 2001;9:97–106.
17. Neusser M, Stanyon R, Bigoni F, Wienberg J, Müller S. Molecular cytotaxonomy of New World monkeys (Platyrrhini): comparative analysis of five species by multi-color chromosome painting gives evidence for a classification of Callimico goeldii within the family of Callitrichidae. Cytogenet Cell Genet 2001;94:206–215.
18. Sherlock JK, Griffin DK, Delhanty JD, Parrington JM. Homologies between human and marmoset (Callithrix jacchus) chromosomes revealed by comparative chromosome painting. Genomics 1996;33:214–219.
19. Gerbault-Serreau M, Bonnet-Garnier A, Richard F, Dutrillaux B. Chromosome painting comparison of Leontopithecus chrysomelas (Callitrichine, Platyrrhini) with man and its phylogenetic position. Chromosome Res 2004;12:691–701.
20. Neusser M, Münch M, Anzenberger G, Müller S. Investigation of marmoset hybrids (Cebuella pygmaea x Callithrix jacchus) and related Callitrichinae (Platyrrhini) by cross-species chromosome painting and comparative genomic hybridization. Cytogenet Genome Res 2005;108:191–196.
21. Richard F, Lombard M, Dutrillaux B. ZOO-FISH suggests a complete homology between human and capuchin monkey (Platyrrhini) euchromatin. Genomics 1996;36:417–423.
22. Garcia F, Nogues C, Ponsa M, Ruiz-Herrera A, Egozcue J, Garcia Caldes M. Chromosomal homologies between humans and Cebus apella (Primates) revealed by ZOO-FISH. Mamm Genome 2000;11:399–401.
23. Garcia F, Ruiz-Herrera A, Egozcue J, Ponsa M, Garcia M. Chromosomal homologies between Cebus and Ateles (primates) based on ZOO-FISH and G-banding comparisons. Am J Primatol 2002;57:177–188.
24. Stanyon R, Consigliere S, Muller S, Morescalchi A, Neusser M, Wienberg J. Fluorescence in situ hybridization (FISH) maps chromosomal homologies between the dusky titi and squirrel monkey. Am J Primatol 2000;50:95–107.
25. Barros RM, Nagamachi CY, Pieczarka JC, et al. Chromosomal studies in Callicebus donacophilus pallescens, with classic and molecular cytogenetic approaches: multicolour FISH using human and Saguinus oedipus painting probes. Chromosome Res 2003;11:327–334.
26. Stanyon R, Bonvicino CR, Svartman M, Seuanez HN. Chromosome painting in Callicebus lugens, the species with the lowest diploid number (2 n=16) known in primates. Chromosoma 2003;112:201–206.
27. de Oliveira EH, Neusser M, Figueiredo WB, et al. The phylogeny of howler monkeys (Alouatta, Platyrrhini): reconstruction by multicolor cross-species chromosome painting. Chromosome Res 2002;10:669–683.

28. de Oliveira EH, Neusser M, Pieczarka JC, Nagamachi C, Sbalqueiro IJ, Müller S. Phylogenetic inferences of Atelinae (Platyrrhini) based on multi-directional chromosome painting in Brachyteles arachnoides, Ateles paniscus paniscus and Ateles b. marginatus. Cytogenet Genome Res 2005;108:183–190.
29. Consigliere S, Stanyon R, Koehler U, Agoramoorthy G, Wienberg J. Chromosome painting defines genomic rearrangements between red howler monkey subspecies. Chromosome Res 1996;4:264–270.
30. Consigliere S, Stanyon R, Koehler U, Arnold N, Wienberg J. In situ hybridization (FISH) maps chromosomal homologies between Alouatta belzebul (Platyrrhini, Cebidae) and other primates and reveals extensive interchromosomal rearrangements between howler monkey genomes. Am J Primatol 1998;46:119–133.
31. Morescalchi MA, Schempp W, Consigliere S, Bigoni F, Wienberg J, Stanyon R. Mapping chromosomal homology between humans and the black-handed spider monkey by fluorescence in situ hybridization. Chromosome Res 1997;5:527–536.
32. Wienberg J, Stanyon R, Jauch A, Cremer T. Homologies in human and Macaca fuscata chromosomes revealed by in situ suppression hybridization with human chromosome specific DNA libraries. Chromosoma 1992;101:265–270.
