

Chromosomal homologies between humans and *Cebus apella* (Primates) revealed by ZOO-FISH

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The chromosome reorganizations that arose during primate evolution have usually been detected by use of banding patterns. The ZOO-FISH technique allows more precise characterization of the chromosome homologies between humans and other non-human primates. This technique is useful when the phylogenetic distance between the species is large and chromosome homologies are difficult to detect by comparing G bands (Sherlock et al. 1996).

The genus *Cebus* (Cebidae, Platyrrhini) has been widely studied from a cytogenetic point of view (Garcia et al. 1983; Matayoshi et al. 1986; Mudry 1990; Ponsà et al. 1995). Results obtained by

comparing the G- or R-banding patterns of this genus and those of other primates allowed us to establish the hypothesis that *Cebus* maintained a primitive karyotype (Dutrillaux and Couturier 1981; Clemente et al. 1990). For this reason, comparison between *Cebus* and the human karyotype is especially interesting.

Homologies between *Cebus capucinus* and human chromosomes have been established by comparing their R-banding patterns (Dutrillaux 1979) and by the ZOO-FISH technique (Richard et al. 1996). Comparison between the G-banding pattern of *Cebus apella* and the human karyotype was also carried out by Clemente et al. (1987) and Borrell (1995). Using ZOO-FISH, we have confirmed the homologies for human Chromosomes (Chrs) 2, 3, 9, and 14 in *C. apella* (Garcia et al. 1999).

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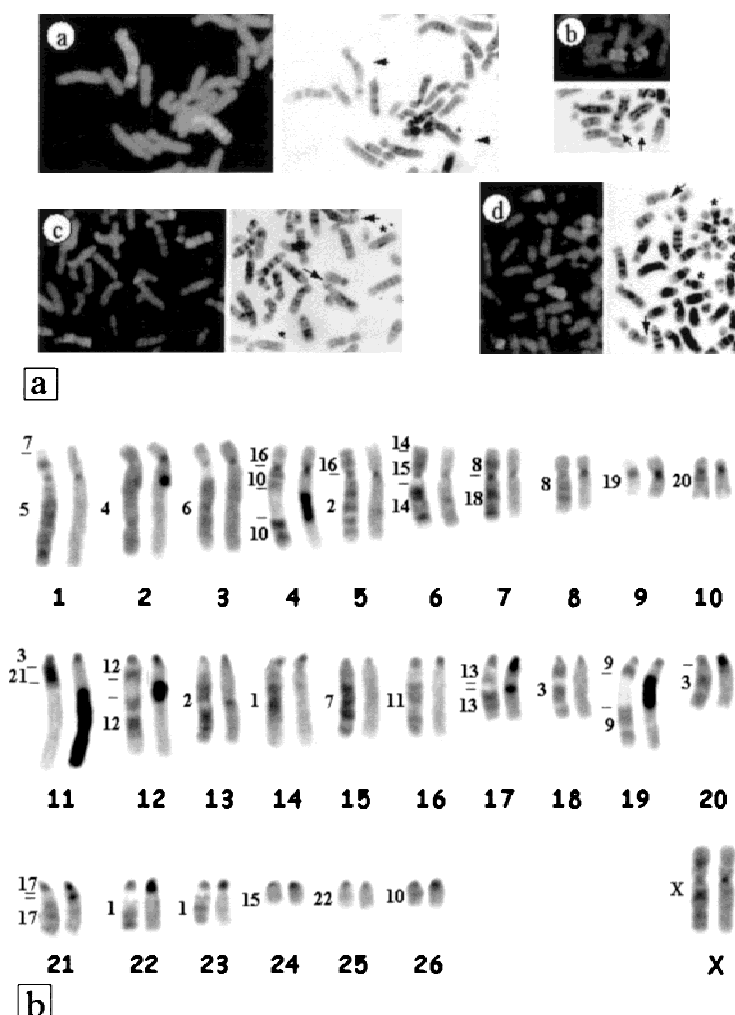


