Molecular Evidence of Y-Autosomal Translocations in Owl Monkeys

N. S.-F. Ma, D. C. Page, and T. S. Harris

Probe pDP1007, which contains highly conserved DNA sequences from the sex-determining region of the human Y chromosome, cross-hybridized with owl monkey EcoRI restriction fragments of 1.8 kb and 6.6 kb. Southern transfer analysis of owl monkey (karyotype VI)–rodent somatic cell hybrids localized the 1.8-kb fragment on the owl monkey X chromosome and the 6.6-kb fragment, which is male specific, on chromosome 14/Y. Regional in situ chromosome mapping of pDP1007 revealed specific sites of hybridization: the distal short arm of the X chromosome of karyotypes IV, VI, and VII; the small metacentric Y of karyotype IV; the C-band positive region on the short arm of chromosome 17/Y (karyotype VII); and the C-band positive region on the long arm of chromosome 14/Y (karyotype VI). These molecular findings reinforce cytological evidence that Y-chromosomal material has been transferred to autosomes 14 and 17 in owl monkeys of karyotypes VI and VII, respectively, in which there are no independently segregating Y chromosomes.

The widely distributed South American owl monkey, *Aotus,* is characterized by diversified karyotypes with diploid chromosome counts ranging from 46 to 58.[2,8,11,13,19] In addition, some owl monkeys are distinguished by an uncommon chromosomal sex determination system. A Y-to-autosome (Y;A) translocation in the males has been observed in at least three isolated populations of owl monkeys inhabiting Bolivia (karotype VI, $2n = 49$ male; 50 female), Peru (karyotype VII, $2n = 51$ male; 52 female), and Brazil ($2n = 49$ male; 50 female).[8,9,12] The initial interpretation of this XX:X,t(Y;A) sex determination system was based on cytological observation of male meiotic pairing of the X chromosome with two autosomes to form a trivalent structure and on the identification of unpaired mitotic chromosomes by band pattern analysis.[9,12] In tracing the evolution of the owl monkey Y chromosome, Ma found that this chromosome is prone to rearrangement and translocation.[6] The Y chromosome, the smallest chromosome in the owl monkey complement, can be metacentric (in karyotypes I, II, III, IV, VIII, and IX), acrocentric (in karyotypes V, X, and XI), translocated onto the short arm of an autosome (in karyotype VII), or interstitially inserted into the long arm of autosome 14 (in karyotype VI).

Recent advances in molecular cloning techniques have led to the isolation of numerous DNA sequences from various regions of the human Y chromosome. A particular focus of interest has been the portion of the short arm of the human Y chromosome (HSA Yp) that has been proposed to contain the sex-determining gene(s).[16,18] Within this region, certain single-copy DNA sequences were found to be evolutionarily conserved by cross-reacting with DNA sequences in a wide range of placental mammals, including the owl monkey.[18] Therefore, these highly conserved HSA Y DNA sequences, which detect male-specific restriction fragments in the placental mammalian genome, can be valuable markers for regional localization of the Y chromosome (or chromosome segment) in the owl monkey chromosome complement with an XX:X,t(Y;A) sex determination system. In our study, one of these conserved single-copy DNA sequences from the human Y chromosome, clone pDP1007, was used to map the autosomally translocated Y chromosome (or chromosome segment) in karyotype VI ($2n = 49$; male) of the Bolivian owl monkey and in karyotype VII ($2n = 51$; male) of the Peruvian owl monkey.

The single-copy DNA probe pDP1007 derives from interval IA2 of the human Y chromosome. It detects two related sequences, one on HSA Yp and another on HSA Xp21–p22.[18] The DNA sequences on HSA Yp, designated *ZFY*,[17] appear to en-

From the New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts (Ma and Harris), and the Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge (Page). This work was supported by NIH Division of Research Resources grant RR 00168 to the New England Regional Primate Research Center, Harvard Medical School. We thank Ms. J. Armstrong and A. Hay for help in the photographic work and Ms. B. Blake and Dr. N. King for reviewing the manuscript. Address reprint requests to Dr. Ma, New England Regional Primate Research Center, Harvard Medical School, 1 Pine Hill Drive, Southborough, MA 01772.

