Y-chromosomal red-green opsin genes of nocturnal New World monkey[1]

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Abstract **The X-chromosomal locality of the red-green-sensitive opsin genes has been the norm for all mammals and is essential for color vision of higher primates. Owl monkeys (*Aotus*), a genus of New World monkeys, are the only nocturnal higher primates and are severely color-blind. We demonstrate that the owl monkeys possess extra red-green opsin genes on the Y-chromosome. The Y-linked opsin genes were found to be extremely varied, in one male appearing to be a functional gene and in other males to be multicopy pseudogenes. These Y-linked opsin genes should offer a rare opportunity to study the evolutionary fate of genes translocated to the Y chromosome.**

Key words: Visual pigment; Opsin; Nocturnal vision; Owl monkey (*Aotus trivirgatus*)

1. Introduction

The red-green opsin is the protein moiety of the visual pigment maximally sensitive to middle to long wavelength light and is responsible for distinguishing colors in the red–green (500–570 nm) region of the spectrum. In humans, apes and Old World monkeys, the red and green opsin genes are tandemly located on the X chromosome and either the red or the green opsin gene is expressed from one X chromosome through a process of stochastic interaction of a locus-control region, situated upstream of the gene array, with a promoter of either of the genes [1,2]. In many New World monkeys, the red-green opsin is encoded in a single-locus gene on the X chromosome with allelic variations [3]. For these higher primates, the X locality of the gene(s) is extremely important for their color vision because only one opsin type is produced in a given red-green cone photoreceptor cell as a result of X chromosome hemizygosity in males and random X inactivation in females [4].

Owl monkeys (*Aotus*) are unique among New World monkeys in being nocturnal, having the monoallelic red-green pigment but lacking a functional blue pigment and being monochromatic [5,6]. In mammals with monomorphic red-green opsins, natural selection to restrict the opsin gene in the X chromosome would be loosened. However, no mammal with an extra-X red-green opsin gene has been reported. Here we show that owl monkeys have the extra red-green opsin gene(s) on the Y chromosome.

2. Materials and methods

2.1. DNA cloning

All owl monkeys (*Aotus trivirgatus*) were from a breeding colony in the Primate Research Institute, Kyoto University. Founder monkeys nos. 24 and 25 (Fig. 1A) have a Bolivian origin while the origins of the other founders, nos. 14 and 23, are not known. Subject no. 25 died before the commencement of the study so no DNA was available. Genomic DNA was extracted from blood samples and genomic libraries were constructed using EMBL3 λ-phage vector as previously reported [7]. Using the human red opsin full-length cDNA [8] as a probe, we screened the library as previously described [7]. The plasmid subclones were sequenced in both strands using the Thermo Sequenase™ Cycle Sequencing Kit (Amersham) with dye-labeled M13 forward and reverse primers. Reactions were run on a LI-COR 4200L-1 automated DNA sequencer.

2.2. Southern hybridization

Approximately 2 μg per lane of the digested DNA was electrophoresed on 0.4% agarose gel, soaked in 0.25 M HCl for depurination and transferred to a positively charged nylon membrane (Biodyne B, Pall) using the VacuGene vacuum blotting system (Amersham). Probe DNA was labeled with [α-^{32}P]dCTP. Hybridization and washing were carried out as previously described [7]. Signal intensity was quantitated using BAS-5000 imaging analyzer and Science Lab 99 Image Gauge V3.45 software (Fuji Film).

2.3. Fluorescence in situ hybridization

Metaphase chromosomes were prepared from phytohemagglutinin-stimulated cultured lymphocytes. Fluorescence in situ hybridization (FISH) was performed using a λDNA clone of 29X (λAT9) as a probe as described previously [7,9].

