Comparative mapping of ZFY in the hominoid apes

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Summary. Within our project of comparative mapping of candidate genes for sex-determination/testis differentiation, we used a cloned probe from the human ZFY locus for comparative hybridization studies in hominoids. As in the human, the ZFY probe detects X- and Y-specific restriction fragments in the chimpanzee, the gorilla, the orangutan, and the gibbon. Furthermore, the X-specific hybridization site in the great apes resides in Xp21.3, the same locus defining ZFX in the human. The Y-specific locus of ZFY maps closely to the early replicating pseudoautosomal segment in the telomeric or subtelomeric position of the Y chromosomes of the great apes, again as found in the human. Thus, despite cytogenetically visible structural alterations within the euchromatic parts of the Y chromosomes of the human species and the great apes, a segment of the Y chromosome defined by the pseudoautosomal region and ZFY seems to be more strongly conserved than the rest of the Y chromosome.

Introduction

The highly heteromorphic mammalian X and Y chromosomes are thought to have evolved from a homologous chromosome pair (Ohno 1967). Consequently, homologous segments should still exist on the highly differentiated mammalian sex chromosomes representing a relic of such an ancestral pair of autosomes. This evolutionary process should form the basis for the bipartite structure of the eutherian Y chromosome: the remaining homologous pairing segment is shared by and recombines between the X and Y chromosomes, thereby ensuring the proper segregation of the sex chromosomes during male meiosis, whereas on the other hand, the Y-specific region, including the testis-determining gene(s) (TDF), must avoid recombination, otherwise the chromosomal basis of sex determination would break down. Indeed, there is cytogenetic and molecular evidence, in a variety of placental mammals, that the heteromorphic X and Y chromosomes share a homologous segment in the telomeric or subtelomeric position, a location that is compatible with meiotic crossing over in the male sex (for review see Schempp et al. 1989).

Molecular evidence for the existence of strictly homologous sequences on the heteromorphic sex chromosomes recombining frequently in male meiosis has been found in the human (Cooke et al. 1985; Simmler et al. 1985), the mouse (Keitges et al. 1985; Soriano et al. 1987), and the chimpanzee (Weber et al. 1988). Because these sex-chromosomal sequences follow a seemingly autosomal mode of inheritance, they have been termed pseudoautosomal (Burgoyne 1982). Although the pseudoautosomal pairing segments of the human and the chimpanzee can be regarded as strictly homologous, that of the mouse differs with respect to chromosomal banding patterns (Somssich et al. 1981) and genetic content (Harbers et al. 1990), and therefore cannot be homologized in a direct linear manner.

Our own chromosomal replication and in situ hybridization studies (reviewed in Schempp et al. 1989) have revealed that, in the human, the chimpanzee, the gorilla, and the orangutan, an early replicating segment in a distal position of the X and Y chromosomes is consistent with the specific localization of a human-derived pseudoautosomal repeat, DXYZ2 (Simmler et al. 1985; Royer et al. 1986). Thus, the human and the great apes share a homologous segment at one end of their sex chromosomes. Moreover, considering the findings on recombination in the human and the chimpanzee, a pseudoautosomal behavior of these early-replicating segments in a distal position on the X and Y chromosomes may also be inferred by analogy for the gorilla and the orangutan (Weber et al. 1987).

In the human, the testis-determining region has been localized close to the pseudoautosomal segment on the short arm of the Y chromosome (Vergnaud et al. 1986). The ZFY gene (zincfinger Y), formerly a candidate testis-determining gene, maps only about 200 kb proximal to the pseudoautosomal segment. A largely homologous DNA sequence also occurs on the human X chromosome, and has been termed by analogy ZFX (Page et al. 1987b; Page 1988). The present candidate testis-determining gene, SRY (sex-determining region Y), maps within a 35-kb interval adjacent to the pseudoautosomal
boundary (Sinclair et al. 1990). As an unfavorable consequence, the great majority of human XX males result from unequal crossing over between the X and Y during paternal meiosis, transferring the testis-determining gene(s) to the X chromosome (Petit et al. 1987). The question arises whether this hazardous proximity of the testis-determining region to the Y pseudoautosomal segment also holds true for the Y chromosomes of the great apes. Assuming that the ZFY gene is in close proximity to the testis-determining region and can thus serve as a marker for this region, we used a cloned probe from the human ZFY locus, pZFY-1 (Jäger et al. 1990), for comparative in situ hybridization on metaphase chromosomes of the great apes.

