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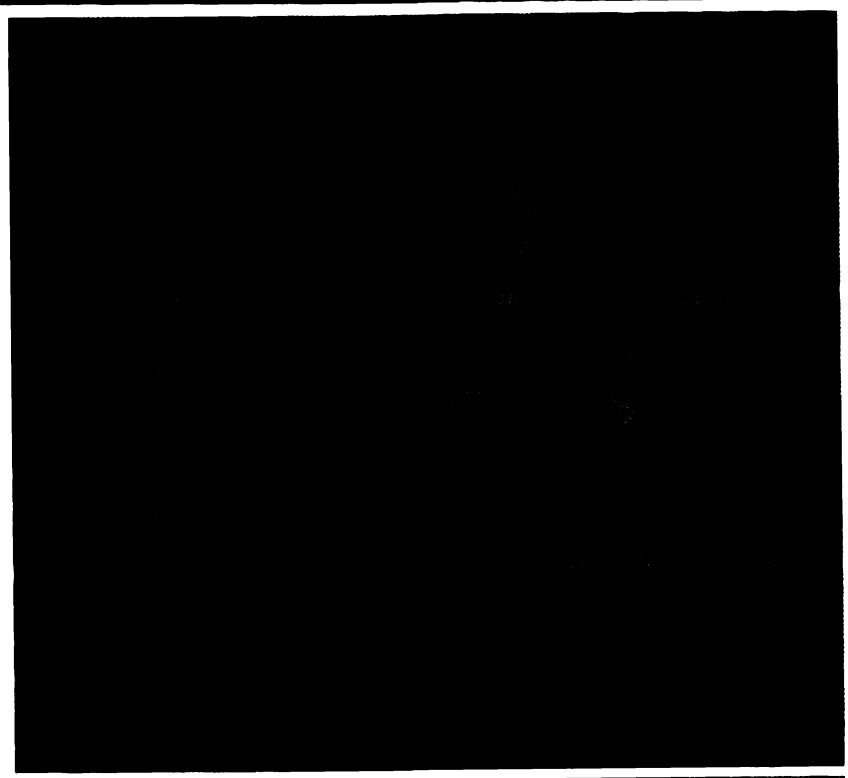

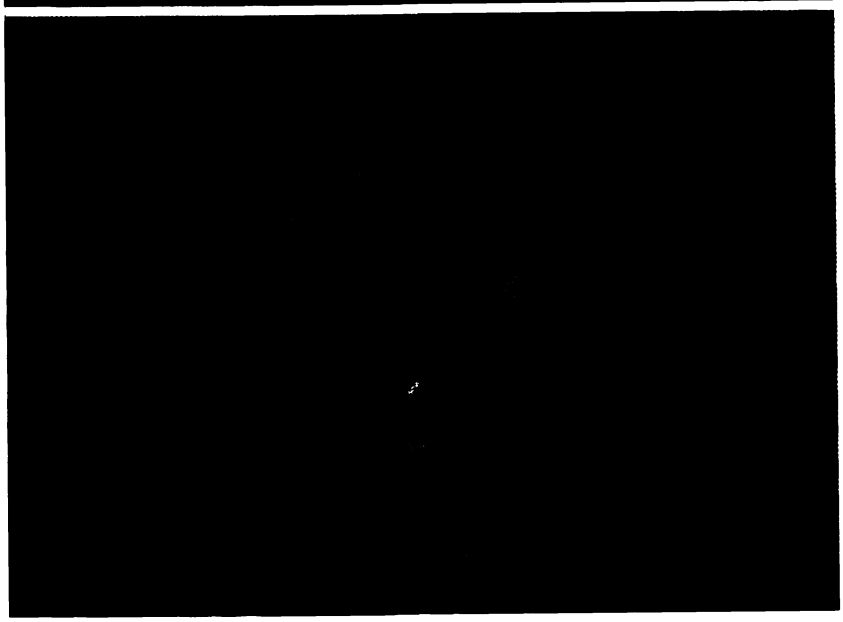

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An evolutionarily conserved early replicating segment on the sex chromosomes of man and the great apes

