Bull. Natl. Health Sci., **117**, 77-90 (1999) **Reviews**

Studies on karyotype evolution in higher primates in relation to human chromosome 14 and 9 by comparative mapping of immunoglobulin Cε genes with fluorescence in situ hybridization[#1]

Hideyuki Tanabe[#2]

FISH 法による免疫グロブリン Cε 遺伝子の比較マッピングに基づいたヒトおよび高等霊長類における 14 番および 9 番染色体の核型進化に関する研究[#1]

田辺秀之[#2]

Karyotypic homologies in relation to human chromosome 14 and 9 were studied through comparative mapping of the immunoglobulin Cε genes in higher primates by fluorescence in situ hybridization (FISH) technique. The Cε genes will be suitable probes for the analysis of evolutionary rearrangements due to that the multiple recombinational events such as gene duplications and deletions have occurred repeatedly in the immunoglobulin CH gene family (IGH@) during the course of primate evolution. IGH@ locating on the terminal region of human chromosome 14 (HSA14), at band HSA14q32.33, has generated multiple pseudogenes and among subclasses of IGH@ the Cε genes have shown most dynamic changes with generating both truncated type (Cε2) and processed type (Cε3) pseudogenes. In this study, chromosomal homologies and rearrangements on HSA14 (Cε1) and HSA9 (Cε3) in relation to the evolutionary genesis of their primate homologous chromosomes in speciation were investigated by comparative mapping with FISH and chromosome painting (ZOO-FISH) techniques. Comparative mapping of the Cε1 gene at HSA14q32.33 was carried out in seven species of nonhuman primates: common chimpanzee (PTR), pygmy chimpanzee (PPA), gorilla (GGO), orangutan (PPY), white-handed gibbon (HLA), agile gibbon (HAG), and Japanese macaque (MFU). The Cε1 gene was assigned to the telomeric region of HSA14 homologues in each species, namely, PTR15q32, PPA15q32, GGO18q16, PPY15q32, HLA17qter, HAG17qter, and MFU7q29, respectively. These results suggested that HSA14 has high degree of syntenic organization with its primate homologues confirmed by ZOO-FISH. Concerning HSA9, comparative mapping of the Cε3 gene at HSA9p24.2→p24.1 was performed. The mapped positions indicated the HSA9 homologous regions detected by ZOO-FISH in each species, namely, PTR11q34, PPA11q34, GGO13q22, PPY13q16, HLA8qter, HAG8qter, and MFU14q22, respectively, suggesting that several dynamic chromosomal rearrangements including at least twice pericentric inversions have occurred during the course of hominoid evolution. The comparison of syntenic groups and painting results has provided a hypothesis of the evolutionary genesis of HSA9 and its homologues with defined breakpoints on the present chromosomes. Likewise, studies on karyotype evolution will be promoted by combining comparative mapping with ZOO-FISH that can more clearly define the chromosomal rearrangements among species.

Keywords: karyotype evolution, FISH, primates, comparative mapping, immunoglobulin Cε genes

1. Introduction

1.1 Comparative cytogenetics in higher primates

The understanding of evolutionary processes in mammals has been greatly facilitated by the development of cytogenetic, cellular and molecular procedures in the last two decades. The advent of differential staining techniques of Q-, G-, R-, and C-bands has permitted the unequivocal identification of human chromosomes as well as other primate chromosomes[1-5]. An example of Q-/G-banded images of human cells is shown in Fig. 1. The application of chromosome banding to cytogenetic studies of mammalian chromosomes has made it possible to monitor more accurately the divergence of chromosome structure in mammalian evolution[6-9].

Cytogenetic analyses of G-banding pattern have carried out in more than 80 primate species, and these results have led to several general conclusions. Perhaps most striking is the

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[#2] To whom correspondence should be addressed: Hideyuki Tanabe; Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan; Phone: +81-3-3700-9874; Fax: +81-3-3707-6950; E-mail: tanabe@nihs.go.jp

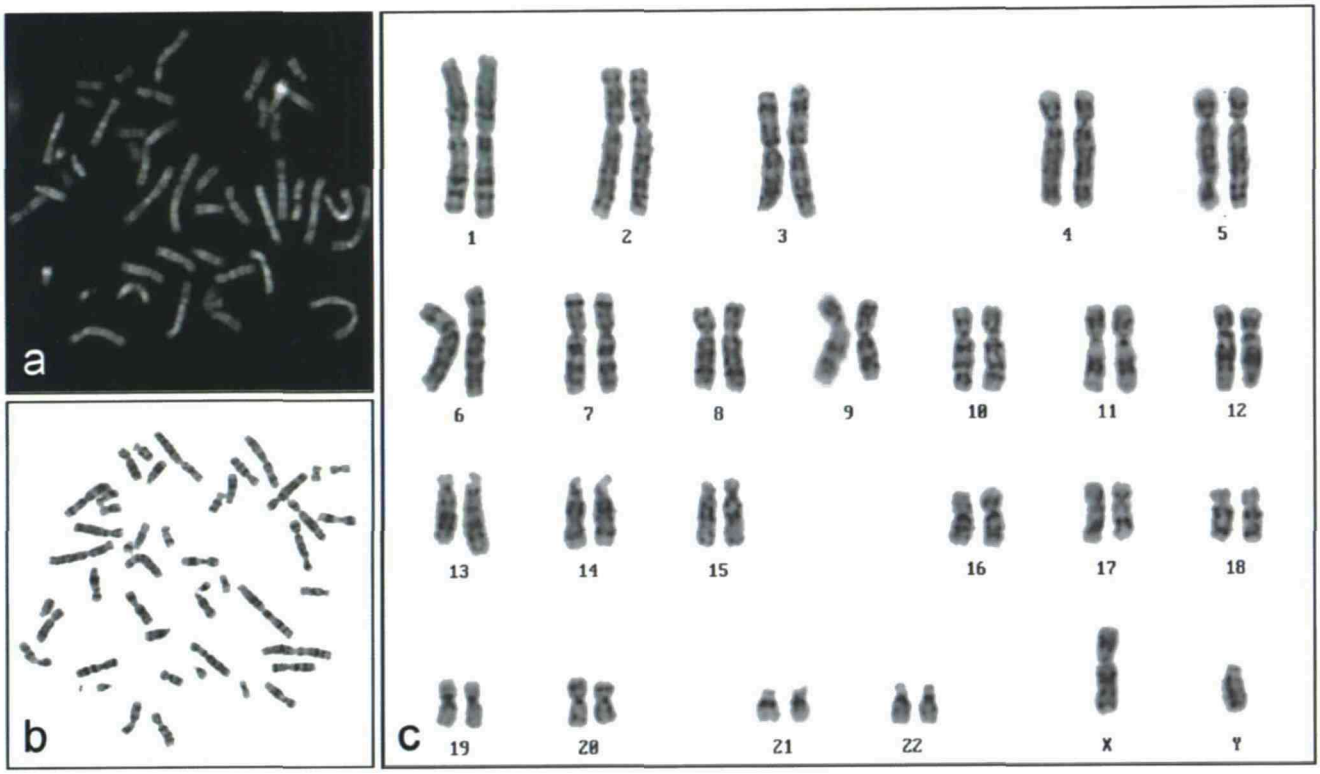

Fig. 1. Chromosome banded images of cells from a human male individual
a) Q-banded metaphase, b) G-banded metaphase, c) G-banded karyotype of the same cell as in b)

extensive conservation of chromosome banding patterns among various primate species[6,8,10-12]. Comparative chromosome analysis has showed that extensive chromosome banding homology exists not only between closely related primates, such as man and the great apes (chimpanzee, pygmy chimpanzee, gorilla, and orangutan)[6,7,11,3], but also to a lesser extent between distantly related primates such as man and the wooly monkey[14,15]. Only a few chromosome rearrangements between man and the great apes have been observed, and all the human chromosomes have tentative homologues in these species[6]. Yunis and Prakash (1982)[13] have used high-resolution banding of the chromosomes of man and the great apes to reconstruct the chromosomal rearrangements that have presumably occurred and become fixed during hominoid evolution. Their report confirmed previous observations[11,16-19] that most of the postulated rearrangements were pericentric inversions, although other rearrangements, such as Robertsonian translocations, paracentric and pericentric inversions, fusions, and fissions, have also occurred in primate species. For example, comparative karyotype analysis between human and the great apes showed the difference in the diploid number of man (2n=46) and the great apes (2n=48). Human chromosome 2 has no single counterpart in the great apes. Instead, there is an acrocentric homologue pair for 2p, and another acrocentric homologue pair for 2q. This chromosome comparison between species suggested that human chromosome 2 emerged as a result of a fusion between two nonhomologous ancestral chromosomes similar to these arm counterparts. This fusion event explains the difference in diploid number between man and the great apes[3,6,11,16,20]. The high extent of chromosome conservation in primates has allowed the construction of chromosome phylogenies, which demonstrate tentative chromosomal changes that have occurred during the simian radiation[6,21,22].

