CAPÍTULO
4

Resultados

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* Par cromosómico nº 4 de *Cebus nigrivittatus*

Trabajo 1

Chromosomal homologies between humans and Cebus apella chromosomes revealed by ZOO-FISH

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Incorporating Mouse Genome

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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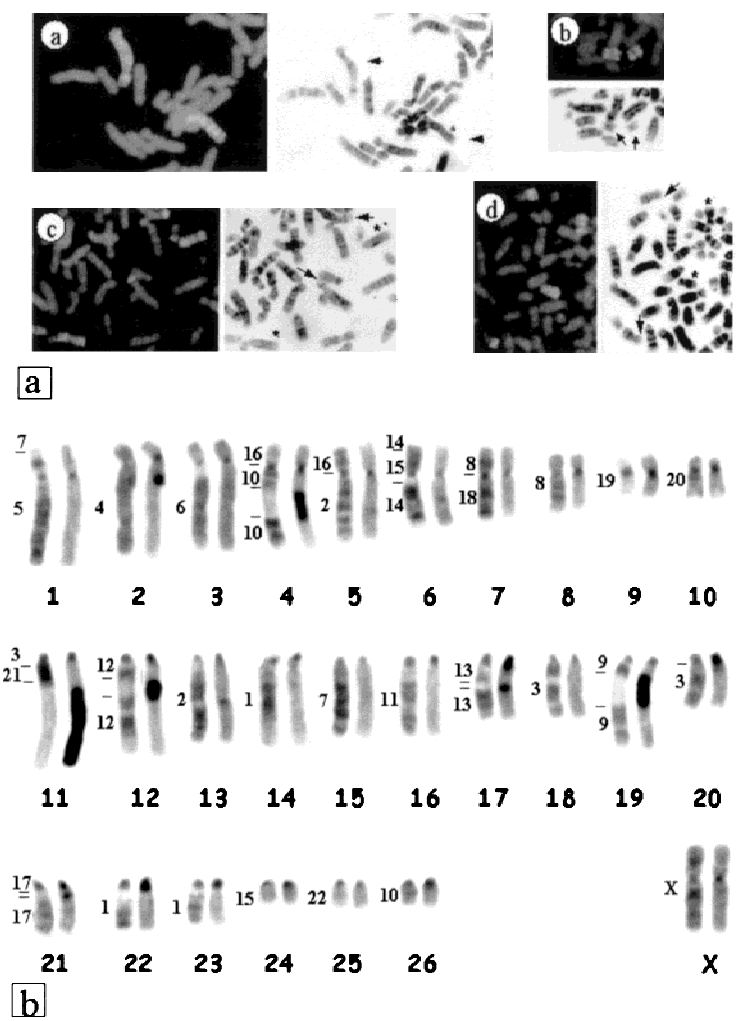

Fig. 1. (a) ZOO-FISH in *Cebus apella* with the probes from human Chrs 5 (a), 19 (b), 7 (c), and 8 (d). Arrows in (a) and (b) indicate a single chromosome pair of *C. apella* painted with human probes from Chrs 5 and 19. Arrows and asterisks in (c) and (d) indicate two chromosome pairs of *C. apella* 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	*Cebus apella* 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]	fusion15qter/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.