33. Finelli P, Stanyon R, Plesker R, Ferguson-Smith MA, O'Brien PC, Wienberg J. Reciprocal chromosome painting shows that the great difference in diploid number between human and African green monkey is mostly due to non-Robertsonian fissions. Mamm Genome 1999;10:713–718.
34. Bigoni F, Koehler U, Stanyon R, Ishida T, Wienberg J. Fluorescene in situ hybridization establishes homology between human and silvered leaf monkey chromosomes, reveals reciprocal translocations between chromosomes homologous to human Y/5, 1/9, and 6/16, and delineates an X1X2Y1Y2/X1X1X2X2 sex-chromosome system. Am J Phys Anthropol 1997;102:315–327.
35. Bigoni F, Stanyon R, Wimmer R, Schempp W. Chromosome painting shows that the proboscis monkey (Nasalis larvatus) has a derived karyotype and is phylogenetically nested within Asian Colobines. Am J Primatol 2003;60:85–93.
36. Jauch A, Wienberg J, Stanyon R, et al. Reconstruction of genomic rearrangements in great apes and gibbons by chromosome painting. Proc Natl Acad Sci USA 1992;89:8611–8615.
37. Koehler U, Arnold N, Wienberg J, Tofanelli S, Stanyon R. Genomic reorganization and disrupted chromosomal synteny in the siamang (Hylobates syndactylus) revealed by fluorescence in situ hybridization. Am J Phys Anthropol 1995;97:37–47.
38. Koehler U, Bigoni F, Wienberg J, Stanyon R. Genomic reorganization in the concolor gibbon (Hylobates concolor) revealed by chromosome painting. Genomics 1995;30:287–292.
39. Yu D, Yang F, Liu R. [A comparative chromosome map between human and Hylobates hoolock built by chromosome painting]. Yi Chuan Xue Bao 1997;24:417–423.
40. Nie W, Rens W, Wang J, Yang F. Conserved chromosome segments in Hylobates hoolock revealed by human and H. leucogenys paint probes. Cytogenet Cell Genet 2001;92:248–253.
41. Müller S, Neusser M, Wienberg J. Towards unlimited colors for fluorescence in-situ hybridization (FISH). Chromosome Res 2002;10:223–232.
42. Müller S, Hollatz M, Wienberg J. Chromosomal phylogeny and evolution of gibbons (Hylobatidae). Hum Genet 2003;113:493–501.
43. Stanyon R, Wienberg J, Romagno D, Bigoni F, Jauch A, Cremer T. Molecular and classical cytogenetic analyses demonstrate an apomorphic reciprocal chromosomal translocation in Gorilla gorilla. Am J Phys Anthropol 1992;88:245–250.
44. Yunis JJ, Prakash O. The origin of man: a chromosomal pictorial legacy. Science 1982;215:1525–1530.
45. Wienberg J, Jauch A, Ludecke HJ, et al. The origin of human chromosome 2 analyzed by comparative chromosome mapping with a DNA microlibrary. Chromosome Res 1994;2:405–410.
46. Arnold N, Wienberg J, Ermert K, Zachau HG. Comparative mapping of DNA probes derived from the V kappa immunoglobulin gene regions on human and great ape chromosomes by fluorescence in situ hybridization. Genomics 1995;26:147–150.
47. Haaf T, Bray-Ward P. Region-specific YAC banding and painting probes for comparative genome mapping: implications for the evolution of human chromosome 2. Chromosoma 1996;104:537–544.
48. Kasai F, Takahashi E, Koyama K, et al. Comparative FISH mapping of the ancestral fusion point of human chromosome 2. Chromosome Res 2000;8:727–735.
49. Müller S, Stanyon R, Finelli P, Archidiacono N, Wienberg J. Molecular cytogenetic dissection of human chromosomes 3 and 21 evolution. Proc Natl Acad Sci USA 2000;97:206–211.

50. Ventura M, Weigl S, Carbone L, et al. Recurrent sites for new centromere seeding. Genome Res 2004;14: 1696–1703.
51. Tsend-Ayush E, Grutzner F, Yue Y, et al. Plasticity of human chromosome 3 during primate evolution. Genomics 2004;83:193–202.