Modern primates include 175 species, and a representative phylogenetic tree of hominoid based on several morphological characters (dentition, skeletal traits, cephalic arterial system, larynges, and others) is presented in Fig. 2[23,24]. Although several methodologies of molecular evolution have been extensively applied to the hominoids (man and apes), relatively less molecular data are available for primate species outside this group[25-27].

1.2 Comparative gene mapping

The genetic map of homologous loci in a number of mammalian species provides a basis for the evolutionary field of comparative gene mapping[28]. The occurrence of homologous linkage groups in related species is generally interpreted to

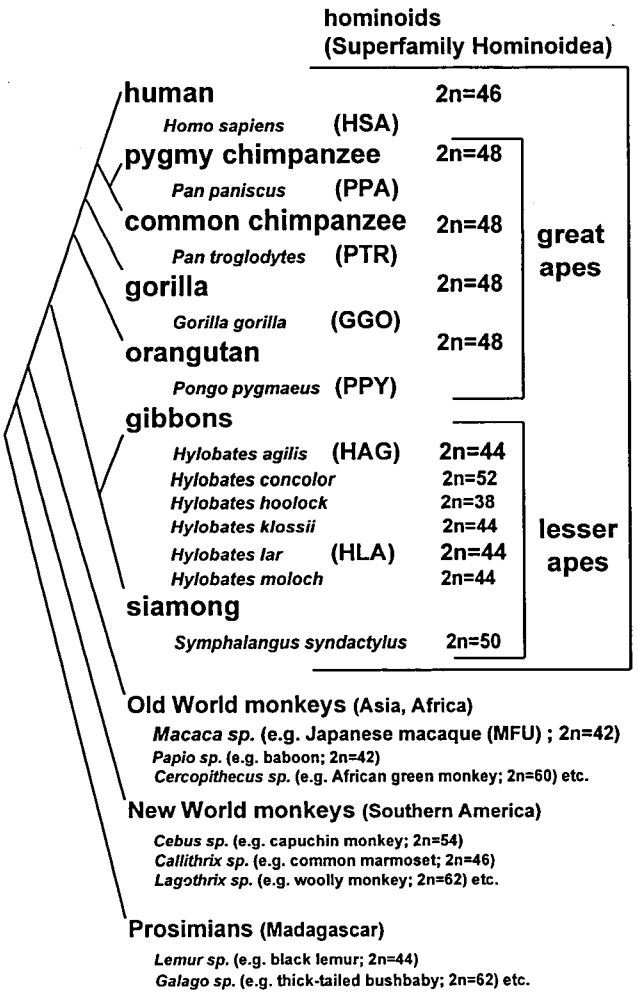

Fig. 2. Primate phylogenetic tree
Classification of the Order Primates. The tree shows phylogenetic relationships and the diploid chromosome number of each species is also described.

reflect the presence of that linkage arrangement in species which were ancestral to the modern species. The genetic maps have been derived primarily by sexual genetic analysis or by parasexual procedures by means of somatic cell hybridization[29,30]. The application of somatic cell hybridization to gene mapping resulted in a remarkable increase in chromosome assignments in man[31,32]. With the development of techniques for molecular cloning, thousands of cloned structural genes and anonymous DNA segments have become available for use in the genetic map as well[33,34]. The molecular clones allow the genetic localization of virtually any gene or DNA segment regardless of whether the locus is expressed or functional. Since human is the most extensively mapped mammalian species[34], technologies similar to those employed in human genetics have been also used to construct comparative gene maps in other mammalian species, and the human genetic map is used as an index for primate and for mammalian comparisons[34,35]. A general conclusion from comparative mapping studies in man and the nonhuman primates is that many syntenic associations have been evolutionarily conserved and that such conservation can be traced to each of the primate suborders[8,11,36-40]. A positive correlation seems to exist between morphological and syntenic changes at the chromosome level. This makes morphological attributes, such as banding patterns useful indicators of syntenic homologies in the primate order.

1.3 Fluorescence in situ hybridization (FISH)

An important advance in human gene mapping has been the development of in situ hybridization of cloned probes to metaphase chromosomes[41]. This method involves the molecular hybridization of radiolabelled cloned probes to homologous DNA segments on a metaphase chromosome, followed by an autoradiography and chromosome banding procedure. Regional localization of genes can be directly visualized on individual chromosomes. More recently, nonisotopic hybridization techniques, particularly fluorescence in situ hybridization (FISH), have become an important tool in studies of genome mapping and chromosome structure and function[42-44]. Currently composite DNA probe sets for delineating whole chromosomes, chromosomal regions such as telomere and centromere, or gene-specific loci are available[45]. In particular whole chromosomes or large chromosomal regions in a metaphase are painted by FISH using an entire chromosomes as probe DNA. This procedure called "chromosome painting" [43,46,47] has become a valuable tool to elucidate karyotype rearrangements in primate evolution, since FISH with human chromosome-specific probes has enabled interspecific chromosome homologies to be much precise. This new approach is called as comparative chromosome painting[48,49], or ZOO-FISH[50]. For example, the well-known fusion-origin of human chromosome 2 was confirmed by the human chromosome 2 library which painted two chromosome pairs in all great apes[51,52]. In contrast to previous comparative gene mapping experiments that have been restricted to single-copy sequences, the chromosome painting approach provides an overall comparison of DNA sequence homologies for complete chromosomes.

Although FISH data clearly show that the human genome is closely related to the great apes by the presence of similar DNA sequences providing another phylogenetic parameter for interspecific comparison, whole chromosome painting does not allow the demonstration of homology among subchromosomal regions or intrachromosomal rearrangements, which are supposed to be prominent in the evolution of human and great ape karyotypes. However, these subchromosomal regions or intrachromosomal rearrangements can be readily detected by

defined human probes that span subregions of chromosomes, especially if one of the assumed breakpoints is included[53-55]. As the human genome analysis is remarkably progressing, an increasing number of DNA probes will become available for comparative gene mapping studies of any region of interest. Therefore, in this study I used both procedures of ZOO-FISH and comparative mapping for detailed analysis of genome alignment and also precise recognition of chromosome rearrangements in homologous segments.