B. Weber,[1] W. Schempp,[1] and H. Wiesner[2]

[1] Institut für Humangenetik und Anthropologie, Universität Freiburg, Freiburg i. Br., and [2] Tierpark Hellabrunn, München

Abstract. Replication studies on prometaphase chromosomes of man, the chimpanzee, the pygmy chimpanzee, the gorilla, and the orangutan reveal great interspecific homologies between the autosomes. The early replicating X chromosomes clearly show a high degree of conservation of both the pattern and the time course of replication. An early replicating segment on the short arm of the X chromosomes of man (Xp22.3) which escapes inactivation can be found on the X chromosomes of the great apes as well. Furthermore, the most early replicating segment on the Y chromosomes of all species tested appears to be homologous to this segment on the X chromosomes. Therefore, these early replicating segments in the great apes may correspond to the pseudoautosomal segment proposed to exist in man. From further cytogenetic characterization of the Y chromosomes it is evident that structural alterations have resulted in an extreme divergence in both the euchromatic and heterochromatic parts. It is assumed, therefore, that, in contrast to the X chromosomes, the Y chromosomes have undergone a rapid evolution within the higher primates.

Despite numerous comparative cytogenetic studies in man and the great apes (see, e.g., Turleau et al., 1972; Dutrillaux, 1975; Egozcue, 1975; Schnedl et al., 1975; Miller, 1977; Seuánez, 1979; Schmid et al., 1981; Yunis and Prakash, 1982), the Y and the late-replicating X chromosomes are only poorly characterized in the great apes. Replication studies on prometaphase human sex chromosomes have revealed a distinct early replicating segment on the distal ends of both Xp and Yp (Müller and Schempp, 1982; Schempp and Müller, 1982), corresponding to the pairing segment in which a synaptonemal complex is formed during male meiosis (Moses et al., 1975; Solari, 1980; Chandley et al., 1984). Furthermore, this early replicating segment on Xp appears to escape inactivation (Race and Sanger, 1975; Mohandas et al., 1979; Müller et al., 1980; Wolf, 1981); therefore, it was suggested that the segments on the distal portions of Xp and Yp are a remnant left unchanged during the differentiation of heteromorphic sex chromosomes from originally homomorphic autosomes (Schempp and Meer, 1983).

The question arises whether the sex chromosomes of the great apes demonstrate a similar replication behavior. Therefore, we have performed a comparative replication study on prometaphase chromosomes, paying special attention to the sex chromosomes of man, the chimpanzee, the pygmy chimpanzee, the gorilla, and the orangutan. In order to draw a comprehensive comparison of the Y chromosomes of man and the great apes, additional staining procedures for the characterization of heterochromatin were applied.

Materials and methods

Chromosome preparations

Chromosome preparations from five normal human males and females (*Homo sapiens* [HSA]), five male and three female chimpanzees (*Pan troglodytes* [PTR], TNO Primate Center, Rijswijk, The Netherlands; Tierpark Hellabrunn, München, FRG; Zoologischer

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Garten, Frankfurt, FRG; Zoologischer Garten, Berlin, FRG), two male pygmy chimpanzees (*Pan paniscus* [PPA], Zoologischer Garten, Köln, FRG), three male and two female gorillas (*Gorilla gorilla* [GGO], Zoologischer Garten, Köln, FRG; Zoologischer Garten, Berlin, FRG), and four male and three female Bornean orangutans (*Pongo pygmaeus* [PPY], Zoologischer Garten, Köln, FRG; Tierpark Hellabrunn, München, FRG) were made from peripheral lymphocytes according to the method of Pfeiffer (1974), with minor modifications (Schempp and Meer, 1983). Briefly, the cells were cultivated in RPMI 1640 (Gibco), supplemented with 15% fetal calf serum and phytohemagglutinin. After 65 h of incubation, bromodeoxyuridine (BrdU, 10 µg/ml) and fluorodeoxyuridine (FdU, 0.5 µg/ml) were added; 6 h later the cultures were treated with Colcemid (0.05 µg/ml) for 30 min. The cells were then harvested by centrifugation, resuspended in hypotonic solution (0.4% KCl) for 30 min at 37 °C, and fixed in 3:1 methanol:acetic acid. Cells were washed three times with fixative and kept at 4 °C overnight. Chromosomes were spread on cold slides by flaming. Slides were air-dried for at least 24 h.