Fig. 1. (a) ZOO-FISH in *Cebus apella* with the probes from human Chrs 5 (a), 19 (b), 7 (c), and 8 (d). Arrows in (a) and (b) indicate a single chromosome pair of *C. apella* painted with human probes from Chrs 5 and 19. Arrows and asterisks in (c) and (d) indicate two chromosome pairs of *C. apella* wholly or partially painted with human probes for Chrs 7 and 8. (b) Composite *Cebus apella* karyotype with sequential G-C bands, with a G-banded chromosome on the left and the same C-banded chromosome on the right. To the left of each G-banded chromosome, the numbers indicate the human probe that hybridizes with each region. Chromosomes were obtained from standard lymphocyte cultures. Sequential ZOO-FISH G-bands have been used to identify *C. apella* chromosomes that had hybridized with each human probe.

Table 1. Chromosome homologies between *Cebus apella* and humans revealed by ZOO-FISH.

Human chromosome	<i>Cebus apella</i> chromosome	Chromosome reorganization
1p	14 ^{a,b}	—
1q	22 ^c	fusion 22qter/23qter
	23 ^{a,c}	—
2p+qprox	5q ^{a,b}	pericentric inversion
2q (except qprox)	13 ^b	pericentric inversion
3p	18 ^{a,c}	paracentric inversion
3qprox*	11qprox ^c	—
3qter	20qter ^{a,c}	—
4	2 ^{a,b}	centromeric shift
5	1 (except pter) ^b	2 paracentric inversions
6	3 ^b	centromeric shift
7 (except qter)	15 ^c	2 pericentric inversions
7qter	1pter ^c	fusion 15qter/1pter
8p	7p ^c	paracentric inversion
8q	8 ^c	pericentric inversion
9	19 ^b	2 pericentric inversions
10p	26 ^{a,c}	—
10q	4qe ^c	paracentric inversion
11	16 ^{a,b}	a pericentric and a paracentric inversion
12	12 ^c	pericentric inversion
13	17 ^c	—
14 (except qprox)	6q (except qprox) ^c	paracentric inversion
14qprox	6pter ^c	pericentric inversion
15 (except qprox)	6qprox+6p (except pter) ^c	—
15qprox	24 ^c	—
16p	4p ^c	—
16q	5p ^{a,c}	—
17	21 ^c	pericentric inversion
18	7q ^b	pericentric inversion
19	9 ^b	—
20	10 ^b	pericentric inversion
21	11qter (except term. heterochromatin) ^b	—
22	25 ^b	—
X	X ^b	—

^a Upside-down.

* See text for more details.

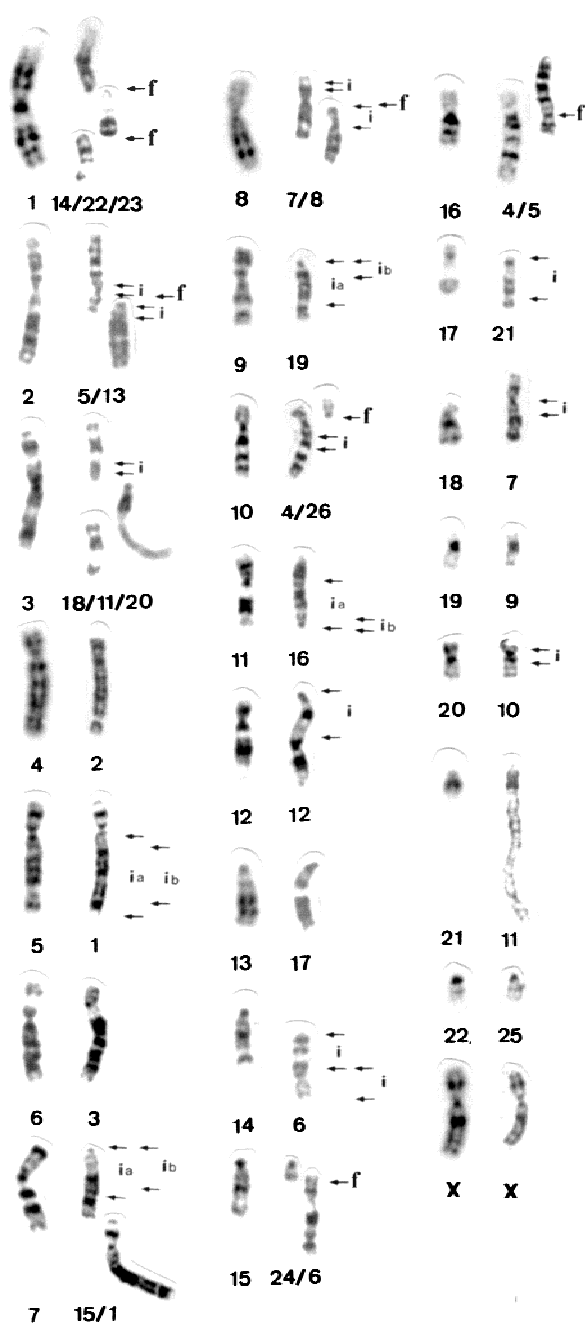
^b Homologies previously detected by G-banding in our laboratory.^c Homologies that have been elucidated by ZOO-FISH (present work).