1.4 Immunoglobulin Cε genes

In the present study, I analyzed localization of genes for immunoglobulin heavy chain in order to obtain additional information about homologies as well as chromosome rearrangements in karyotypes of primate species at the molecular cytogenetic level for better understanding of their karyotype evolution. Immunoglobulins are the effector molecules of the immune system and they are composed of two identical light (L) and heavy (H) chains, each of which consists of variable (V) and constant (C) regions, and have a Y-shaped structure connected by the hinge region[56]. The antigen-binding sites that are formed by a complex of their V regions including three small hypervariable regions of both L and H chains could bind numerous different antigens. There are five classes of H chains, $\alpha, \delta, \varepsilon, \gamma$, and μ, which form the different classes of antibodies, IgA, IgD, IgE, IgG, and IgM, respectively, that determine the each effector function. The human H chain genes, which are divided into the ~300 variable (VH), ~20 diversity (D), 6 joining (JH), and 11 constant (CH) gene segments, are arrayed in a direction from telomere to centromere to build up a huge gene cluster at the distal region of chromosome 14 (HSA14q32.33) and the total length is estimated to encompass 2.5 to 3 megabases DNA, making it one of the largest gene clusters in the mammalian genome[57-61]. In this gene cluster the combinatorial somatic recombination of the VDJ-segments greatly increases the diversity of antibody that occurs in the B lymphocyte in which the antibody is expressed and the process called class switching allows the antibodies with varied biological properties[62,63]. During the switch recombination, DNA deletion occurs by cutting and rejoining between the assembled VDJ sequence and the upstream of the particular CH gene segment. From the evolutionary view point, likewise, the dynamic genetic rearrangements such as deletions, duplications, insertions, and gene conversions as well as point mutations have occurred in the genesis of CH genes (defined as to IGH@[64]) during the course of primate evolution[65-71](Fig. 3). The human IGH@ contains 9 functional CH genes and 2 pseudogenes with the order of 5'-Cμ-Cδ-Cγ3-Cγ1-Cε2(Ψ)-Cα1-ΨCγ-Cγ2-Cγ4--Cα2-3'. It seems that a Cγ gene must have been duplicated to give the subcluster of Cγ-Cγ-Cε-Cα, after which the entire group was then duplicated[58,72]. IGH@ contains another pseudogene, the processed pseudogene Cε3 (IGHEP2)[73], which is located on a different chromosome from HSA14[74], suggesting

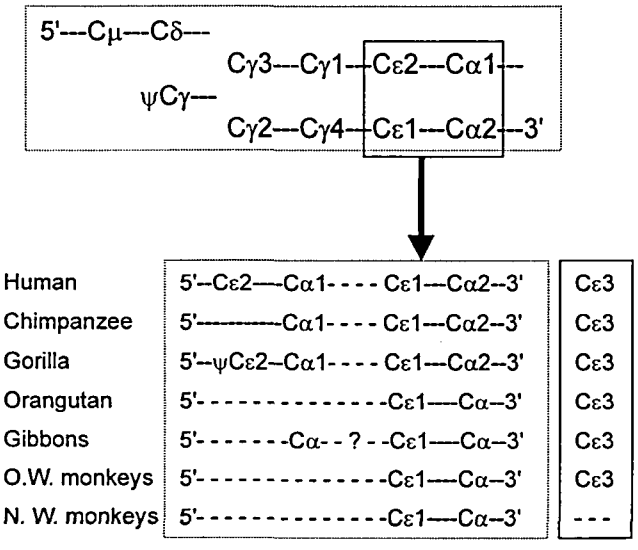

Fig. 3. Organization of IGH@ in higher primate genomes
IGH@ has evoluved by the gene duplication involving the Cγ-Cγ-Cε-Cα region after Old World monkeys branched out. Organization of the Cε and Cα genes is described in the lower dotted rectangle with Cε gene at each side from human to New World monkeys.

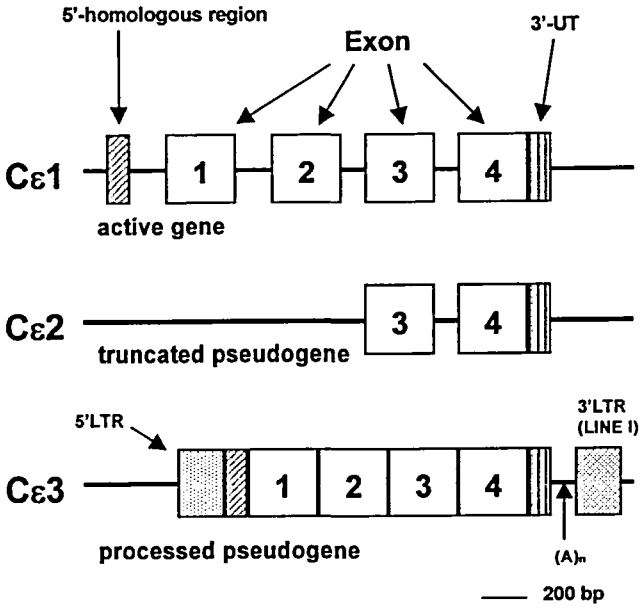

Fig. 4. Schematic representation of the three Cε genes
The Cε2 gene lacks the 5' upstream region including exon 2. The Cε gene lacks the three introns entirely and has an A-rich sequence followed by the 3'-untranslated region so far indicating a processed pseudogene.

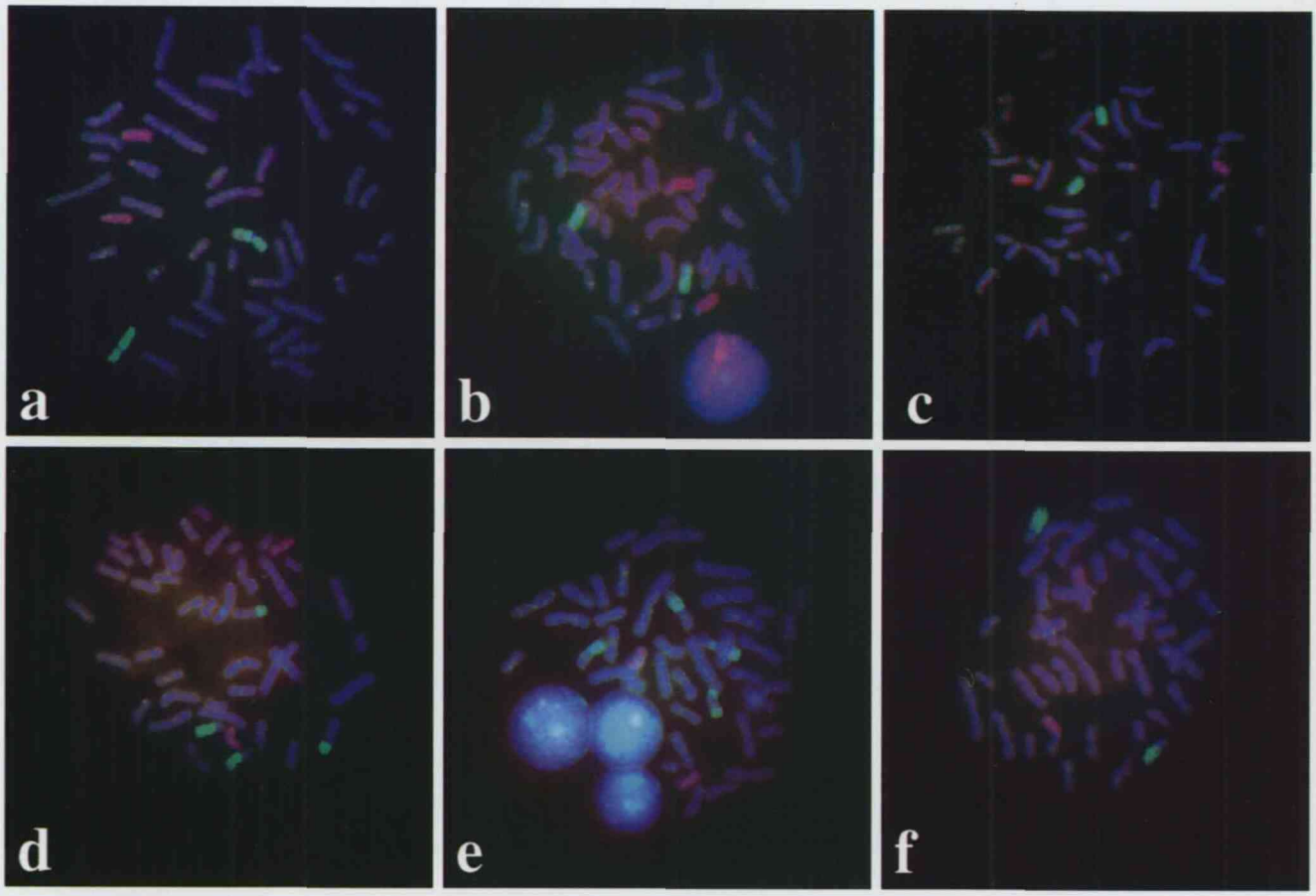

Fig. 5. ZOO-FISH images
Cy3-labelled WCP#14 and SpectrumGreen-labelled WCP#9 probes were hybridized to human and primate metaphases: a) human, b) common chimpanzee, c) orangutan, d) white-handed gibbon, e) agile gibbon, and f) Japanese macaque.