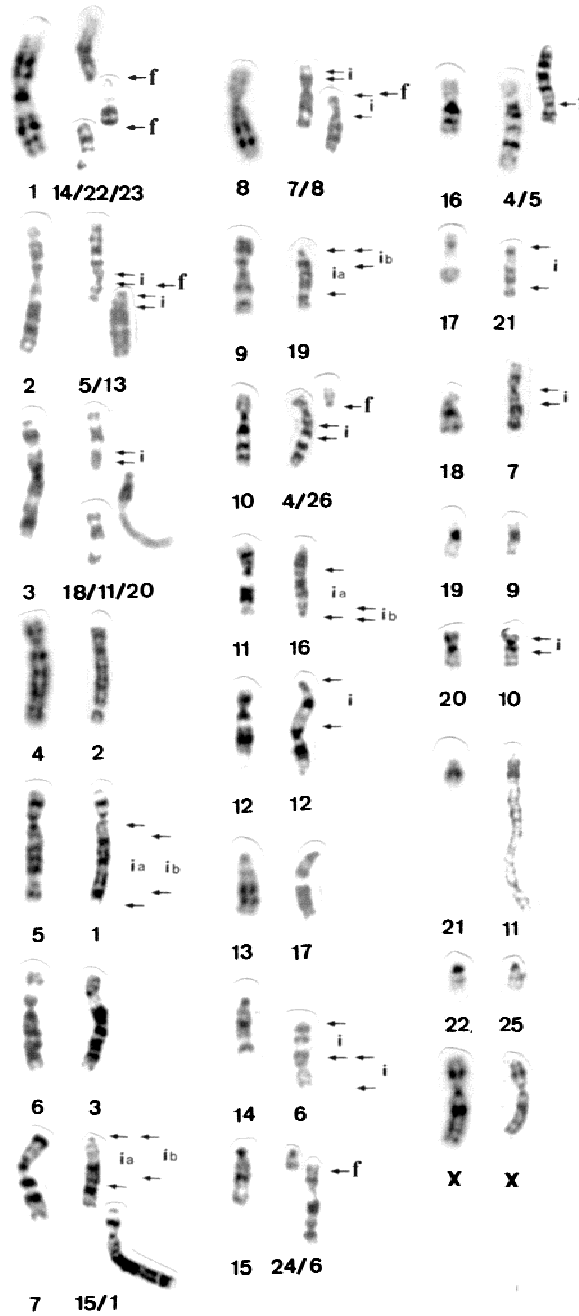

Fig. 2. Comparison of human and *Cebus apella* G-banded chromosomes. i = inversion; f = fusion/fission

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 (Yunish 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 (*Alu*I, *Hae*III, and *Rsa*I) shows that this heterochromatin is different in both species (Garcia 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 (Garcia 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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Trabajo 2

Fragile sites in human and Macaca fascicularis chromosomes are breakpoints in chromosome evolution

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Fragile sites in human and *Macaca fascicularis* chromosomes are breakpoints in chromosome evolution

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Key words: aphidicolin, chromosome, evolution, fragile site, human, *Macaca fascicularis,* Primates

Abstract

We have analysed the expression of aphidicolin-induced common fragile sites at two different aphidicolin concentrations (0.1 μmol/L and 0.2 μmol/L) in three female and one male crab-eating macaques (*Macaca fascicularis*, Cercopithecidae, Catarrhini). A total of 3948 metaphases were analysed: 1754 in cultures exposed to 0.1 μmol/L aphidicolin, 1261 in cultures exposed to 0.2 μmol/L aphidicolin and 933 in controls. The number of breaks and gaps detected ranged from 439 in cultures exposed to 0.1 μmol/L aphidicolin to 2061 in cultures exposed to 0.2 μmol/L aphidicolin. The use of a multinomial FSM statistical model allowed us to identify 95 fragile sites in the chromosomes of *M. fascicularis*, of which only 16 are expressed in all four specimens. A comparative study between the chromosomes of *M. fascicularis* and man has demonstrated that 38 human common fragile sites (50%) are found in the equivalent location in *M. fascicularis*. The analysis of the rearrangements that have taken place during chromosome evolution has revealed that the breakpoints involved in these rearrangements correspond significantly ($p < 0.025$) to the location of *M. fascicularis* fragile sites.

Introduction

Fragile sites are heritable loci, located in specific chromosomal regions, that are expressed as gaps or breaks when the cells are exposed to specific culture conditions or to certain chemical agents, and occur only in a limited number of metaphases, depending on the chemical or the culture conditions used (Sutherland 1979).

Fragile sites are classified according to the frequency with which they are expressed and also according to the culture conditions that induce their expression. Rare fragile sites have a very low frequency of expression and not all chromosomes are affected; they segregate in a Mendelian fashion. Common fragile sites, which are probably homozygous in all individuals, are characteristic of the normal structure of the chromosome (Sutherland & Richards 1999).

Little is known about the molecular structure and functional significance of fragile sites. They have been regarded: as indicative of chromosome instability that may be the basis for structural rearrangements and/or genetic amplifications

(Coquelle *et al.* 1997); as sites for the integration of foreign DNA (Mishmar *et al.* 1998); as breakpoints involved in some human neoplasias (Yunis & Soreng 1984, Yunis *et al.* 1987, Mimori *et al.* 1999); and are usually found in the vicinity of genes implicated in carcinogenesis (Jones *et al.* 1995, Engelman *et al.* 1998). They may also be involved in oncogenesis by producing chromosomal rearrangements under the influence of clastogenic agents (Sutherland & Richards 1999).

The significance of fragile sites in the processes of chromosomal evolution and speciation is uncertain. Comparative studies of rodents and man have shown some conservation of fragile sites between the two groups (Djalali *et al.* 1987). In primates, some inducible fragile sites are also conserved (Yunis & Soreng 1984, Schmid *et al.* 1985, Smeets & Klundert 1990) and some of them have been related to rearrangements known to have taken place during chromosome evolution (Miró *et al.* 1987, Fundia *et al.* 2000). These data suggest that fragile sites may have been conserved and may have participated in evolutionary processes in the same way as some genes or chromosomal regions.

Certain chromosome regions are especially sensitive to ionizing radiation, and are more affected by breaks and chromosome rearrangements than others. *In-vitro* studies of radiation-induced effects on chromosomes in humans (Dutrillaux *et al.* 1983, Kano & Little 1986, Barrios *et al.* 1989) and other primates (Muleris *et al.* 1984, Paravatou-Petsota *et al.* 1985, Borrell *et al.* 1998) show a non-random distribution of induced breakpoints. Many authors have suggested that induced and evolutionary breakpoints coincide with bands where fragile sites are located (Yunis & Soreng 1984, Yunis *et al.* 1987, Barrios *et al.* 1989, Clemente *et al.* 1990).