52. Marzella R, Viggiano L, Miolla V, et al. Molecular cytogenetic resources for chromosome 4 and comparative analysis of phylogenetic chromosome IV in great apes. Genomics 2000;63:307–313.
53. Nickerson E, Nelson DL. Molecular definition of pericentric inversion breakpoints occurring during the evolution of humans and chimpanzees. Genomics 1998;50:368–372.
54. Marzella R, Viggiano L, Ricco AS, et al. A panel of radiation hybrids and YAC clones specific for human chromosome 5. Cytogenet Cell Genet 1997;77:232–237.
55. Müller S, Wienberg J. "Bar-coding" primate chromosomes: molecular cytogenetic screening for the ancestral hominoid karyotype. Hum Genet 2001;109:85–94.
56. Eder V, Ventura M, Ianigro M, Teti M, Rocchi M, Archidiacono N. Chromosome 6 phylogeny in primates and centromere repositioning. Mol Biol Evol 2003;20:1506–1512.
57. Müller S, Finelli P, Neusser M, Wienberg J. The evolutionary history of human chromosome 7. Genomics 2004;84:458–467.
58. Montefalcone G, Tempesta S, Rocchi M, Archidiacono N. Centromere repositioning. Genome Res 1999;9:1184–1188.
59. Carbone L, Ventura M, Tempesta S, Rocchi M, Archidiacono N. Evolutionary history of chromosome 10 in primates. Chromosoma 2002;111:267–272.
60. Wimmer R, Kirsch S, Rappold GA, Schempp W. Evolutionary breakpoint analysis on Y chromosomes of higher primates provides insight into human Y evolution. Cytogenet Genome Res 2005;108:204–210.
61. Ijdo J, Baldini A, Ward DC, Reeders ST, Wells RA. Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci USA 1991;88:9051–9055.
62. Avarello R, Pedicini A, Caiulo A, Zuffardi O, Fraccaro M. Evidence for an ancestral alphoid domain on the long arm of human chromosome 2. Hum Genet 1992;89:247–249.
63. Fan Y, Linardopoulou E, Friedman C, Williams E, Trask BJ. Genomic structure and evolution of the ancestral chromosome fusion site in 2q13-2q14.1 and paralogous regions on other human chromosomes. Genome Res 2002;12:1651–1662.
64. Fan Y, Newman T, Linardopoulou E, Trask BJ. Gene content and function of the ancestral chromosome fusion site in human chromosome 2q13-2q14.1 and paralogous regions. Genome Res 2002;12:1663–1672.
65. Stankiewicz P, Park SS, Inoue K, Lupski JR. The evolutionary chromosome translocation 4;19 in Gorilla gorilla is associated with microduplication of the chromosome fragment syntenic to sequences surrounding the human proximal CMT1A-REP. Genome Res 2001;11:1205–1210.
66. Stankiewicz P, Shaw CJ, Withers M, Inoue K, Lupski JR. Serial segmental duplications during primate evolution result in complex human genome architecture. Genome Res 2004;14:2209–2220.
67. DeSilva U, Massa H, Trask BJ, Green ED. Comparative mapping of the region of human chromosome 7 deleted in williams syndrome. Genome Res 1999;9:428–436.
68. Fujiyama A, Watanabe H, Toyoda A, et al. Construction and analysis of a human-chimpanzee comparative clone map. Science 2002;295:131–134.
69. Kehrer-Sawatzki H, Sandig H, Goidts V, Hameister H. Breakpoint analysis of the pericentric inversion between chimpanzee chromosome 10 and the homologous chromosome 12 in humans. Cytogenet Genome Res 2005;108:91–97.
70. Ventura M, Mudge JM, Palumbo V, et al. Neocentromeres in 15q24-26 map to duplicons which flanked an ancestral centromere in 15q25. Genome Res 2003;13:2059–2068.
71. Locke DP, Archidiacono N, Misceo D, et al. Refinement of a chimpanzee pericentric inversion breakpoint to a segmental duplication cluster. Genome Biol 2003;4:R50.
72. Kehrer-Sawatzki H, Schreiner B, Tanzer S, Platzer M, Müller S, Hameister H. Molecular characterization of the pericentric inversion that causes differences between chimpanzee chromosome 19 and human chromosome 17. Am J Hum Genet 2002;71:375–388.