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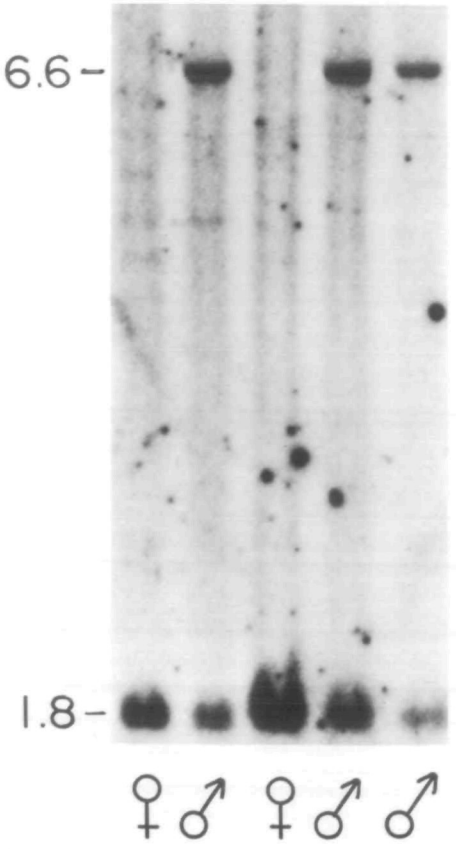


Figure 1. Southern transfer of EcoRI-digested DNA from male and female owl monkeys hybridized with the human pDP1007 probe.

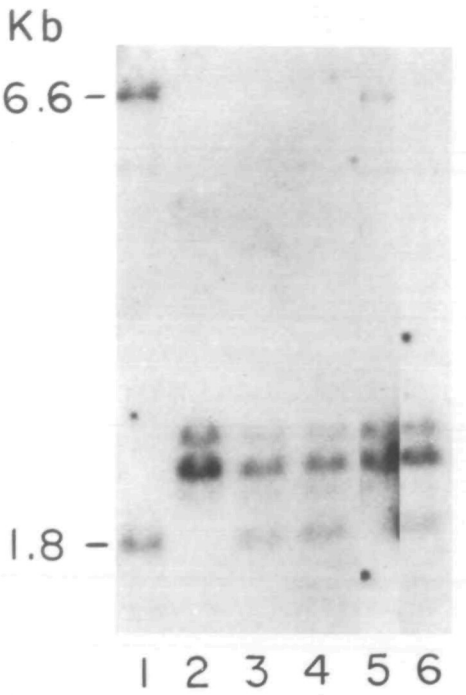


Figure 2. Southern transfer analysis of DNA from rodent–owl monkey (karyotype VI) cell hybrids containing the *ZFX* (1.8-kb fragment) and *ZFY* (6.6-kb fragment) gene loci. Lane 1, owl monkey (male) control; lane 2, mouse control; lanes 3, 4, and 6, hybrid clones containing the 1.8-kb fragment but lacking the 6.6-kb fragment; lane 5, hybrid clone that has the 6.6-kb fragment but not the 1.8-kb fragment.

code a zinc-finger protein and may prove to be the testis-determining factor.[18] The closely related sequences on the HSA Xp chromosome, the X-linked homologue of *ZFY*, are referred to as *ZFX*.[17] The pDP1007 probe and its homologues also have been used in analyzing the sex-determining region on the mouse Y chromosome.[15,18]

Materials and Methods

The human insert of plasmid pDP1007 is a 1.3-kb genomic Hind III fragment purified from phage λOX82. This fragment, which derives from the human Y chromosome, is inserted into the Hind III site of pUC13.

Rodent–owl monkey karyotype VI somatic hybrid cell lines containing various subsets of owl monkey chromosomes, previously used for gene mapping studies,[7] are included here for mapping the owl monkey homologue of pDP1007 DNA sequences. The construction, propagation, and chromosomal identification of these hybrid lines have been described previously.[14]

Southern Transfer Analysis

Cellular DNAs from owl monkey lymphocytes, rodent fibroblast cell lines, and rodent–owl monkey (karyotype VI) somatic cell hybrids were digested to completion with restriction endonuclease EcoRI, separated on an 0.8% agarose gel, transferred to a nylon membrane (Amersham), and hybridized with the ^{32}P-labeled pDP1007 in sodium phosphate buffer (pH 7.2) at 65°C. We subsequently rinsed the membrane in sodium phosphate buffer (pH 7.2) with 1% SDS and exposed it to Kodak XAR-5 film at −70°C with intensifying screens for 1 to 10 days.