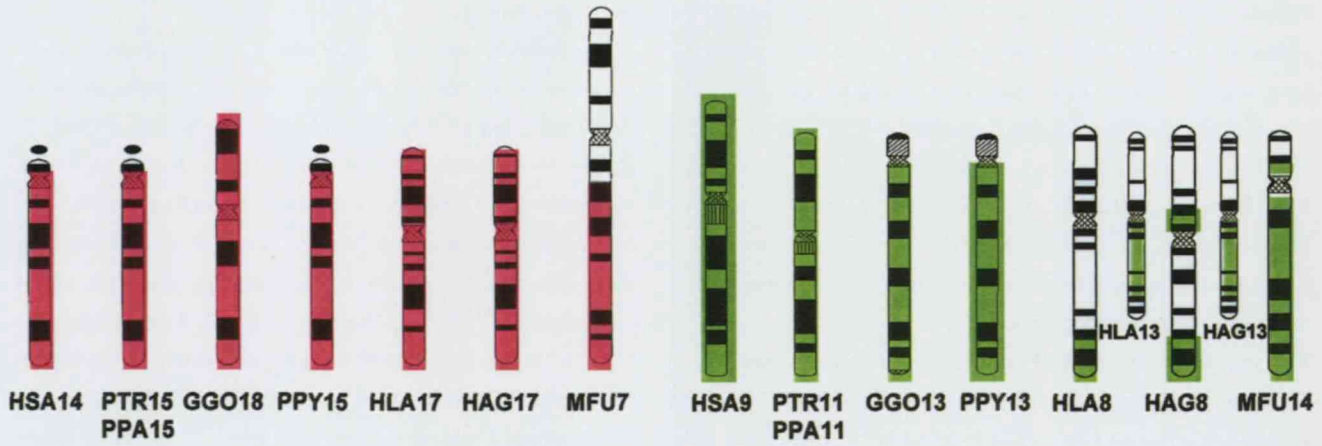

Fig. 6. Idiogramatic summary of ZOO-FISH results
Painted chromosomal regions of both WCP probes in human and the primates were indicated by respective colors, pink and green. Note the difference between HLA8 and HAG8.

extensive reorganization of IGH@ loci. The presence of homologues of the processed pseudogene Cε3 has been demonstrated in catarrhine primate species (Old World monkeys, apes, and human) so far examined[75]. Thus, detailed comparative mapping of IGH@ loci between human and nonhuman primates will reveal a dynamic evolutionary trail of this gene family in relation to chromosome rearrangements. In this study among IGH@ a class of the Cε genes is adopted since the most dynamic reorganization might be occurred in the Cε loci by the existence of two types of pseudogenes, namely, Cε2 (IGHEP1) which is a truncated-type pseudogene and Cε3 (IGHEP2) which is a processed-type pseudogene (Fig. 4). Moreover, the phylogenetic relationships among human and the great apes based on the gene trees in relation to the processed pseudogene Cε3 were documented[75,76].

2. Syntenic organization of HSA14 in the primate homologues

In the present study I used the following seven primate species by employing the systems for the great apes[5], gibbons[77,78], and Japanese macaque[79,80]: common chimpanzee (*Pan troglodytes*; PTR), pygmy chimpanzee (*Pan paniscus*; PPA), gorilla (*Gorilla gorilla*; GGO), orangutan (*Pongo pygmaeus*; PPY), white-handed gibbon (*Hylobates lar*; HLA), agile gibbon (*Hylobates agilis*; HAG), and Japanese macaque (*Macaca fuscata*; MFU).

2.1 Comparative chromosome painting (ZOO-FISH) with HSA14 DNA probe

I employed FISH with a HSA14 specific composite DNA probe, WCP#14, to demonstrate homology between HSA14 and its counterpart in seven primate species. This probe produced uniform hybridization signals in one pair of whole chromosomes in each species of six primates except for Japanese macaque whose hybridization signals appeared on only a distal part of the long arm of a chromosome pair (Figs. 5 and 6). Each painted chromosome was identified by Q-banding as PTR15 and PPA15 for both common and pygmy chimpanzees, GGO18 for gorilla, PPY15 for orangutan, HLA17 and HAG17 for both white-handed and agile gibbons, and MFU7q for Japanese macaque, respectively (Fig. 6). The present painting results confirmed the homology of the previous works[5,51].

2.2 Comparative mapping of the Cε1 gene in higher primates

I performed comparative mapping at the single gene level by FISH using a human Ch4A-H-Ig ε -12 probe[81] for the human Cε1 gene. This probe gave hybridization signals on HSA14q32.3 (Fig. 7), confirming the localization of the IGH@ gene cluster. This probe also hybridized to specific loci on metaphase chromosomes of each primate species, indicating existence of homologous chromosome regions among these species. After chromosome identification by Q-banding, the primate Cε1 genes were mapped to bands PTR15q32, PPA15q32, GGO18q16, PPY15q32, HLA17qter, HAG17qter, and MFU7q29, respectively (Fig. 7)[82]. These mapped positions were all the telomeric regions of long arms of each homologue of HSA14 that corresponding to the position of HSA14q32.33. A summary of FISH mapping data is given in Fig. 8.

2.3 Comparative mapping and ZOO-FISH reveal syntenic organization of HSA14 in the primate homologues

Comparative chromosome banding analysis has shown homology between HSA14 and its equivalent chromosome of the great apes, but homologies with the white-handed gibbon, agile gibbon, and Japanese macaque were unclear until the application of comparative chromosome painting technique, in another word, ZOO-FISH[49,51]. As proposed after these studies, HSA14 corresponds to PTR15, PPA15, GGO18, PPY15, HLA17, HAG17, and a distal part of MFU7q and these were confirmed in this study. Although ZOO-FISH method is effective for identification of chromosome homologies, further approach for more detailed analysis to identify syntenic chromosome segments for intrachromosomal rearrangements is necessary. As shown in Fig. 8, the primate Cε1 genes localized at the terminal region of the long arms of HSA14 homologues. This assignment provides us a new information of syntenic segments at telomeric region and that human genome is closely similar to these primate species at the single gene level. The present data also suggest that GGO18 which is only a submetacentric chromosome among the homologues of HSA14 in the great apes might have been derived from at least one pericentric inversion during the evolution (Fig. 6). Further analyses will clarify the rearrangements.