Staining methods

BrdU-replication patterns. Prometaphase chromosome preparations of the BrdU-treated cultures were differentially stained with acridine orange (50 µg/ml), resulting in RBA patterns (ISCN, 1985). By adding BrdU to the cultures during the last 6.5 h, thymidine is incorporated into early replicating chromosome sites, resulting in bright fluorescence (light bands), while the incorporation of BrdU into late-replicating chromosome material produces dull-red staining (dark bands).

Q-banding. Quinacrine mustard was used according to the technique of Caspersson et al. (1970).

Distamycin A/DAPI banding. A slight modification of the technique originally described by Schweizer et al. (1978) was applied. In brief, the slides were incubated in distamycin A (50 µg/ml in McIlvaine's buffer, pH 7.0) for 20 min at room temperature. The slides were then lightly washed in buffer and stained with DAPI (1 µg/ml in McIlvaine's buffer, pH 7.0) for a further 20 min. After a rinse in buffer, the preparations were mounted in equal parts of glycerol and McIlvaine's. For fluorescence microscopy, a BP 365/FT 395/LP397 (Zeiss) filter combination was used.

C-banding. Constitutive heterochromatin was stained according to the method of Sumner (1972).

Chromosome length measurements

Centromere indices of the Y chromosomes of man and the great apes were determined by length measurements of 30 Q-banded metaphase plates of each species. For interspecific comparison of the Y chromosomes, the X-chromosome length was used as an internal standard.

Results

Replication pattern of the autosomes

High-resolution early replication patterns (RBA bands) on prometaphase chromosomes reveal great homologies between the chromosomes of man (HSA), chimpanzee (PTR), pygmy chimpanzee (PPA), gorilla (GGO), and orangutan (PPY) (Fig. 1). Differences between interspecific homologous chromosomes can easily be explained by structural rearrangements. Besides the well-known telomeric fusion event between two great ape chromosomes, resulting in HSA 2, a reciprocal translocation between GGO 4 and GGO 19 and a telomeric R-band deletion in the short arm of PPY 21 must have occurred. All other differences in the early replication pattern between homologous human and great ape chromosomes can be attributed to pericentric and/or paracentric inversions.

Replication pattern of the X chromosomes

A band-by-band analysis of the early replicating X chromosomes of man and the great apes clearly demonstrates the high degree of conservation of both the pattern and the time course of replication. This is most evident from the reduced fluorescence intensity of bands Xp11.21, Xq11.2, and Xq13.3, which are the latest replicating bands, in the early S-phase, on the X chromosomes of all species (Fig. 2a, d).

The heterochromatic "caps" on PTR Xq, GGO Xp, and GGO Xq will not be considered here because of their nonfunctional character.

On the inactivated X chromosomes of each of the higher primates is a single band, Xp22.3, that replicates early in the S-phase and, consequently, should escape inactivation (Fig. 2e). Since the later replicating segments of the inactivated X chromosomes display slight intraindividual variation in each species, a stringent representation of the time course of replication does not seem to be feasible. The pattern most often observed in each species is depicted in Fig. 2f.

Replication pattern of the Y chromosomes

The temporal course of early replication of the Y chromosome in man and the great apes is demonstrated in Fig. 2b and 2c. The most early replicating segment, characterizing the onset of replication, is situated on the telomere of the short arm in man and the orangutan, whereas it is located on the telomere of the long arm in the chimpanzee and pygmy chimpanzee. In the gorilla this most early replicating segment is located proximal to an additional heterochromatic cap on Yp (Fig. 2b).