Chromosome in situ Hybridization

We cultured leukocytes of male owl monkey karyotypes IV ($2n = 52$), VI ($2n = 49$), and I + VII ($2n = 52$) in F10 nutrient medium supplemented with 15% fetal bovine serum and 1% phytohemagglutinin (Wellcome). We treated the cells with bromodeoxyuridine and harvested them to obtain prometaphase and metaphase spreads as described by Harper and Saunders.[4] We air-dried slides with good mitotic chromosomes for at least 3 days before subjecting them to in situ hybridization treatment.

We labeled the probe to a specific activity of 6–8 × 10^7 cpm/μg DNA using tritiated ^{3}H-TTP, ^{3}H-dATP, and ^{3}H-dCTP (NEN) and the random primer technique of Feinberg and Vogelstein.[3]

We treated slides with good mitotic spreads with ribonuclease, exposed them to a short denaturation treatment with formamide, and hybridized them overnight with 10 ng/slide of the ^{3}H-labeled probe. We then rinsed them to remove the excess probe, coated them with a nuclear track emulsion, and stored them at 4°C in the dark for 7 to 21 days. The slides were then developed, fixed, and air-dried as described previously.[10]

To obtain C-banded chromosomes, we stained the slides with Hoechst and Giemsa stains using a modified procedure described by Lin et al.[5] We treated additional slides by the sodium borate method[1] to obtain G-banded chromosomes. Mitotic spreads on each slide were screened with a Nikon Plan 100 × dry NCG objective. We recorded silver grains in contact with chromosomes from each definable metaphase by tabulating them on an idiogram with the nomenclature designated for owl monkey karyotypes IV, VI, and VII as described previously.[11] In addition, we photographed some mitotic spreads and printed them for confirmational analysis.

Results

DNAs extracted from a total of 34 (16 male, 18 female) owl monkeys of karyotypes I through VII were probed with pDP1007. The data, as illustrated in Figure 1, are consistent with previous findings.[18] Cross-reactivity of the human Y probe pDP1007 with EcoRI-digested DNAs from the 16 male owl monkeys was revealed by the presence of two nonpolymorphic bands of 6.6 kb and 1.8 kb. The 6.6-kb fragment was absent from DNAs prepared from the female owl monkeys and present in those prepared from the male owl monkeys. The intensity of the nonpolymorphic 1.8-kb fragments in the female samples was twice that in the male samples.

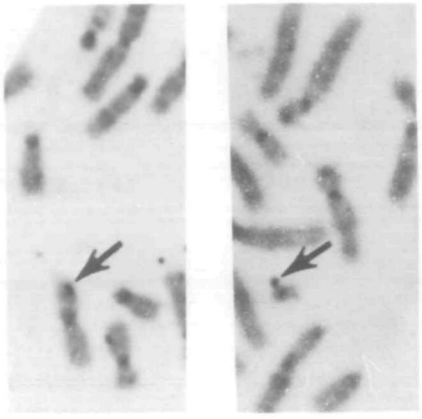
EcoRI-digested DNAs from a panel of 16 rodent–owl monkey (karyotype VI, male) somatic cell hybrids were hybridized with the same probe. We scored them separately for the presence of the 1.8-kb and 6.6-kb fragments (Figure 2), as summarized in Table 1. The 1.8-kb fragment segregated concordantly with the X chromosome. The assignment of the 1.8-kb DNA sequences to the owl monkey X chromosome was confirmed by the high discordancy rate (13% to 69%) with all other chromosomes. Therefore, this 1.8-kb fragment assigned to the X chromosome rep-

Table 1. Segregation pattern of the owl monkey 1.8-kb and 6.6-kb fragments with DNAs from rodent–owl monkey (karyotype VI) somatic cell hybrids containing different subsets of owl monkey chromosomes