3. Results

From genomic libraries constructed from two male owl monkeys (no. 14 and no. 29 in Fig. 1A), we isolated two (denoted 14X and 14Y) and five genes (29X, 29Y-1, 29Y-2, 29Y-3 and 29Y-4) (Fig. 1B), respectively, that are highly homologous to the human red opsin gene [8] (94.3–95.1% identity in the coding region). The human red opsin gene consists of six exons and five introns [8]. The same exon–intron structure was identified in the cloned genes except in genes 29Y-2, 29Y-3 and 29Y-4 which were cloned partially (Fig. 1B). All cloned genes except 29Y-4 were assigned to the hybridization

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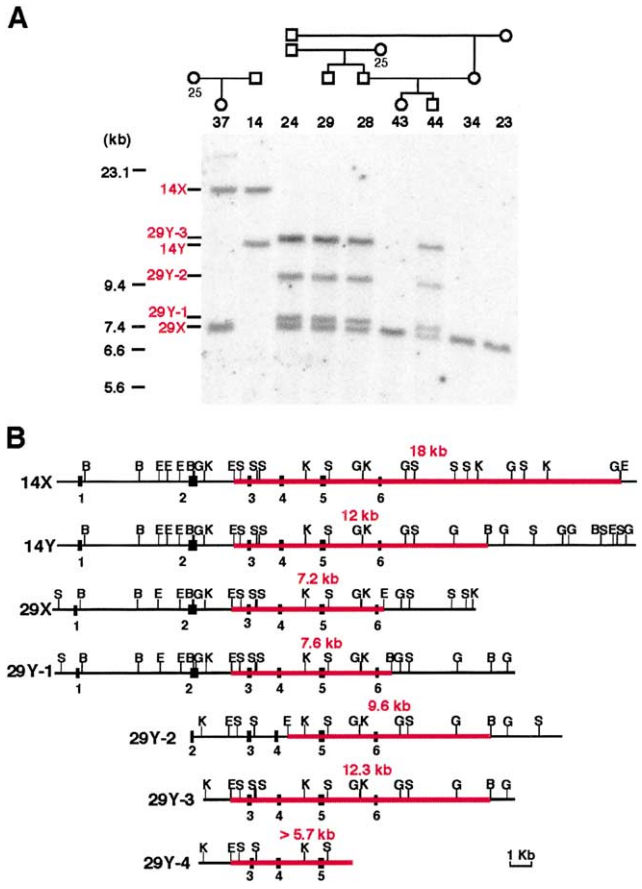

Fig. 1. Genomic organization of the red-green opsin genes of owl monkeys. A: Genomic DNA digested with *Bam*HI and *Eco*RI was hybridized with the human red opsin exon 5 as previously described [7]. Subject numbers and familial relationships are given: circles, females; squares, males. λ*Hin*dIII size standards are indicated in kb. B: The six exons are depicted with solid boxes. The *Bam*HI–*Eco*RI double-digested DNA fragments containing exon 5 are shown in red. The cloned genes are assigned to hybridization bands in A according to fragment size. B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; K, *Kpn*I; S, *Sac*I.

bands in the genomic Southern blot according to band size (Fig. 1A,B). The banding pattern in no. 29 also appeared in its male relatives (nos. 24, 28 and 44; denoted 29-type males) (Fig. 1A). In each 29-type male, the intensity ratio of 29X, 29Y-1, 29Y-2, and 29Y-3 bands was approximately 1:1:1:2. Thus we assigned gene 29Y-4 to the same band as 29Y-3. We detected no hybridization signal other than those to the cloned genes in both no. 14 and the 29-type males.

Nucleotide sequencing revealed that genes 29Y-1, 29Y-2, 29Y-3, and 29Y-4 are interrupted in exon 5 by a nucleotide substitution (C to G) creating a premature stop codon (TAG) at the amino acid position corresponding to residue 284 of the human red opsin [8]. In exon 4 of 29Y-1, 29Y-3 and 29Y-4, another premature stop codon (TGA) by a base substitution (C to T) was observed at putative residue position 247. These stop codons must render the four genes non-functional because the resulting truncated proteins would lack the last two transmembrane domains and the chromophore binding site. On the other hand, 14Y, as well as 14X and 29X, appeared to have no structural defect and their deduced amino acid sequences are identical.