The expression of fragile sites induced by aphidicolin (APC) in *Macaca fascicularis* (Cercopithecidae, Catarrhini) has been analysed here. This species was chosen because of its cytogenetic characteristics. All *Macaca* species, as well as most other Catarrhini, have extremely stable karyotypes, which differ from the human karyotype by a limited number of simple rearrangements. In this work, the homologies between *M. fascicularis* and human chromosomes are established, the common aphidicolin-induced fragile sites in *M. fascicularis* are described and are compared to bands, previously described in the literature, that show a significantly high number of breaks after X-irradiation. The *M. fascicularis* fragile sites are compared with common fragile sites and bands significantly affected by X-irradiation in humans, and the possible participation of these regions in chromosome evolution is discussed.

Materials and methods

Blood samples

Heparinized peripheral blood samples were taken from three female and one male *Macaca fascicularis,* $2n = 42$

Media and chromosome preparations

RPMI-1640 medium (Gibco), supplemented with phytohaemagglutinin, pokeweed, 25% fetal bovine serum, L-glutamine, penicillin, streptomycin, heparin and Hepes buffer, was used for the blood cultures. A volume of 0.5 ml of each blood sample was cultured in 5 ml of medium for 96 h at 37°C. After 72 h, some cultures were washed in fresh medium, and 25 μl or 50 μl of aphidicolin (0.02 μmol/L dissolved in DMSO) was added to each 5 ml of medium to give a final concentration of 0.1 μmol/L and 0.2 μmol/L, respectively. After 24 h, Colcemid (10 μg/ml) was added to the cultures for the last 20 min. Cells were harvested and chromosomal preparations obtained using a standard protocol.

All specimens were chromosomally characterized using the control cultures, and an idiogram was constructed according to the standard karyotype for *Macaca fascicularis* (Borrell *et al.* 1998). All metaphases were stained homogenously with Leishman solution for the detection of chromosome aberrations (gaps and breaks), and then G-banded with Wright's stain for precise localization of breakpoints. Chromatid and chromosomal breaks and gaps were treated equally as single chromosome events.

Fragile site analysis

Between 200 and 400 metaphases were analysed per specimen for each aphidicolin concentration.

In order to identify which chromosomal bands could be considered fragile sites, an MS-DOS program for the statistical identification of chromosomal fragile sites, FSM (Version 995) was used (Böhm *et al.* 1995, McAllister & Greenbaum 1997). The standardized χ^2 test and G^2 test statistics were used for all chromosomal bands that significantly expressed non-random breaks or gaps ($\alpha = 0.05$). The fragile sites were identified for each of the four specimens analysed.

Fluorescent in-situ *hybridization (ZOO-FISH)*

Whole human chromosome-specific probes (Oncor, Inc.) were used for FISH on *Macaca fascicularis* metaphases. To identify which *M. fascicularis* chromosomes had hybridized with each human probe, G-banding was used after the ZOO-FISH technique (García *et al.* 2000).

Results

Analysis of aphidicolin-induced fragile sites in M. fascicularis

Most of the chromosome abnormalities detected were breaks and gaps (Figure 1). However, other, more complex, chromosome associations were also observed. With the higher dose of aphidicolin (0.2 μmol/L), the number of gaps and breaks per metaphase increased by more than 6-fold (Table 1).

In the cultures treated with 0.1 μmol/L aphidicolin, the number of metaphases analysed per specimen ranged from 347 to 524; 439 breaks and gaps were mapped to 149 different G-bands. In the cultures treated with 0.2 μmol/L aphidicolin, the number of metaphases analysed per specimen ranged from 255 to 372; 2061 breaks and gaps were mapped to 226 different G-bands.