Fragment	Segregation pattern	Chromosome 1	2	3	4	5	6	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	X	14/Y
1.8 kb	+/+	9	6	4	8	10	8	6	7	7	6	8	6	4	10	4	3	8	9	11	12	12	8	3	10	13[a]	2
	+/−	4	7	9	5	3	5	7	6	6	7	5	7	9	3	9	10	5	4	2	1	1	5	10	3	0[a]	11
	−/+	1	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	3	0	0	0	0[a]	0
	−/−	2	3	3	3	2	2	3	3	3	3	3	3	3	2	3	3	3	3	3	2	0	3	3	3	3[a]	3
% Discordancy		31	44	56	31	25	38	44	38	38	44	31	44	56	25	56	63	31	25	13	13	25	31	63	19	0	69
6 6 kb	+/+	2	1	1	1	1	0	1	1	1	0	1	1	0	1	1	0	1	1	1	2	1	1	0	1	2	2[a]
	+/−	0	1	1	1	1	2	1	1	1	2	1	1	2	1	1	2	1	1	1	0	1	1	2	1	0	0[a]
	−/+	8	5	3	7	10	9	5	6	6	6	7	5	4	10	3	3	7	8	10	11	14	7	3	9	11	0[a]
	−/−	6	9	11	7	4	5	9	8	8	8	7	9	10	4	11	11	7	6	4	3	0	7	11	5	3	14[a]
% Discordancy		50	38	25	50	69	69	38	44	44	50	50	38	38	69	25	31	50	56	69	69	94	50	31	63	69	0

The number of hybrid cell lines that retained the DNA sequences and chromosome (+/+), retained the DNA sequences but lost the chromosome (+/−), lost the DNA sequences but retained the chromosome (−/+), and lost both the DNA sequences and chromosome (−/−) are indicated.

[a] Independent hybrids showing concordant segregation.



A B

Figure 3. Representative autoradiograms showing hybridization of the pDP1007 probe to (A) the X chromosome and (B) the Y chromosome of owl monkey karyotype IV ($2n = 52$).

resents the owl monkey homologue of the human *ZFX* gene.

The segregation pattern of the 6.6-kb fragment is 100% concordant with owl monkey chromosome 14/Y (karyotype VI). The discordancy rate of all other chromosomes, ranging from 25% to 94%, provides evidence for the assignment of the 6.6-kb fragment to chromosome 14/Y. The 6.6-kb fragment, therefore, represents the owl monkey homologue of the human *ZFY* gene.

In order to identify the precise chromosomal locations of the owl monkey homologues of the *ZFX* and *ZFY* loci, mitotic spreads prepared from a male owl monkey of karyotype IV ($2n = 52$) were hybridized with the labeled probe. One hundred and eighteen silver grains were in contact with chromosomes from a total of 35 mitotic spreads selected for the analysis. Eighteen (15%) silver grains were associated with the X chromosome, with a peak of 12 grains (67%) deposited at the distal end of the short arm. In addition, a second peak of 15 grains (13%) was concentrated on the Y chromosome (Figure 3). Because of its size, no specific site of hybridization could be determined on this tiny metacentric Y chromosome from preliminary data. However, the high percentage of silver grain deposition on the Y chromosome provides evidence for the assignment of the *ZFY* locus to the Y chromosome of owl monkey karyotype IV. No noticeable regions of grain deposition could be defined over the autosomes.

To confirm the chromosomal assignment of the owl monkey *ZFX* locus and to map the *ZFY* locus regionally on chromosome 14/Y (karyotype VI), we probed mitotic spreads from two male owl monkeys of karyotype VI ($2n = 49$) with the tritiated pDP1007 sequences. In situ hybridization with pDP1007 on 169 mitotic cells revealed a total count of 493 silver grains associated with chromosomes. Sixty-eight grains (13.7%) were over the X chromosome, with a peak of 53 grains (78%) deposited at the distal end of the short arm, identical to the site on the X chromosome of karyotype IV. Moreover, a second peak of 86 (17.4%) silver grains was localized on chromosome 14/Y, of which 52 (60%) were deposited on the interstitial C-band positive region of the long arm (Figure 4). The remaining grains were scattered over the other chromosomes; there were no noticeable grain deposition regions. Statistical evaluation of the silver grains indicated that probe pDP1007 hybridized to specific sites on the X and 14/Y chromosomes of owl monkey karyotype VI—precisely, to the distal short arm of the X chromosome and to the interstitial C-band positive region of the long arm of chromosome 14/Y.