The fully expressed R-type replication pattern of the Y chromosome in man consists of four early replicating bands, two on Yp and two on the proximal portion of Yq. In the chimpanzee, as well as the pygmy chimpanzee, two early replicating bands are located on the distal long arm. The different sizes of

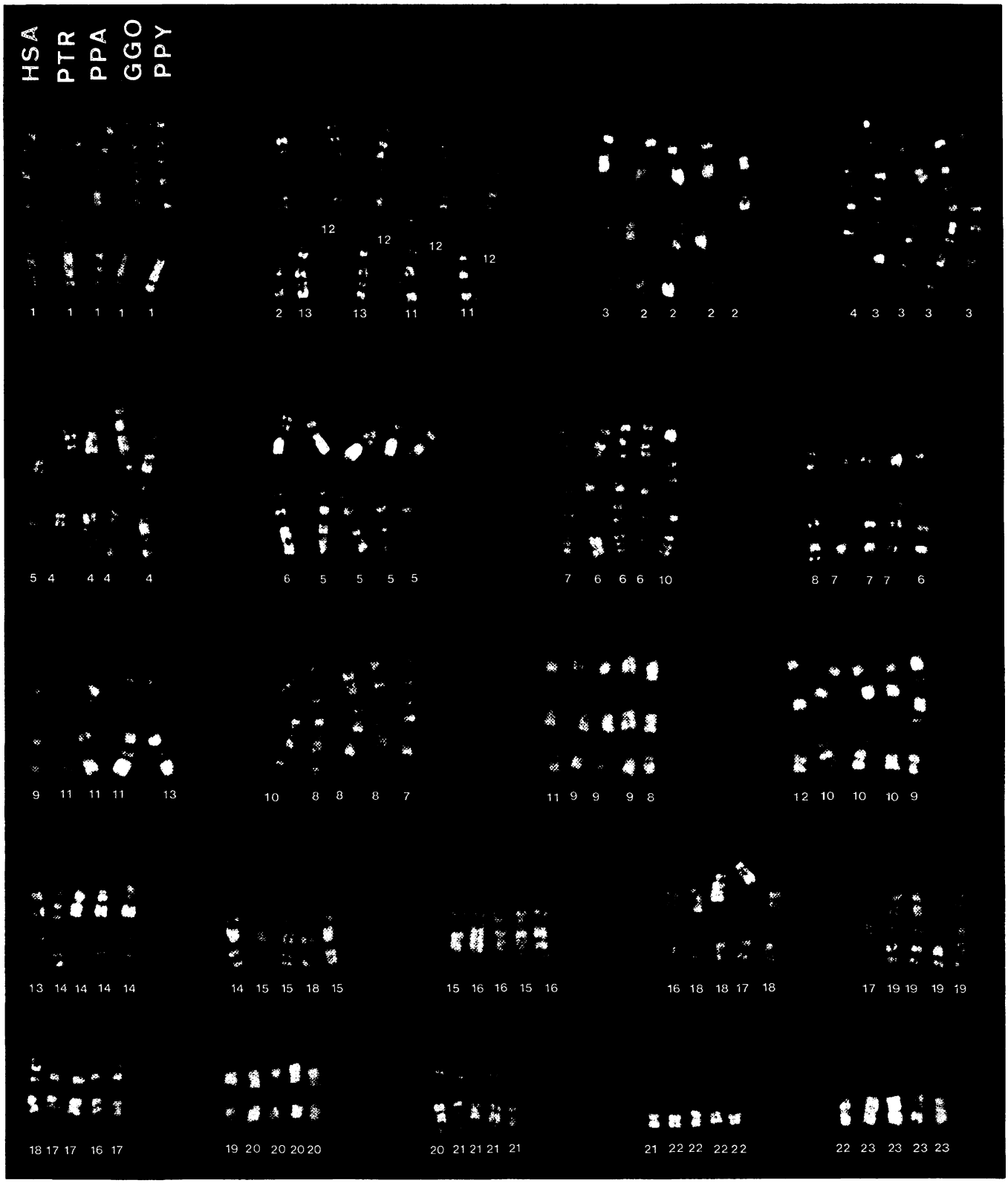

Fig. 1. High-resolution early replication banding patterns (RBA bands) of the autosomes of man (HSA), the chimpanzee (PTR), the pygmy chimpanzee (PPA), the gorilla (GGO), and the orangutan (PPY). The presumptive homologous chromosomes are arranged according to ISCN (1985) conventions, each being indicated by its standard nomenclature.