In contrast to the great apes which have diploid chromosome numbers of 48, the lesser apes show karyotypic variation: *Hylobates agilis* (2n=44), *Hylobates concolor* (2n=52), *Hylobates hoolock* (2n=38), *Hylobates klossii* (2n=44), *Hylobates lar* (2n=44), *Hylobates moloch* (2n=44), and *Symphalangus syndactylus* (2n=50). Moreover, these lesser apes show little similarity of chromosome banding patterns to human chromosomes[19,77,78,83-87]. Painting with 22 human autosome libraries to these hylobatid metaphases showed considerably complicated chromosomal rearrangements including numerous translocations in hylobatid chromosomes[51,88,89]. These results demonstrated that the 22 autosomes have been divided into 51 elements to composing the 21 gibbon autosomes (2n=44)[51], while at least 33 translocations have occurred in the siamong (*Symphalangus syndactylus*, recently changed to *Hylobates*

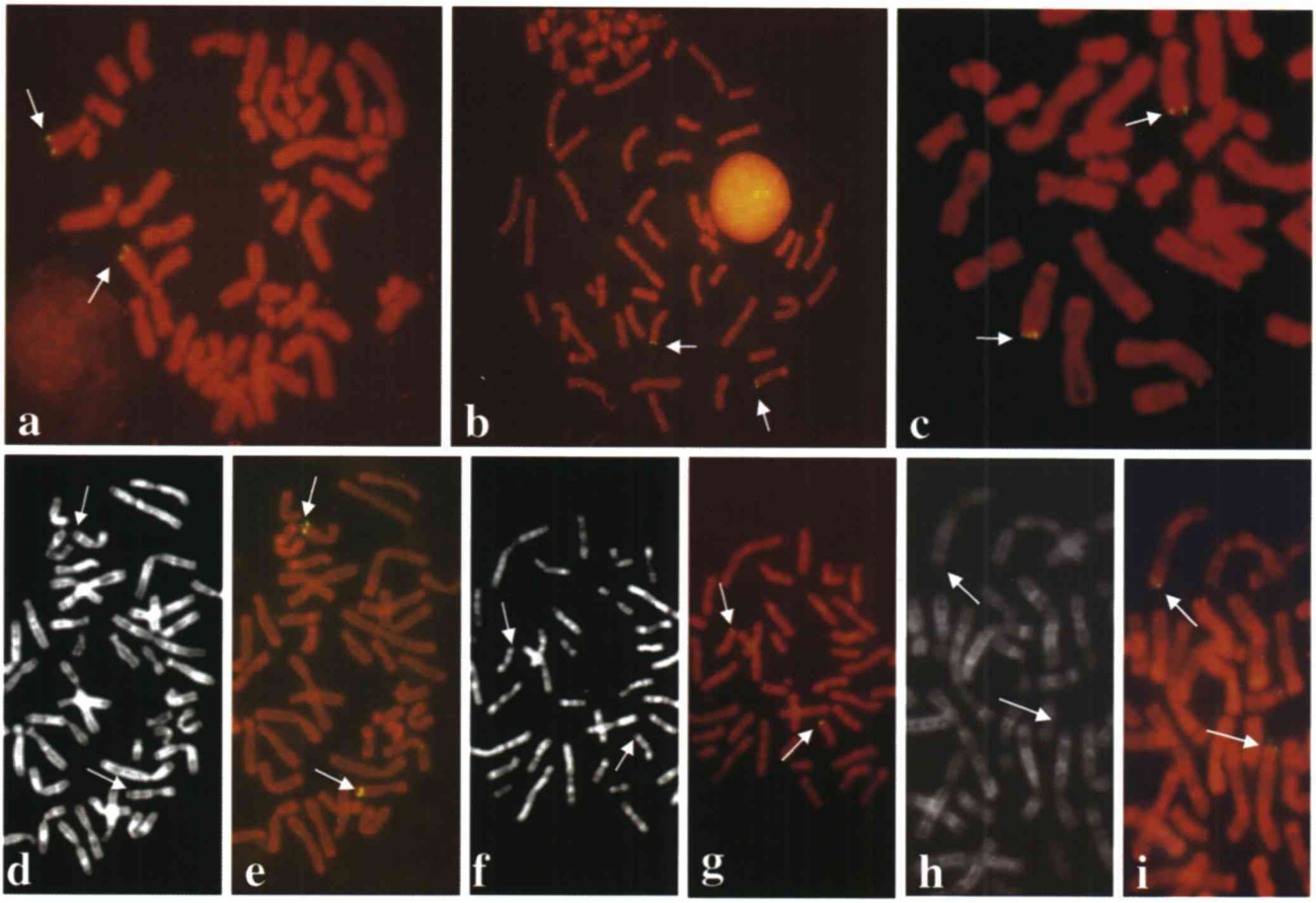

Fig. 7. FISH images of metaphases hybridized with the C ε 1 gene
Localization of the human and primate C ε 1 genes: a) human, b) common chimpanzee, c) pygmy chimpanzee, d)-e) gorilla, f)-g) white-handed gibbon, and h)-i) Japanese macaque. Pre-Q-banded images showed in d), f), and h) indicating the same images as in e), g), and i), respectively.

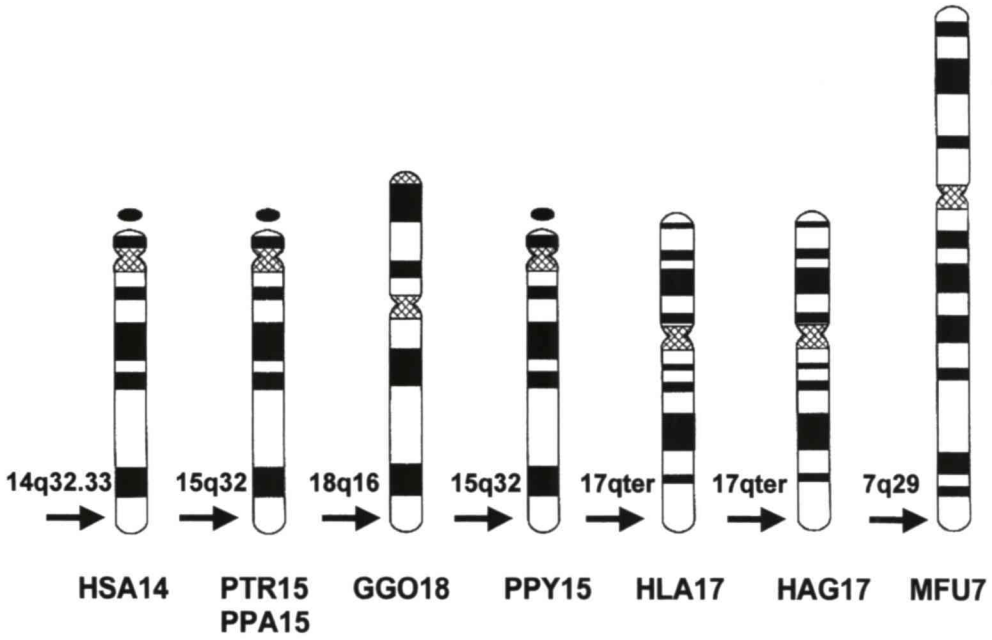

Fig. 8. Idiogramatic representation of the localization of the primate C ε 1 genes
Arrows indicate the position of the C ε 1 genes and chromosome band numbers of each species are given.

syndactylus; 2n=50)[88]. In spite of many rearrangements between human and gibbon chromosomes, the present ZOO-FISH studies showed that only HLA17 and HAG17 was painted indicating no interchromosomal rearrangements. However, the present between HSA14 and HLA17/HAG17 indicate the occurrence of complicated intrachromosomal rearrangements including at least one pericentric inversion event, since HSA14 is acrocentric and both HLA17 and HAG17 are submetacentric, and no banding similarities is found between them. It is interesting to know why HSA14 homologues in gibbon karyotypes are highly conserved without interchromosomal rearrangements, because extremely high evolutionary rate and interchromosomal rearrangements have been noted in gibbon genomes[51,77,78,90,91].

In Japanese macaque, HSA14 corresponded to the distal part of MFU7q by the present ZOO-FISH confirming the previous painting studies[49,92] as well as chromosome banding studies[12,39,80,93-95]. The present comparative mapping data confirmed syntenic segment between HSA14 and MFU7q at the single gene level.

Collectively, the comparative mapping of the Cε1 genes in higher primates supports the previous chromosome banding and painting studies and suggests that HSA14 has a high degree of syntenic organization with its homologues of the great apes, white-handed and agile gibbons, and Japanese macaque. The combined procedure of ZOO-FISH and comparative mapping here indicates interspecies chromosome homologies as well as syntenic segments at the DNA level.