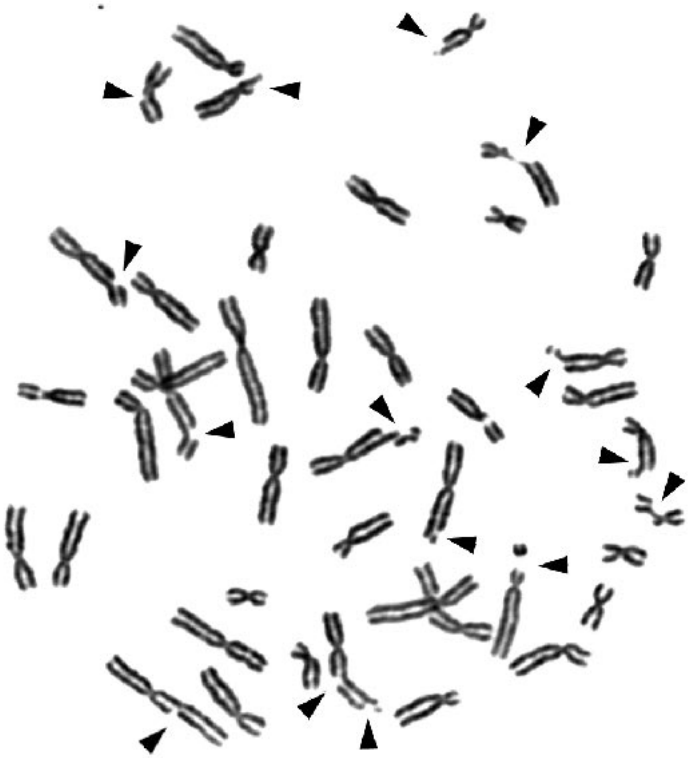

Figure 1. Homogeneously stained metaphase chromosome spread from *Macaca fascicularis* showing chromatid and chromosome breaks and gaps (arrows). Scale bar = 10 μm.

Table 1. Number of metaphases analysed, chromosomal breaks (gaps included) and breaks (gaps included)/metaphase observed in aphidicolin-treated and control cultures from the four *Macaca fascicularis* specimens.

Specimens	Aphidicolin-treated 0.1 μmol/L			Amphidicolon-treated 0.2 μmol/L			Controls		
	Metaphases	Breaks	Breaks/ Metaphase	Metaphases	Breaks	Breaks/ Metaphase	Metaphases	Breaks	Breaks/ Metaphase
1	524	145	0.27	372	471	1.26	262	3	0.01
2	370	174	0.47	330	502	1.52	192	1	0.005
3	513	42	0.08	255	723	2.83	206	4	0.02
4	347	78	0.22	304	365	1.20	273	2	0.01
Total	1754	439	0.25	1261	2061	1.63	933	10	0.01

In control cultures, the number of metaphases analysed per specimen ranged from 192 to 272, and the total number of breaks and gaps was 10.

To determine which breaks or gaps could be regarded as fragile sites, the statistical program FSM (Version 995) was applied to the breaks and gaps detected after exposure to both 0.1 μmol/L and 0.2 μmol/L aphidicolin (Table 2). For each data set, FSM analysis provides a critical value C_α (the minimum number of breaks needed for a site to be considered as fragile in that given data set). This value was 3 for cultures treated with 0.1 μmol/L aphidicolin, and 3–4 for cultures treated with 0.2 μmol/L aphidicolin.

The number of fragile sites detected per specimen ranged from 4 to 14 in cultures treated with 0.1 μmol/L aphidicolin, and a total of 20 sites were regarded as fragile (Table 2). In cultures treated with 0.2 μmol/L aphidicolin, the number of fragile sites detected per specimen ranged from 34 to 53, and a total of 95 sites were regarded as fragile (Table 2, Figure 2). All fragile sites detected in cultures treated with 0.1 μmol/L aphidicolin were also detected in cultures treated with 0.2 μmol/L aphidicolin. For this reason, the sites detected after treatment with 0.2 μmol/L aphidicolin will be considered as fragile sites for *M. fascicularis*. Among them, 16 sites (1p35, 1q22, 2q22, 2q24, 3q21, 3q22, 3q23, 3q32, 4q31, 5q33, 6p14, 7q25, 18q23, 20q15, Xp22 and Xq22) were observed in all four specimens, and 47 were found only in one specimen. Fragile sites were found in all chromosomes except the Y (Figure 2).

Results of ZOO-FISH and comparison with G bands

The homologies between the chromosomes of *M. fascicularis* and those of man have been established using ZOO-FISH technique. The results are in agreement, except for the Y chromosome, with those observed by Wienberg *et al.* (1992) in *M. fuscata.* Using the ZOO-FISH technique combined with G-banding it has been possible to characterize the rearrangements that differentiate both karyotypes and to identify the bands in which the breakpoints were located (Figure 3, Table 3). The chromosome homologies between MFA and human, and the rearrangements involved, are listed in Table 3. In MFA, in contrast to *M. fuscata,* there is no cross-hybridization of HSA Y, indicating that it corresponds only to MFA Y.

The ZOO-FISH technique has allowed us to identify three different kinds of relationships between human and MFA chromosomes: (1) human chromosomes (1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19 and X) represented as a whole MFA chromosome; (2) MFA chromosomes that contain material from two human chromosomes (7/21, 15/14, 20/22); and (3) human chromosomes homologous to more than one MFA chromosome (HSA 2 contains material from two MFA chromosomes).

Based on ZOO-FISH and G-banding results, human chromosomes can be classified into three different groups: (1) those that are homologous

Table 2. Chromosome breaks and gaps at sites determined to be fragile (FSM analysis, χ^2 test, G^2 test, $\alpha = 0.05$) in cultures treated with 0.1 μmol/L and 0.2 μmol/L aphidicolin (APC) in all specimens of *M. fascicularis* analysed in this study.