In this report we describe the analysis of *Cebus apella* chromosomes by ZOO-FISH with probes for each human chromosome. The aims of this work are to establish the chromosome homologies between both species and to detect the chromosome reorganizations that would explain these homologies.

The ZOO-FISH technique has allowed us to establish homologies between human (HSA) and *Cebus apella* (CAP) chromosomes (Fig. 1, Table 1) and to determine three different kinds of relations between human and CAP chromosomes: (a) human chromosomes represented as a whole CAP chromosome: 4, 6, 9, 11, 12, 13, 17, 19, 20, 22, and X; (b) human chromosomes represented as part of a CAP chromosome, but associated with another HSA chromosome: 5, 14, 18, and 21; and (c) human chromosomes represented in more than one CAP chromosome: 2, 7, 8, 10, 15, and 16 (in two CAP chromosomes) and 1 and 3 (in three CAP chromosomes).

Based on the ZOO-FISH and G-banding sequential results, we have proposed the G-banding homologies between CAP and human chromosomes and the chromosomal reorganizations that would explain these homologies (Fig. 2, Table 1).

From the results obtained, we have classified human chromosomes into three different groups: (a) those that do not need any chromosome reorganization to be homologous to CAP chromosomes: 13, 19, 22, and X; (b) those that need only a single chromosome reorganization to be homologous to CAP: 4 and 6 (centromeric shift), 12, 17, and 20 (pericentric inversion), and 21 (fission); and (c) those that need more than one chromosome reorganization to be homologous to CAP: 1, 2, 3, 5, 7, 8, 9, 10, 11, 14, 15, 16, and 18.

**Fig. 2.** Comparison of human and *Cebus apella* G-banded chromosomes. i = inversion; f = fusion/fission

In this report we present, for the first time, the results obtained by applying ZOO-FISH, using all human chromosome probes, on *Cebus apella* (CAP) chromosomes. At present, the only results published applying ZOO-FISH to the genus *Cebus* are in *C. capucinus* (CCA) (Richard et al. 1996). Karyotypes from both species (CAP and CCA) are not identical. Even if they have the same fundamental number ($2n = 54$), they show some differences in three chromosome pairs, which could be explained either by pericentric inversions or by changes in the localization and amount of constitutive heterochromatin (unpublished results). The ZOO-FISH technique could not detect these chromosome reorganizations; therefore, the results in both species are coincident, even if their karyotypes are not identical.

The combined use of ZOO-FISH and G-banding allowed us to confirm homologies that had been previously established in our

laboratory using only G-banding, and to delineate more precisely the breakpoints involved in the evolutionary chromosome rearrangements that explain the homologies between CAP and HSA (Table 1, Fig. 2). The ZOO-FISH technique has been extremely useful to establish the homologies between human Chrs 3, 7, and 11 and CAP chromosomes. These homologies were extremely difficult to determine with only G-banding, owing to the complex reorganizations that have taken place during primate evolution.