3. Evolutionary genesis of HSA9 and its primate homologues

3.1 ZOO-FISH with HSA9 DNA probe in higher primates

FISH with HSA9 DNA probe (WCP#9) to metaphases of each species resulted as expected in a specific delineation of chromosome homology. These painted chromosomes were PTR11, PPA11, GGO13, PPY13, distal portion of HLA8q and proximal portion of HLA13q, centromeric and distal regions of HAG8q and proximal portion of HAG13q , and MFU14q, respectively (Figs. 5 and 6), in accordance with the previous painting data[51]. According to Stanyon *et al.* (1987)[78], balanced inversion and translocation polymorphisms have been reported for different gibbon species, and three forms of inversion of gibbon chromosome 8 are noted as 8a, 8b, and 8c. The presented gibbon species here also indicated polymorphism to form 8b/8b in HLA and 8c/8c in HAG (Fig. 6). WCP#9 probe indicated that the form 8b had one painted part of distal q subregion whereas the form 8c showed two painted parts of centromeric and distal q subregions (Figs. 5 and 6).

3.2 High resolution mapping of the human Cε3 gene to HSA9p24.2→p24.1

FISH using WES-H-Igε-31 probe[81] for the Cε3 gene to human metaphases resulted in assignment of the gene to the telomeric region of HSA9p, band 9p24 (Fig.9). The locus for the Cε3 gene was further examined by high-resolution G-banding at approximately 600 or more band-stage resulting the subregional assignment to 9p24.2→p24.1 (Fig. 9)[96].

3.3 Comparative mapping of the Cε3 gene in higher primates

The DNA probe, Ch28-PTR-Igε-301, for the chimpanzee genomic Cε3 gene[76] used to comparative mapping in higher primates hybridized to specific chromosomes of each species. The primate Cε3 genes localized to PTR11q34, PPA11q34, GGO13q22, PPY13q16, HLA8qter, HAG8qter, and MFU14q22, respectively (Figs. 9 and 10), suggesting that several dynamic chromosomal rearrangements including at least twice pericentric inversions have occurred during the course of hominoid evolution. To investigate the interchromosomal rearrangements with more precise chromosomal breakpoints in speciation, three other cosmid markers of cCI9-37, cCI9-135 and cCI9-208 on HSA9 were used for further comparative mapping in the great apes. The idiogramatic summary of all the results of comparative mapping indicated in Fig. 10.

3.4 Evolutionary genesis of HSA9 and its primate homologues

Comparative banding analysis between human and primates has been used for a number of years as a basis for studying primate phylogenies[6,13,16]. Yunis and Prakash (1982)[13] have used high resolution banding techniques to chromosomes of human and the great apes to reconstruct chromosome rearrangements that have presumably occurred and become fixed during hominoid evolution. They reported that the orangutan lineage was first branched off after 6 karyotypic changes and subsequently each lineage of the gorilla after 14 karyotypic changes, chimpanzee after 15 karyotypic changes, and human after 11 karyotypic changes has occurred. Most of the postulated chromosomal rearrangements were pericentric inversions, although other rearrangements of paracentric inversions, fusions, etc., have also occurred. The presumed common ancestors of human and the great apes shared a substantially identical genetic set, which might have possessed a more-or-less orang-gorilla type of chromosomes equivalent to human chromosomes. However, the details of the evolutionary scenario of HSA9 were still unclear, even with high-resolution banding techniques. The present comparative mapping of the Cε 3 genes and the other three markers combined with ZOO-FISH data supports the hypothesis of Yunis and Prakash (1982)[13] for the origin of HSA9. I defined the breakpoints of the pericentric

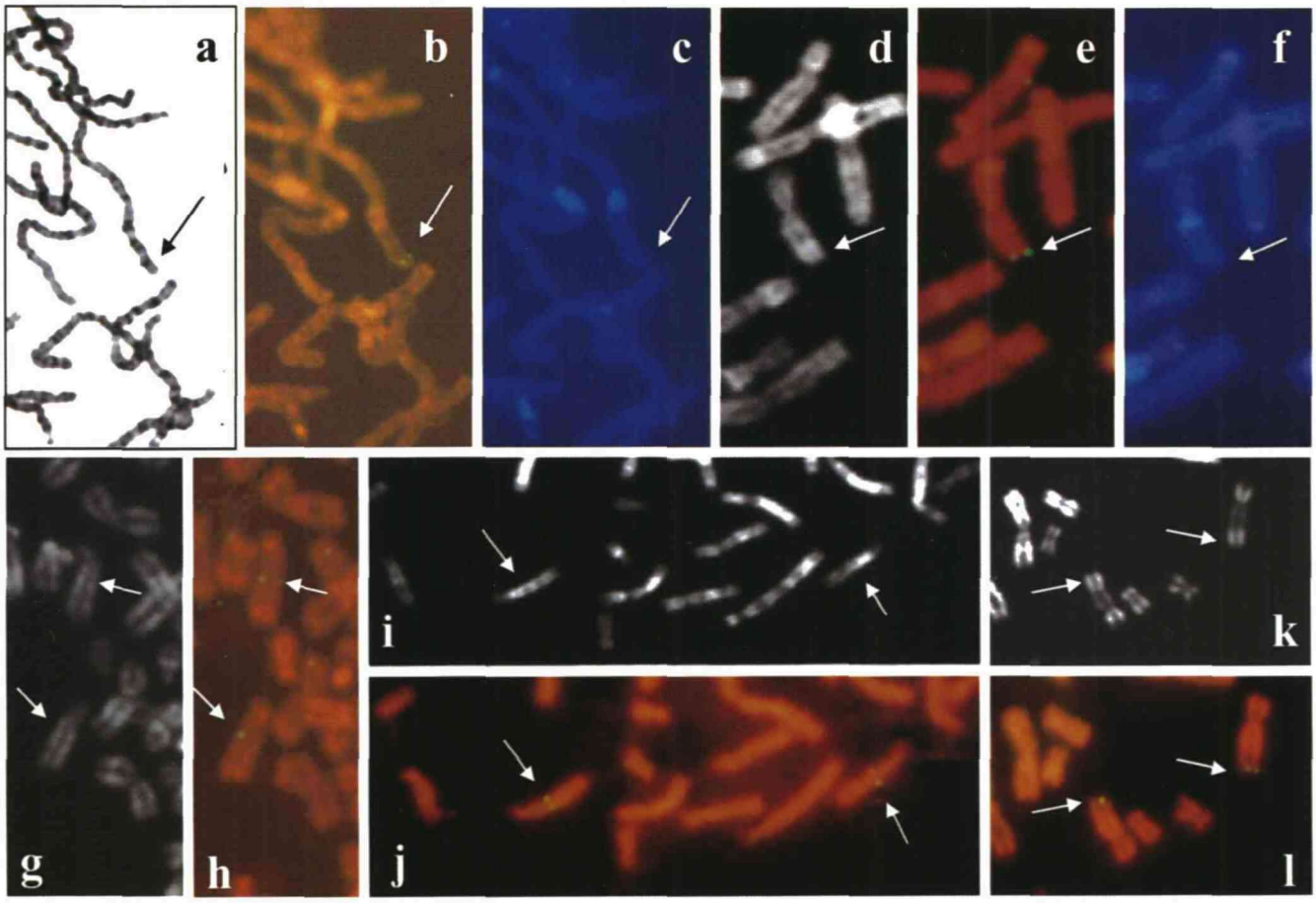

Fig. 9. FISH images of metaphases hybridized with the Cε3 gene
Localization of the human and primate Cε3 genes: a)-c) human, d)-f) pygmy chimpanzee, g)-h) gorilla, i)-j) orangutan, and k)-l) white-handed gibbon. High resolution G-banded image was shown in a) and DAPI images showed in c) and f). Pre-Q-banded images showed in d), g), i) and k) indicating the same images as in e), h), j) and l), respectively.