Bands	0.1 μmol/L APC	0.2 μmol/L APC
1p35	3	30
1q11	—	3
1q14	—	8
1q22	8	72
1q32	—	11
2p23	—	6
2p22	—	12
2q11	—	4
2q13	5	17
2q15	4	20
2q18	—	7
2q22	39	128
2q23	4	12
2q24	29	129
2q25	4	11
3p21	—	11
3q12	—	9
3q13	—	7
3q21	19	78
3q22	6	87
3q23	3	29
3q25	—	5
3q32	5	61
4p24	—	8
4p21	—	6
4q21	—	6
4q23	—	6
4q24	—	4
4q31	—	31
4q32	—	8
5p14	—	11
5p13	—	10
5p12	—	6
5q17	—	25
5q21	—	10
5q31	—	4
5q33	—	15
5q34	—	13
5q35	—	4
6p14	11	110
6q14	—	13
6q23	—	15
7q13	—	3
7q14	—	6
7q21	—	6
7q23	—	17
7q25	4	35
8q22	—	11
8q24	—	6
9q12	—	6
9q16	—	7
9q22	—	15
9q23	—	4
9q24	—	10
10q12	—	7
10q14	—	12
10q23	—	13
11p21	—	15
11p13	—	6
11p12	—	12
11q21	—	12
12p12	—	7
12q11	—	8
12q12	—	5
12q13	—	8
13p23	—	12
13p12	—	7
13q12	—	6
13q21	—	8
13q23	—	21
14q22	—	11
15q21	—	6
15q23	—	14
15q25	—	6
16p15	3	4
16p13	—	7
16q13	—	11
16q23	—	12
16q24	3	7
17q14	3	12
17q16	—	9
18q12	—	9
18q13	—	11
18q22	—	9
18q23	—	41
19q15	—	8
20q12	—	11
20q15	21	80
20q16	—	7
Xp22	5	37
Xq12	—	7
Xq22	15	51
Xq23	—	7
Xq24	—	14
Xq26	—	4

to MFA chromosomes without any chromosome rearrangements (5, 8, 12, 16, 17 and 19); (2) those that need only a single rearrangement to be homologous to MFA chromosomes (1, 9, 11 and 18 [pericentric inversion], 2 [fusion], 4, 6 and 13 [centromeric shift], 10 [paracentric inversion], 14 and 15, 21 and 22 [fission]); and (3) those that need more than one chromosome rearrangement to be homologous to MFA (3, 7 and 20).