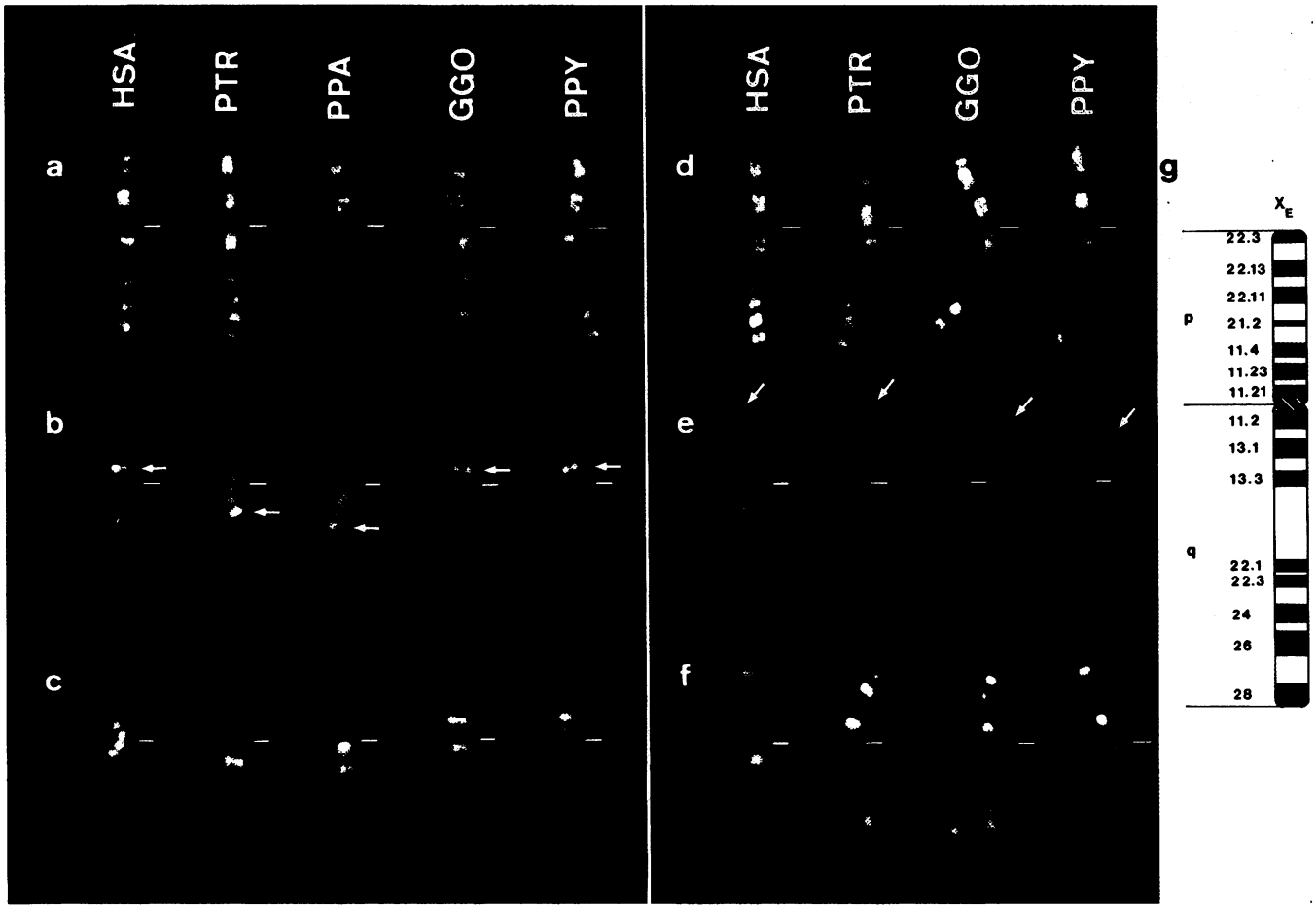

Fig. 2. Detailed replication analysis of the sex chromosomes of man and the great apes: **(a–c)** X and Y chromosomes from male specimens of man (HSA), the chimpanzee (PTR), the pygmy chimpanzee (PPA), the gorilla (GGO), and the orangutan (PPY): **(a)** RBA pattern of the X chromosomes; **(b)** Y chromosomes of man and the great apes displaying the most early replicating segment (arrows); **(c)** Y chromosomes showing the fully expressed R-type replication pattern. **(d–f)** early and late-replicating X chromosomes of the female specimens of HSA, PTR, GGO, and PPY. **(d)** RBA pattern of the early replicating X chromosomes; **(e)** late-replicating X chromosomes showing only one early replicating segment on the distal portion of Xp (arrows); **(f)** inactivated X chromosomes representing a later stage of replication. **(g)** Diagrammatic representation of the early replicating human X chromosome. The band designations follow ISCN (1985) conventions.