73. Dennehey BK, Gutches DG, McConkey EH, Krauter KS. Inversion, duplication, and changes in gene context are associated with human chromosome 18 evolution. Genomics 2004;83:493–501.
74. Goidts V, Szamalek JM, Hameister H, Kehrer-Sawatzki H. Segmental duplication associated with the human-specific inversion of chromosome 18: a further example of the impact of segmental duplications on karyotype and genome evolution in primates. Hum Genet 2004;115:116–122.

75. Dixkens C, Klett C, Bruch J, et al. ZOO-FISH analysis in insectivores: "evolution extols the virtue of the status quo." Cytogenet Cell Genet 1998;80:61–67.
76. Breen M, Thomas R, Binns MM, Carter NP, Langford CF. Reciprocal chromosome painting reveals detailed regions of conserved synteny between the karyotypes of the domestic dog (Canis familiaris) and human. Genomics 1999;61:145–155.
77. Gibbs RA, Weinstock GM, Metzker ML, et al. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nature 2004;428:493–521.
78. Stanyon R, Stone G, Garcia M, Froenicke L. Reciprocal chromosome painting shows that squirrels, unlike murid rodents, have a highly conserved genome organization. Genomics 2003;82:245–249.
79. Pevzner P, Tesler G. Human and mouse genomic sequences reveal extensive breakpoint reuse in mammalian evolution. Proc Natl Acad Sci USA 2003;100:7672–7677.
80. Nadeau JH, Taylor BA. Lengths of chromosomal segments conserved since divergence of man and mouse. Proc Natl Acad Sci USA 1984;81:814–818.
81. Murphy WJ, Froenicke L, O'Brien SJ, Stanyon R. The origin of human chromosome 1 and its homologs in placental mammals. Genome Res 2003;13:1880–1888.
82. Ruiz-Herrera A, Ponsa M, Garcia F, Egozcue J, Garcia M. Fragile sites in human and Macaca fascicularis chromosomes are breakpoints in chromosome evolution. Chromosome Res 2002;10:33–44.
83. Lupski JR. Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. Trends Genet 1998;14:417–422.
84. Stankiewicz P, Lupski JR. Molecular-evolutionary mechanisms for genomic disorders. Curr Opin Genet Dev 2002;12:312–319.
85. Samonte RV, Eichler EE. Segmental duplications and the evolution of the primate genome. Nat Rev Genet 2002;3:65–72.
86. Eichler EE, Sankoff D. Structural dynamics of eukaryotic chromosome evolution. Science 2003;301:793–797.
87. Bailey JA, Liu G, Eichler EE. An Alu transposition model for the origin and expansion of human segmental duplications. Am J Hum Genet 2003;73:823–834.
88. Khaitovich P, Muetzel B, She X, et al. Regional patterns of gene expression in human and chimpanzee brains. Genome Res 2004;14:1462–1473.
89. Tuzun E, Bailey JA, Eichler EE. Recent segmental duplications in the working draft assembly of the brown Norway rat. Genome Res 2004;14:493–506.
90. Bailey JA, Church DM, Ventura M, Rocchi M, Eichler EE. Analysis of segmental duplications and genome assembly in the mouse. Genome Res 2004;14:789–801.
91. Toder R, Xia Y, Bausch E. Interspecies comparative genome hybridization and interspecies representational difference analysis reveal gross DNA differences between humans and great apes. Chromosome Res 1998;6:487–494.
92. Snijders AM, Nowak N, Segraves R, et al. Assembly of microarrays for genome-wide measurement of DNA copy number. Nat Genet 2001;29:263–264.
93. Locke DP, Segraves R, Carbone L, et al. Large-scale variation among human and great ape genomes determined by array comparative genomic hybridization. Genome Res 2003;13:347–357.
94. Fiegler H, Gribble SM, Burford DC, et al. Array painting: a method for the rapid analysis of aberrant chromosomes using DNA microarrays. J Med Genet 2003;40:664–670.
95. Gribble SM, Fiegler H, Burford DC, et al. Applications of combined DNA microarray and chromosome sorting technologies. Chromosome Res 2004;12:35–43.
96. Tanabe H, Müller S, Neusser M, et al. Evolutionary conservation of chromosome territory arrangements in cell nuclei from higher primates. Proc Natl Acad Sci USA 2002;99:4424–4429.