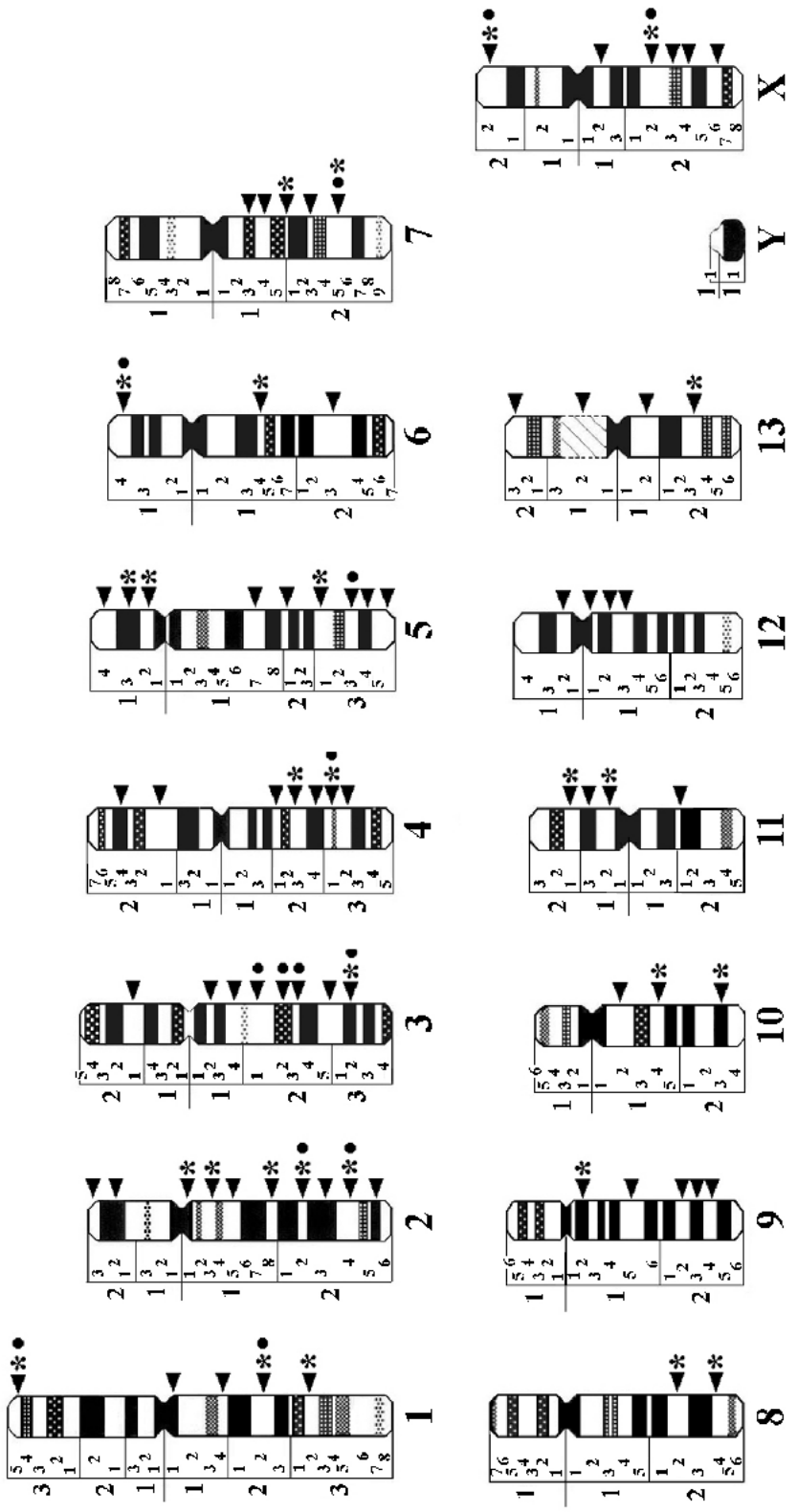

Figure 2. Idiogram of *Macaca fascicularis* chromosomes showing the location of fragile sites (arrowheads) found in one or more specimens. * Fragile sites in MFA homologous to HSA fragile sites; ● fragile sites in all four specimens.

Discussion

In this report, the inter- and intrachromosomal rearrangements that explain the homologies between the chromosomes of *M. fascicularis* and those of man are described (Table 3; Figure 3). The combined use of ZOO-FISH and G-banding allowed the characterization of 11 intrachromo-