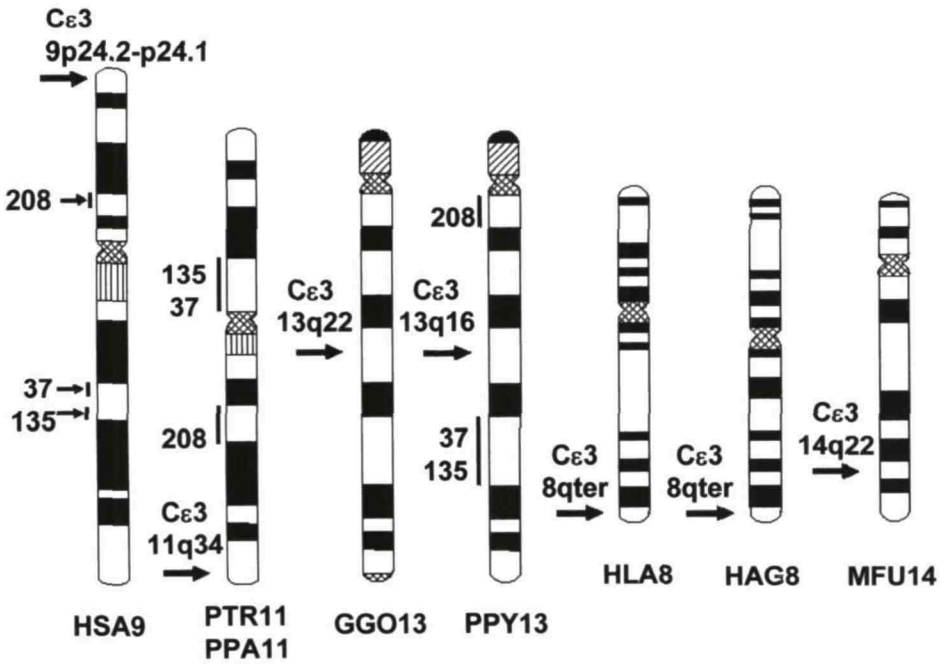

Fig. 10. Idiogramatic summary of the localization of the primate Cε3 genes and other comparative mapping results
Arrows indicate the position of the Cε3 genes and other markers on HSA9. Chromosome band numbers of each species of the Cε3 genes are given.

inversions that occurred in the human-chimp ancestor of the present orangutan chromosome 13 (PPY13p13 and PPY13q16) and gorilla chromosome 13 (GGO13p13 and GGO13q22), and in the chimp ancestor of the present chimpanzees chromosome 11 (the proximal region between PTR/PPA11p11 and PTR/PPA11q22). Putting these data together with other comparative mapping data involving the ABL1 proto-oncogene[97], it is hypothesized that HSA9 genesis took place as follows; 1) The human-chimp-gorilla-orang ancestral HSA9 chromosome was an orang-gorilla type acrocentric chromosome (PPY13/GGO13), and nucleolar organizer regions appeared on PPY13p only after branching from a human-chimp-gorilla common ancestor. 2) After the gorilla branched off, the first pericentric inversion occurred in a human-chimp ancestor with breakpoints at the regions of the present PPY13q16 and PPY13p13. 3) Centromeric heterochromatin subsequently accumulated in the human-chimp ancestral chromosome. 4) The second pericentric inversion took place in the chimp branch, with breakpoints in the middle region of the present PTR/PPA11p11 and the proximal portion of the PTR/PPA11q22. 5) Further accumulation of centromeric heterochromatin and G-positive band on the short arm occurred in the human branch, generating the present HSA9. The inversion breakpoints that occurred in the human-chimp ancestral chromosome were proximal to the markers cCI9-208 and cCI9-37, corresponding to the present HSA9 proximal region between HSA9p13.3 and HSA9q22.2 (Figs. 11 and 12). This model will be refined and confirmed by the analysis of further comparative mapping of other DNA markers of HSA9 to the great apes[98].

As for the gibbon species, the Cβ genes were mapped to HLA8qter and HAG8qter, and HSA9 homologues were divided into two or three segments detected by ZOO-FISH suggesting interchromosomal rearrangements in these species. Indeed, ZOO-FISH with HSA5, 16, 17, 22, and HSA9 specific probes hybridized to the gibbon chromosomes 8 demonstrated the presence of complex interchromosomal rearrangements as well as intraspecific heteromorphisms in the lar gibbon group (2n=44) (Figs. 5, 6, 10 and 12)[51]. Further analysis will clarify the gibbon chromosomal reconstitution.

For Japanese macaque, the Cε3 gene was mapped to MFU14q22 and HSA9 homologue was hybridized to one chromosome pair of MFU14 detected by ZOO-FISH. However, chromosome banding homology between HSA9 and MFU14 shows little similarities suggesting that only intrachromosomal rearrangements have occurred on MFU14 with syntenic conservation during the macaque speciation (Figs. 6 and 12).

3.5 Evolutionary aspects of chromosome breakpoints

A cohesive picture of the patterns of chromosomal evolution among mammals is beginning to take shape, largely from the combination of comparative studies of cytogenetics and gene mapping studies. In general, the standard observation is a relatively conserved mode of chromosome change. The occurrence of chromosomal exchange is so slow that ancestral karyotypes of living families or even certain orders (primates, carnivores, marsupials) can be deduced from ZOO-FISH analysis of living species[50]. The syntenic predictions of these cytological conclusions usually have been affirmed.

The rule of chromosomal conservation has a number of exceptional species in every group thus far studied. In primates, the gibbons and owl monkeys have highly rearranged karyotypes relatively to the ancestral forms. Stanyon and Chiarelli (1983)[77] hypothesized that changes in the hylobatid karyotype are characterized by an extremely high evolutionary rate compared with other primates. Recently this was confirmed by chromosome painting studies[51,88,89]. The mechanism of this rapid rate of chromosomal evolution in gibbons remains still unanswered, but the molecular data suggested that the evolutionary rate of genes or whole genome in gibbons is within the range of other primates[27,72,99,100].

Relatively few reports have been published on the breakpoints in evolutionary chromosome changes in primates. Previously, fragile site expression was studied in human and the great apes in relationships to a potential involvement of the site in chromosome rearrangements in evolutionary changes. However, no apparent correlation was found between the expression of a certain fragile site and the sites of chromosome rearrangement in evolutionary changes[101,102], although there was a report of the breakpoint of pericentric inversion between Bornean and Sumatran orangutans at PPY2q14 which corresponds to a conserved fragile site in human (FRA3B at HSA3p14) and primate species[103].

4. Concluding remarks

Karyotypic homologies in higher primates were studied through comparative mapping of the immunoglobulin Cε genes by FISH combined with ZOO-FISH techniques. Here, I analyzed loci of the Cα1 gene at HSA14q32.33 and the Cβ gene at HSA9p24.2→p24.1, evolutionary genesis in relation to HSA14 and HSA9 and their primate homologues were considered. Combined method of both comparative mapping and ZOO-FISH procedures suggested the highly syntenic organization of HSA14 and provided the refined hypothesis of the genesis of HSA9 and its primate homologues in speciation. During the course of hominoid evolution, at least twice