the Y chromosomes of the two chimpanzee species can be explained by an additional early replicating euchromatic portion in the proximal long arm of PPA Y. The replicating pattern in GGO Y shows two early replicating bands, one in the short arm and the other in the proximal region of the long arm, whereas in PPY Y there are two early replicating bands located in the short arm (Fig. 2c). Thus, on the cytogenetic level, the human Y chromosome reveals the highest content of early replicating chromatin material when compared to the Y chromosomes of the great apes.

Characterization of the heterochromatin of the Y chromosomes

A comparison of the heterochromatic regions of the Y chromosomes of man and the great apes after QFQ-, distamycin A/DAPI-, and CBG-staining is shown in Fig. 3.

A brilliant Q-fluorescent region is visible only in the long arm of HSA Y and GGO Y. Although in man this heterochromatic portion is positively stained with DA/DAPI, in the gorilla the Q-brilliant heterochromatin is DA/DAPI negative.

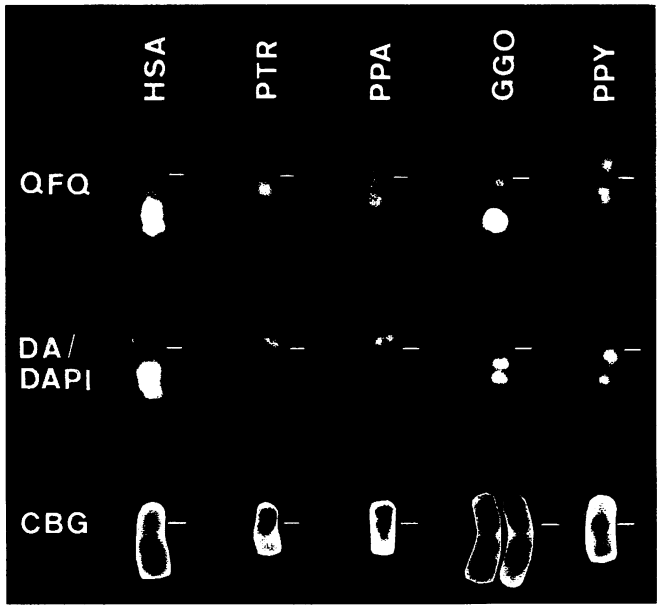

Fig. 3. A comparison of the Y-heterochromatin of the higher primates using QFQ-, distamycin A/DAPI-, and CBG-staining (from top to bottom). The two types of CBG-stained gorilla Y chromosomes can be found on the same slide.