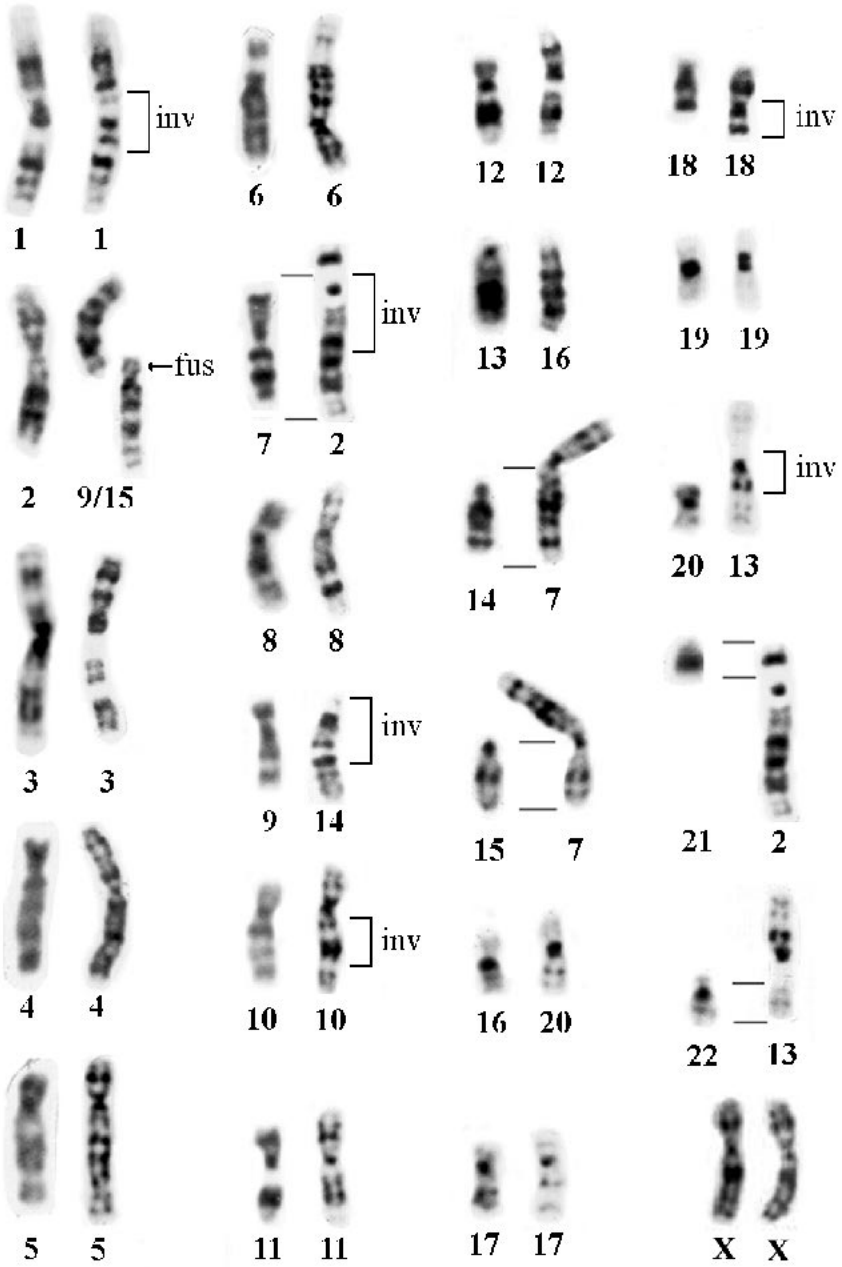

Figure 3. Comparison of human and *Macaca fascicularis* G-banded chromosomes. Homologies were established by ZOO-FISH and breakpoints located by comparison with G-bands. *Macaca fascicularis* chromosomes are on the right and human chromosomes on the left. inv = inversion; fus = fusion.

Table 3. Chromosome homologies between *Macaca fascicularis* and humans revealed by the combined use of ZOO-FISH and G-banding.

Human chromosome	*Macaca fascicularis* chromosome	Chromosome rearrangement	*Macaca fascicularis* chromosome band
1	1[a]	Pericentric inversion	1q14[b];1p12
2p	9[a]	Fusion	9p15
2q	15	Fusion	15p13
3	3	Centromeric shift and unidentified structural rearrangements	3q13[b]
4	4	Centromeric shift	4p21[b]
5	5	—	
6	6[a]	Centromeric shift	6q15
7	2q+(2cen/2p12)	Pericentric inversion	2p12;2q18[b]
8	8	—	
9	14	Pericentric inversion and other unidentified rearrangements	14q22[b];14pter
10	10	Paracentric inversion	10q12[b];10q14[b]
11	11	Pericentric inversion	11p12;11q12
12	12	—	
13	16[a]	Centromeric shift	16qter
14	7q	Fission	
15	7p[a]	Fission	
16	20	—	
17	17	—	
18	18[a]	Pericentric inversion	18q12[b];18pter
19	19	—	
20	13q+(13cen/13p12)	Pericentric inversion	13q23[b];13p12
21	2p12/2pter	—	
22	13p12/13pter[a]	—	
X	X	—	

[a]Upside-down.
[b]Chromosome bands that contain fragile sites (FSM analysis).

somal rearrangements (6 pericentric inversions, 1 paracentric inversion and 4 centromeric shifts), and 4 interchromosomal rearrangements (1 fusion and 3 fissions). The chromosome rearrangements detected in *M. fascicularis* are mainly intrachromosomal rearrangements, such as inversions, followed by centromere activation or inactivation. The breakpoints involved in the rearrangements described are listed in Table 3.

Evolutionary conservation of fragile sites

We have described, for the first time, the common aphidicolin-induced fragile sites in *M. fascicularis*. Out of 95 common fragile sites mapped in the chromosomes of MFA, 38 (40%) coincide with common fragile sites mapped to the equivalent site in their HSA homologue (Human Gene Mapping 11, 1991), and 38 of the 76 common fragile sites (50%) described in the literature for HSA have also been found in MFA (Table 4, Figure 2). The χ^2 test indicates that the coincidence of the location of MFA common fragile sites with the location of human ones is significant ($p < 0.005$).

Relationship between fragile sites and bands affected by X-irradiation

Certain chromosomal regions are preferentially involved in the genotoxic effects (breaks, gaps, translocations, dicentrics) produced by ionising radiation (Dutrillaux *et al.* 1983, Kano & Little, 1986, Barrios *et al.* 1989). Thirteen (65%) of the 20 bands in the human karyotype that show a significant number of X-ray-induced breaks (Barrios *et al.* 1989) correspond ($p < 0.005$) with the location of fragile sites in human chromosomes (Table 5).

In *M. fascicularis*, Borrell *et al.* (1998) described 19 bands which are significantly affected by

Table 4. Homologies between human aphidicolin-inducible common fragile sites (HSA c-FS) and *Macaca fascicularis* common fragile sites (MFA c-FS) and the relationship between these fragile sites and the bands involved in chromosome evolution.