It was noted that the daughter of no. 14 did not inherit one of the two father-originated genes (14Y) (Fig. 1A). The daughters of 29-type males also did not inherit four of the five father genes (29Y-1, 29Y-2, 29Y-3, and 29Y-4). This indicates that 14Y and 29Y-1, 29Y-2, 29Y-3, and 29Y-4 are not located on the X chromosome. From the segregation data, it is possible that the translocated genes are either on the Y chromosome or an autosome that happens to segregate in a small number of individuals in a manner consistent with a Y chromosome pattern. In order to establish the chromosomal locality of the extra genes, FISH to chromosome preparations was carried out. FISH directly located hybridization signals on the X chromosomes of both females and males at the distal region of the long arm (Fig. 2A,B, arrowheads), consistent with other mammals [7,10,11]. In male no. 14, an additional signal appeared on the Y chromosome at the distal region of the long arm (Fig. 2A, arrow). The 29-type males were found to be a karyotypical variant (karyotype VI) [12] showing autosome 14-Y translocation and showed multiple signals on the translocated Y (Fig. 2B, arrow). These observations demonstrate that the owl monkeys have one potentially functional (14Y) or four pseudocopies (29Y-1, 29Y-2, 29Y-3, and 29Y-4) of the Y-chromosomal red-green opsin genes in addition to one functional X-chromosomal gene (14X or 29X).

4. Discussion

This is the first report on the Y-chromosomal red-green opsin genes in vertebrates. While all exons have been se-

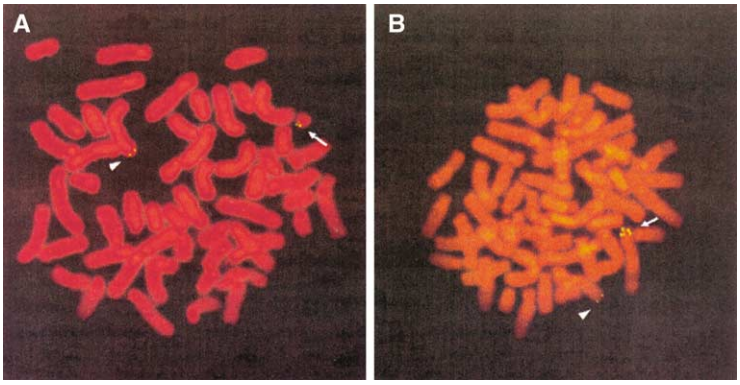

Fig. 2. Chromosomal localization of the owl monkey red-green opsin genes. A: Metaphase plate showing twin-spot signals on the X (arrowhead) and Y (arrow) chromosomes in a male (subject no. 14). B: Metaphase plate showing a twin-spot signal on the X chromosome (arrowhead) and multiple signals on the Y-autosome (arrow) in a 29-type male (subject no. 28) (karyotype VI).

quenced with their flanking non-coding regions for 14X, 14Y and 29X genes, only exons 3, 4 and 5 have been sequenced for the four 29Y pseudogenes. The phylogenetic relationship among 29Ys and other genes has not been resolved with high statistical significance (data not shown) and a more extensive sequence comparison should be carried out to establish the origin and evolutionary history of the Y-chromosomal red-green opsin genes. It should be noted, however, that the restriction site distributions in the downstream region in 14Y and 29Ys (excluding 29Y-4 for which the region has not been cloned) are similar to one another but distinct from those in 14X and 29X (Fig. 1B). This strongly suggests a common origin of the Y translocation between 14Y and 29Ys. After the translocation, it appears that an owl monkey lineage now represented with 29Ys has experienced a nonsense mutation in exon 5 in the original Y-translocated gene and, after the subsequent duplication of the Y gene, another nonsense mutation in exon 4 occurred in one of the duplicates that experienced further duplications which resulted in 29Y-1, 29Y-3 and 29Y-4. It is not clear whether the autosome 14-Y translocation had any impact on the occurrence of nonsense mutations and gene duplications.

It is noted that the Y-linked red-green opsin genes are extremely varied as described above. Genes located on the Y chromosome outside the pseudoautosomal region do not recombine with those on the X chromosome and are predicted to either undergo selection for male function or gradually degenerate because of an accumulation of deleterious mutations [13]. While the occurrence of 29Y pseudogenes appears to be consistent with this general scenario on Y-gene degeneration, could any male function be ascribed to a potentially functional 14Y gene? Considering that social behavior of owl monkeys occurs most often under bright moonlight and that males carry out most parental activities [14], acquisition of an additional cone pigment by males could become favorable through increasing their visual sensitivity. Although it needs to be clarified whether the additional pigment genes on the Y are pseudoautosomal or not and whether the potentially functional Y-linked opsin gene is expressed in the retina, these genes should offer a rare opportunity to study the evolutionary fate of the genes that jumped into the Y chromosome.

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