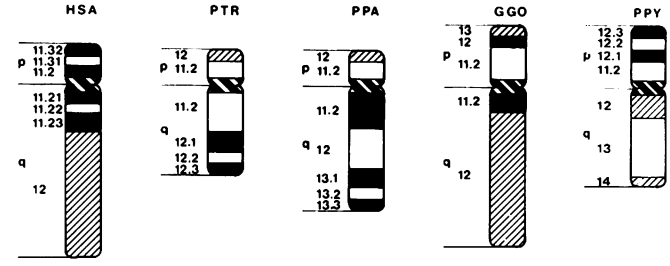

Fig. 4. Diagrammatic representation of the Y chromosomes of man (HSA), the chimpanzee (PTR), the pygmy chimpanzee (PPA), the gorilla (GGO), and the orangutan (PPY). Light and dark bands correspond to euchromatic material, with the dark bands indicating the early replicating segments (RBA-staining). The C-band-positive regions are cross-hatched, the heavily cross-hatched segments corresponding to the centromeric regions. A nomenclature system is proposed following the recommendations of ISCN (1985).

In PPY Y a paracentromeric region and a small distal segment on the long arm stain positively with DA/DAPI, as well as CBG. The long arms of PTR Y and PPA Y chromosomes are DA/DAPI and C-band negative.

DA/DAPI-positive heterochromatin is absent from the short arms of HSA Y and PPY Y. In PTR Yp and PPA Yp a DA/DAPI-positive band is located in homologous distal positions. A small DA/DAPI-positive band can be visualized in the middle of the short arm of GGO Y. In contrast to the two chimpanzee species, the DA/DAPI-positive segment in GGO Yp is C-band negative.

Furthermore, the centromeric regions of the Y chromosomes exhibit different staining behaviors. The centromeric region of HSA Y reveals distinct DA/DAPI fluorescence, whereas the centromeric regions of the great ape Y chromosomes are DA/DAPI negative. After CBG-staining, the centromere of HSA Y is only faintly visible, whereas the centromeric C-bands on the Y chromosomes of the great apes are similar to those of the other chromosomes.

A peculiarity in C-banding patterns was observed in the long arm of GGO Y. Two types of C-banded Y chromosomes can be found on the same slide. The first C-band type resembles the QFQ-staining pattern, whereas the second is similar to the DA/DAPI pattern of Yq. This peculiar staining behavior of gorilla Y heterochromatin was also observed by Schmid et al. (1986). Taken together, these findings suggest that the long arm of the gorilla Y chromosome consists of constitutive heterochromatin, except for the positive Q-banded segment in the paracentromeric long arm.

Diagrammatic representation of the Y chromosomes

The cytogenetic findings on the Y chromosomes of man and the great apes are summarized in Fig. 4. A nomenclature system for the Y chromosomes of the great apes is proposed, following ISCN (1985) recommendations.

Discussion

Earlier cytogenetic studies employing various banding techniques (e.g., Turleau et al., 1972; Dutrillaux, 1975; Egozcue, 1975; Miller, 1977; Seuánez, 1979; Yunis and Prakash, 1982) revealed an identical banding pattern among the X chromosomes of man and the great apes. The great variability of the Y chromosomes among these species was believed to be due only to different amounts of heterochromatic material, whereas the euchromatic parts were assumed to share a basic homology.

Application of the BrdU-replication technique to prometaphase chromosomes in the present study clearly demonstrates the complete conservation of