We have shown (Müller and Schempp 1989), by in situ hybridization with the pZFY-1 probe, that the human ZFY gene maps within an early replicating segment in Yp11.32, which is also the specific site of hybridization of the pseudoautosomal marker repeat DXYZ2 (Simmler et al. 1985). Furthermore, this probe detects another specific hybridization site in Xp21.3, defining the ZFX locus (Müller and Schempp 1989). This localization of ZFX is in good agreement with the linkage studies of Affara et al. (1989), Page et al. (1990), and Leung et al. (1990).

The aim of this study was: (1) to look for the evolutionary conservation of the X- and Y-specific restriction fragments detected by the pZFY-1 probe using Southern blot analysis, in the great apes, (2) to map ZFY relative to the pseudoautosomal region on the Y chromosomes of the great apes (in this regard, the early replicating segments in a telomeric or subtelomeric position can serve as a marker for the pseudoautosomal region on the Y chromosomes of the great apes [Weber et al. 1987]), and (3) to ensure the conservation of the ZFX locus in Xp21.3 of the great apes.

Materials and methods

Blood samples

Heparinized blood samples of the chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orangutan (Pongo pygmaeus), and white-handed gibbon (Hylobates lar) were kindly provided by Dr. Rietischel, Zoologisch-Botanischer Garten Wilhelma, Stuttgart, (FRG).

Chromosome preparations and staining methods

Chromosome preparations were made from peripheral lymphocytes according to standard methods, with minor modifications (Schempp and Meer 1983). During the last 8 h before harvesting, BrdU was added to cultures. As a result, thymidine was incorporated into early replicating and BrdU into late replicating chromosomal segments. Chromosome preparations of BrdU-treated lymphocyte cultures were differentially stained with acridine orange to produce RBA bands (Dutrillaux et al. 1973), or with Hoechst 33258 and Giemsa to produce RBG bands (Perry and Wolff 1974).

Southern blot analysis

DNA was prepared from white blood cells according to published methods (Kunkel et al. 1977). The DNA samples, in aliquots of 5 µg, were digested to completion with the restriction endonuclease TaqI. The resulting fragments were fractionated on a 0.8% agarose gel and then transferred to a Gene Screen nylon membrane (NEN), followed by UV cross-linking of the DNA and hybridization with probe insert pZFY-1 (Jäger et al. 1990). The insert was labeled with 32P to a specific activity to 1.4 × 109 cpm/µg using the random primer technique of Feinberg and Vogelstein (1984). The blots were washed three times at 65°C for 30 min each, in 20 mM sodium phosphate buffer (pH 7.2), 1% SDS, and exposed to Fuji RX film with intensifying screens at −70°C for 1 day.

In situ hybridization

Chromosome spreads derived from BrdU-treated lymphocytes cultures were prestained with acridine orange and photographed; chromosomes were then hybridized in situ as described in Müller and Schempp (1989).

Results

Southern blot analysis

To detect the presence of X- and Y-specific restriction fragments of the human ZFY sequence in the genomes of the hominoids, Southern blot analyses of TaqI-digested DNA of male and female specimens of the human, the chimpanzee, the gorilla, the orangutan, and the gibbon were performed using the human-derived pZFY-1 (Jäger et al. 1990) as a probe (Fig. 1). In the human, pZFY-1 detects one Y-specific TaqI fragment of

![Fig. 1. Southern blot analysis of TaqI-digested DNA of male (M) and female (F) specimens of the human, the chimpanzee, the gorilla, the orangutan, and the gibbon with the human-derived probe pZFY-1. Note that, in all hominoids, pZFY-1 detects X- and Y-specific TaqI fragments. Sizes in kb of phage lambda HindIII fragments used as size markers are indicated on the left](image-url)