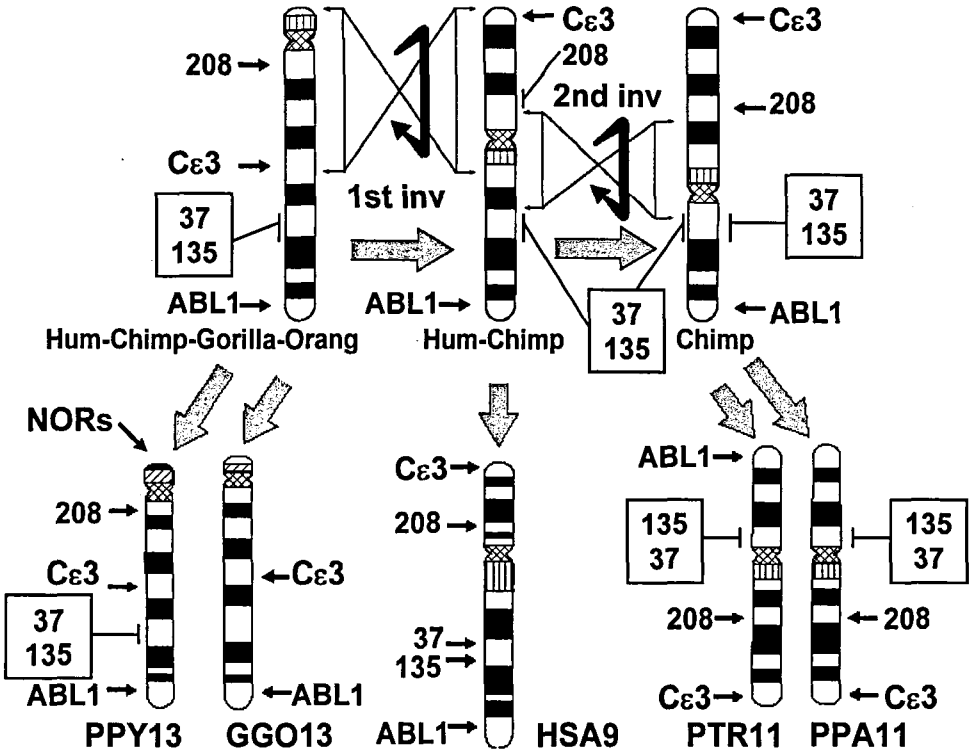

Fig. 11. Schematic representation of an evolutionary scenario on HSA9 genesis in the great apes and human
Small arrows combined with the vertical bar indicate the region of the pericentric inversions (see text for details). Arrows or vertical bars indicate the positions of assigned genes.

pericentric inversions have occurred as dynamic chromosomal rearrangement and their inversion breakpoints have been subregionally determined by the present procedures.

It is noteworthy that recently developed novel molecular cytogenetic approaches such as comparative genomic hybridization (CGH) procedure[104,105] and spectral karyotyping (SKY) method[106]. CGH procedure provides information on gains or losses of DNA sequences in targeted cells by measuring FISH signals on each chromosome and utilized to various tumor cells. Analysis of interspecies genomes by CGH may be possible to show species specific chromosomal subregions with amplifications or deletions in several megabases. On the other hand, SKY method allows that all human chromosomes have been visualized simultaneously in 24 different colors by combinatorial chromosome painting probes labelled with 5 kinds of fluorchromes utilizing Fourier spectroscopy, which can identify the specific emission spectral patterns of each fluorochrome combination corresponding to each human chromosome. This new technique will shed light on the molecular cytogenetic analysis of highly rearranged karyotypes with numerous marker chromosomes such as in tumor cells and facilitate the comparative cytogenetic studies among interspecies since so far only two or three different colored painting probes can be applied at a time. Moreover, recently developed "DNA chip" that is a small solid substrate comprising an array of numerous short DNA pieces enables us to examine the gene expression and/or mutation patterns of an entire genome at one time[107-116]. This epoch-making DNA microarray technology will be applicable to various fields such as mutagenesis, tumorigenesis, developmental biology, and evolutionary comparative studies[109-111,114-116]. The last one has been undertaken as the Great Ape genome project[111].

No matter how excellent these novel technologies are in the application to interspecies ZOO-FISH analysis that will allow us to see the patterns of genome exchange by direct observation, there are still intriguing questions unanswered in the evolutionary breakpoints of chromosomes at the fine structure level. Are they random or do they represent evolutionary "fragile sites" that tend to divide an ancestral mammalian genome into discrete conserved units? Is there a relationship between chromosomal breakpoints in primate evolution and the specific breakpoints observed in human tumorigenesis? Are there interesting genes or sequences at these breakpoints? These questions, of course, require maps of much greater density around the evolutionary junctions in order to determine their relative similarities in different mammalian taxa. Eventually, of

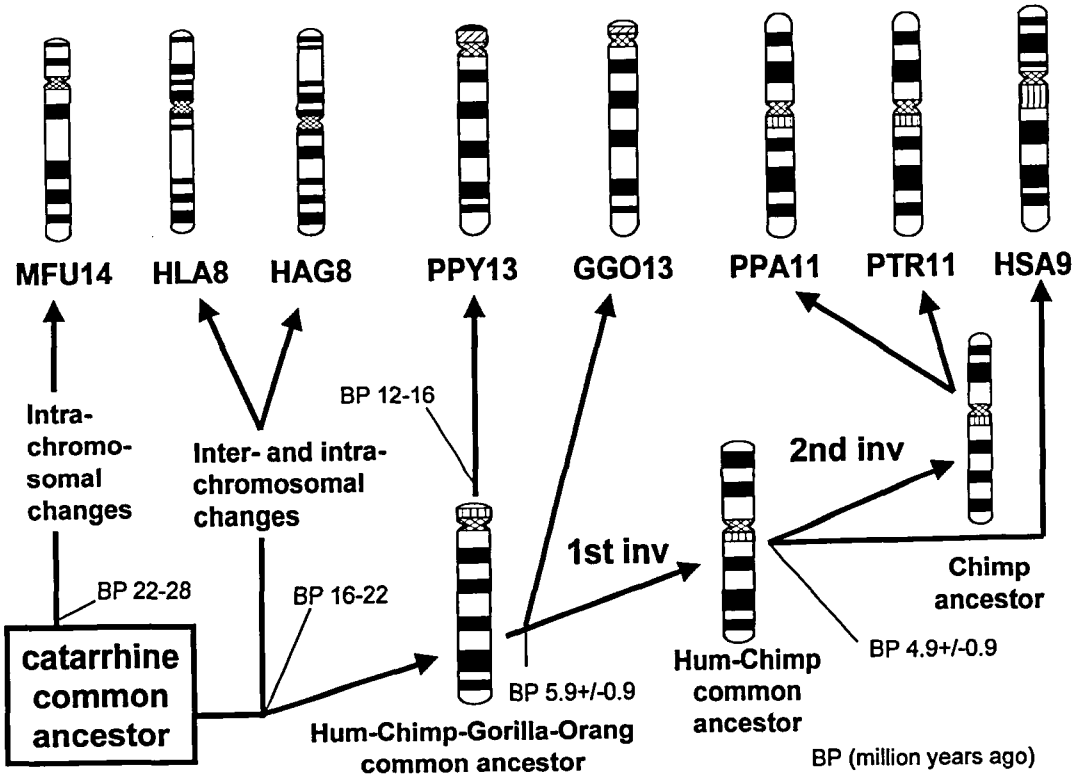

Fig. 12. Evolutionary tree of the HSA9 genesis in higher primates
The schematic tree shows the evolutionary relationships from the catarrhine common ancestor to the present species, respectively.

course, they must be defined at the nucleotide level—now a realistic expectation from the human genome sequencing initiative, especially if it is accompanied by concentrated gene mapping of prototype species from other mammalian families. This might be also facilitated by the other approach, Great Ape genome project, using DNA chips. Rapid technological advances driven by human genome mapping have clearly facilitated progress in comparative gene mapping in other species. As the human genome project provides nearly 6000 genes and 16000 expressed sequence tags in linear order on 24 chromosomes, making high resolution gene-dense map, comparative mapping will be more and rapidly extended by use of these human genome data for phylogenetic description of the genomes of mammalian ancestors.

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*1 Professor emeritus, Chromosome Research Unit, Faculty of Science, Hokkaido University
*2 ex-Division Head, Division of Genetics and Mutagenesis
*3 Graduate School of Science, The University of Tokyo
*4 Primate Research Institute, Kyoto University

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