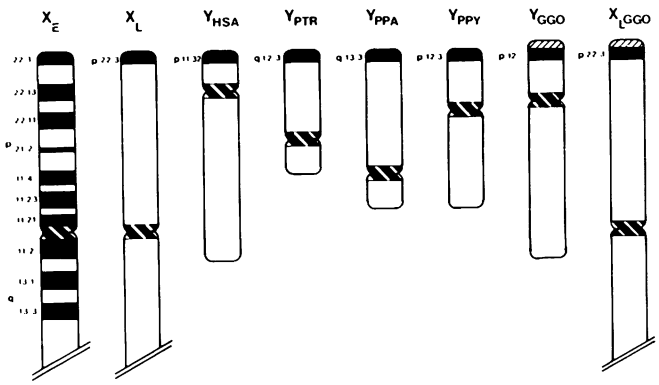

Fig. 5. Schematic representations of the proposed pseudoautosomal segment on the sex chromosomes of man and the great apes. The most early replicating segment on the Y chromosomes and the segment escaping inactivation on the X chromosomes (Xp22.3) of man and the great apes are demonstrated. The synchronously replicating segments are arranged in a line by turning the Y chromosomes of the chimpanzee (Y_{PTR}) and pygmy chimpanzee (Y_{PPA}) upside down. Note that the heterochromatic caps (lightly crosshatched segments) on the distal ends of the short arms of the gorilla X and Y chromosomes would not interfere with a possible pseudoautosomal behavior of Xp22.3 and Yp12 in the gorilla.

both the pattern and the time course of replication of the X chromosomes. With regard to the autosomes of comparable size to the X chromosomes, this high degree of conservation can be demonstrated only for HSA 6 and the corresponding chromosomes of the great apes (Fig. 1).

Based on our cytogenetic data, it is evident that structural alterations have caused enormous differences in both the euchromatic and heterochromatic regions of the Y chromosomes. In contrast to the X chromosomes, the Y chromosomes have apparently undergone a very rapid evolution within the higher primates. Even within such closely related species as the chimpanzee and pygmy chimpanzee, the Y chromosomes differ in the amount of euchromatic material they possess. In regard to the heterochromatic regions, it is well known that the Y chromosome in the higher primates is a major site of hybridization with human satellite DNA (Gosden et al., 1977; Mitchell et al., 1977). On the other hand, recent molecular data have shown that the human Y-specific component of the 2.47-kb *Hae*III repeat is not found on the gorilla Y chromosome, whereas in the chimpanzee related sequences behave as a "dispersed" repeat (Cooke et al., 1982); moreover, the human Y-specific component of the 3.4-kb *Hae*III repeat is not Y-specific in the chimpanzee, gorilla, or orangutan (Kunkel and Smith, 1982). Furthermore, a 5.5-kb *Eco*RI fragment was found to be characteristic of a Y-specific alphoid repeat in man, but was not seen in gorilla, orangutan, or chimpanzee male DNA (Wolfe et al., 1985). This diversity of the DNA sequences may explain the different staining behaviors of the Y heterochromatin in man and the great apes.

Possible homologies in the euchromatic parts of the Y chromosomes cannot easily be ascertained cytogenetically, since striking differences in the amount of early replicating chromatin material are visible. Thus, the four early replicating segments on the human Y chromosome obviously represent the highest amount within the higher primates.

The earliest replicating segment on the distal portion of HSA Yp is well known to replicate synchronously with the most distal segment on Xp known to escape X-inactivation (Xp 22.3); furthermore, these segments on the distal part of Yp correspond well to the pairing region in male meiosis (Müller and Schempp, 1982; Schempp and Meer, 1983).

By analogy to man, in the great apes only the earliest replicating segment on each of the Y chromosomes and the synchronously replicating segment escaping inactivation on the X chromosomes may be homologous (summarized schematically in Fig. 5). Consequently, these synchronously early replicating segments on sex chromosomes of the great apes could correspond to the pseudoautosomal segment proposed to exist in man (Burgoyne, 1982; Polani, 1982). The heterochromatic caps on the short arms of the gorilla sex chromosomes do not interfere with this view.

Recently, human Y chromosome–derived sequences which are located in the pairing region of the human sex chromosomes have been shown to undergo recombination with the sexual phenotype and can therefore be described as "pseudoautosomal" (Cooke et al., 1985; Simmler et al., 1985). In situ hybridization of such human-derived pseudoautosomal sequences will show if these sequences were conserved and are also located in the synchronously early replicating segments of the great ape sex chromosomes (manuscript in preparation).

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