The same hybridization procedure was repeated with metaphase spreads from a male owl monkey hybrid (karyotype I × VII) sired by a male Peruvian owl monkey of karyotype VII ($2n = 51$). Results from the analysis of silver grain deposition on 100 metaphase spreads revealed similar findings: two peaks of grain counts, one on the X chromosome and the other on chromosome 17/Y. On the X chromosome, 76% of the grains were deposited on the distal short arm at a site identical to the one reported for karyotypes IV and VI. Concurrently, 70% (24 of 34) of the silver grains localized on chromosome 17/Y (karyotype VII) were concentrated at the C-band positive region of the distal short arm (Figure 5).

Discussion

Analysis of the Southern transfer hybridization of DNAs from 34 owl monkey samples with the human Y-specific probe pDP1007 revealed two nonpolymorphic bands of 6.6 kb and 1.8 kb in the males and only the 1.8-kb fragment in the females. These findings provide additional data supporting an earlier proposal[18] that the 6.6-kb fragment is Y specific and the related 1.8-kb fragment derives from the X chromosome. This interpretation also is confirmed by the segregation of these fragments with owl monkey chromosomes in a panel of rodent-owl monkey (karyotype VI) somatic cell hybrids. The 6.6-kb fragment, the *ZFY* homologue, was assigned to chromosome 14/Y, and the 1.8-kb fragment, the *ZFX* homologue, was mapped on

Figure 4. Idiogram of the silver grain deposition on 169 metaphase spreads of owl monkey karyotype VI ($2n = 49$) after in situ hybridization with the pDP1007 probe. The silver grains are clustered in the short arm of the X chromosome and in the interstitial long arm of chromosome 14/Y.

the X chromosome of owl monkey karyotype VI.

Analysis of the silver grain deposition counts on chromosomes of male owl monkey karyotypes IV, VI, and I × VII revealed a unanimous regional localization of the *ZFX* gene on the distal short arm of the X chromosome. These independent assignments of the *ZFX* locus on the X chromosome of three owl monkey karyotypes confirmed the location of this gene. This is the first X-chromosomal gene that has

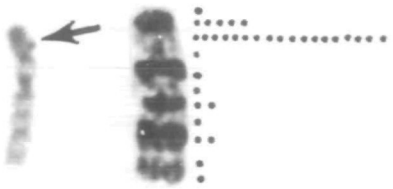


Figure 5. (Right) Distribution of silver grains on chromosome 17/Y from an analysis of 100 metaphase spreads of karyotype VII after in situ hybridization with pDP1007. (Left) C-banded chromosome 17/Y (karyotype VII) with a silver grain indicating the location of the *ZFY* gene locus (arrow).

been regionally localized on both the owl monkey and the human X chromosomes.

The assignment of the homologue of the human *ZFY* gene to the Y chromosome of owl monkey karyotype IV suggested that this conserved gene sequence could be used as a marker for locating the Y-derived material in other owl monkey karyotypes. In owl monkey karyotype VII, the Y-chromosome marker is regionally localized on the distal short arm of chromosome 17/Y at a site that stains positively after C-banding treatment. In owl monkey karyotype VI, the Y-chromosome marker is assigned to the interstitial region of the long arm of chromosome 14/Y, a region that is also C-band positive. These findings provide the first molecular evidence confirming our previous cytological observations that the Y chromosome was translocated to the short arm of autosome 17 in karyotype VII of the Peruvian owl monkey.[12] In karyotype VI of the Bolivian owl monkey, the Y chromosome was inserted interstitially into the long arm of autosome 14 (the homologue of autosome 17 of karyotype VII). Additional assignment of gene markers to autosome 14 (and 14/Y) will be of assistance in supporting or refuting the postulated inversional rearrangement responsible for the interstitial insertion of the Y chromosome or chromosome segment onto this autosome.

Although we localized the Y-specific sequences regionally on autosome 17 (karyotype VII) of the Peruvian owl monkey and homologue 14 (karyotype VI) of the Bolivian owl monkey, it remains unclear how large a portion of the Y chromosome, whether a segment or the entire chromosome, was involved in the translocation. Nevertheless, the identification of the homologous, highly conserved Y-chromosome region in at least two divergent primate species (human and owl monkey) reinforces the conclusion that this region on HSA Yp contains the sex-determining gene(s).

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