In the same way as in other primates (Wienberg and Stanyon 1997), HSA 2 is present in CAP as two different chromosomes (5q and 13; Fig. 1b, Table 1). In the Hominidae (Yunis and Prakash 1982), the Cercopithecidae (Clemente et al. 1990), CAP (present work) and *Saimiri boliviensis boliviensis* (SBB; unpublished results), the fusion between the two pairs of homologous chromosomes to produce HSA 2 would take place in the same band, HSA 2q13.

Human Chrs 4 and 6 are homologous to CAP 2 and CAP 3. These homologies can be explained by a centromeric shift (Fig. 2). This is not the first time that a morphological change in homologous chromosomes of different species can be explained by this mechanism (Dutrillaux et al. 1982; Clemente et al. 1987; Tihy et al. 1996). The presence of latent centromeres that can be activated and inactivated is a well-known phenomenon (Holmquist and Dancis 1980). In addition, the morphology of CAP 2 and 3 chromosomes is similar to that of the chromosomes of *Callithrix jacchus* (CJA), which are also homologous to HSA 4 and 6 (Sherlock et al. 1996). Therefore, in this case, the chromosome reorganization needed to relate HSA 4 and 6 with CJA would also be a centromeric shift.

Human Chr 9 is homologous to a whole chromosome or to a chromosome segment in other primates (Wienberg and Stanyon 1997). HSA 9 is homologous to CAP 19. It must be pointed out that the pericentromeric heterochromatin of HSA 9 seems to be located in the same region in its homolog CAP 19 (in this case, interstitial location). However, the use of *in situ* digestion with restriction enzymes (*AluI*, *HaeIII*, and *RsaI*) shows that this heterochromatin is different in both species (García et al. 1999).

Human Chr 12 is homologous to CAP 12 with a pericentric inversion (Figs. 1b and 2). The same kind of inversion involving the same HSA band would explain the homology between HSA 12 and *Aotus nancymae* 2q (unpublished results). It is not possible to generalize the presence of this inversion in the rest of the platyrrhini, because in SBB, HSA 12 is homologous to Chr 5 (except for the p terminal region that is heterochromatic), without evident chromosome reorganizations (unpublished results).

Human Chr 13 is homologous to CAP 17 without evident chromosomal reorganizations. CAP 17 shows interstitial heterochromatin in the same region that in the chromosome of *Pan troglodytes* (PTR) is homologous to HSA 13. However, the use of *in situ* digestion with restriction enzymes on PTR and CAP chromosomes reveals that this interstitial heterochromatin is different in both species (García et al. 1999).

The chromosome rearrangements detected when comparing CAP and HSA chromosomes are mainly inversions, followed by fusions/fissions, translocations, and centromeric shifts. These kinds of evolutionary reorganizations have also been described by Clemente et al. (1987) and Rumpler and Dutrillaux (1990) as the most frequent reorganizations found in the platyrrhini.

We have found in *C. apella* the following associations: 2/16, 3/21, 5/7, 8/18, 10/16, and 14/15. Two of these associations (3/21 and 14/15) have already been described in other primates and even in other mammals. According to Wienberg and Stanyon (1997), these two associations are ancestral in primates; thus, CAP could also be included in the list of New World monkeys that present these associations in their karyotype. On the other hand, the presence of associations 8/18 and 10/16 is a characteristic that would

link the living New World monkeys (Stanyon 1999), including CAP. Concerning the associations 2/16 and 5/7 found in CAP, they are not present in all platyrrhini; thus, they are not a common character of this group of primates (Stanyon 1999).

Finally, according to our results of sequential ZOO-FISH and G-banding comparison, we can not conclude that all the human euchromatin is represented in CAP as Richard et al. (1996) considered for *C. capucinus*. When G-banding from HSA 3 is compared with the banding pattern of the CAP regions that show hybridization signals with human chromosome 3 probe, the region corresponding to HSA 3 q proximal cannot entirely be found.

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