HSA c-FS (Human Gene Mapping 1991)	MFA c-FS	Evolutionary change	Reference
1p32[a]	1q32	cen CNI7	Clemente *et al.* 1990
1p31	—	cen MTA2	Clemente *et al.* 1990
1p22[a]	1q22		
1q21	—	inv PSP1, CNI1, EPA1	Clemente *et al.* 1990
		inv PTR1	Yunis & Prakash 1982
		inv MFA1	
1q42	1p35		
2p13	9q12	cen LLA2	Miró *et al.* 1987
2q21.3	—	cen PSP9, CNI8, EPA 3, MTA5, CMC3, CPE5, CCE4	Clemente *et al.* 1990
		cen PTR2, GGO2	Yunis & Prakash 1982
		cen MFA15	
2q33[a]	15q21[b]		
2q37.3	15q25		
—	3p21[b]		
3q37	3q32		
4p16.1	—		
4q27	4q23		
4q31.1	4q31		
5p13	5p12	inv PTR5	Yunis & Prakash 1982
5p14	5p13		
5q31.1[a]	5q31		
6q25.1	6p14		
6q15	6q14		
—	6q23[b]		
7p22[a]	2q18	inv MFA2	
7p13	2q13		
7p14.2	—		
7p11.2	2q11		
7q11[a]	—	inv MFA2	
7q22	2q22		
7q32.3[a]	2q24		
8q22	8q22	inv GGO8	Yunis & Prakash 1982
8q24.1[a]	8q24		
9q22.1	—	inv PTR9	Yunis & Prakash 1982
10q22	10q14	inv MFA 10	
10q25	10q23		
11p13	11p21		
11q13[a]	11p12	inv PSP11, CNI15, EPA16, MTA15, CMC4, CPE8	Clemente *et al.* 1990
		inv PPY11	Yunis & Prakash 1982
		inv MFA11	
—	12p12[b]		
13q13.1	16q23		
13q21.1	16q13		
13q32	16p13		
—	7q23[b]		
14q21	7q21	inv GGO14	Yunis & Prakash 1982
14q24.1	7q25		
16q22.1	20q15		
16q23.2	20q16		
17q23.1	17q14	inv PTR17	Yunis & Prakash 1982
18q12.3	18q22		

Table 4. continued

HSA c-FS (Human Gene Mapping, 1991)	MFA c-FS	Evolutionary change	Reference
18q21.3	18q12	inv MFA18	
20p12.2	13q23		
Xp22.31	Xp22		
Xq22.1	Xq22		

Abbreviations: cen, centromere; inv, inversion; CAL, *Cebus albifrons*; CAP, *Cebus apella*; CCE, *Cercopithecus cephus*; CMC, *Cercopithecus mona campbelli*; CNI, *Cercopithecus nictitans*; CPE, *Cercopithecus petaurista*; EPA, *Erythrocebus patas*; GGO, *Gorilla gorilla*; LLA, *Lagothrix lagothricha*; MFA, *Macaca fascicularis*; MTA, *Miopithecus talapoin*; PTR, *Pan troglodytes*; PSP, *Papio sphinx*; PPY, *Pongo pygmaeus.*

[a]Human chromosome bands with a significantly high number of breaks after X-irradiation (Barrios *et al.* 1989).

[b]*Macaca fascicularis* chromosome bands with a significantly high number of breaks after X-irradiation (Borrell *et al.* 1998).

Table 5. Comparison between chromosome bands with a significantly high number of breaks after X-irradiation and common fragile sites (c-FS) in humans (HSA) and *Macaca fascicularis* (MFA).

HSA bands (Barrios *et al.* 1989)	HSA c-FS (Human Gene Mapping 1991)	MFA bands (Borrell *et al.* 1998)	MFA c-FS
1p36	+	1p12	−
1p32	+	1q36	−
1p22	+	2q16	−
1p13	−	3p25	−
1q21	+	3p21	+
1q32	−	4p27	−
2q33	+	5q11	−
3q21	−	5q15	−
5q31	+	6q23	+
7p22	+	7p12	−
7q11	+	7q23	+
7q32	−	8p16	−
8q24	−	10p16	−
9q32	+	12p12	+
9q34	−	13p23	−
11p15	+	13q26	−
11q13	+	15q21	+
12q24	+	17p14	−
14q32	+	19p12	−
17q25	−		

X-irradiation ($p < 0.001$). If these data are compared with the fragile sites described in this work, only five fragile sites detected in MFA (3p21, 6q23, 7q23, 12p12, 15q21) correspond to chromosome bands affected by X-irradiation and, in contrast to what happens in humans, these bands are not significantly colocalized with fragile sites in MFA (Table 5). If human and MFA chromosomes are compared, only a single band containing a fragile site and significantly affected by X-irradiation (MFA15q21 and HSA2q33) is conserved in both species (Table 4).

Fragile sites involved in evolutionary chromosome rearrangements

Nine of the 17 MFA chromosome bands involved in evolutionary rearrangements correspond to fragile sites (Table 3). The χ^2 test indicates that the breakpoints involved in evolutionary

rearrangements correspond to fragile sites ($p < 0.025$).

Table 4 shows the fragile sites found in human chromosomes that are also found in their homologues in MFA, and the evolutionary rearrangements in which these fragile sites are involved. The data show that:

(1) Twenty-one HSA fragile sites are found as fragile sites in MFA chromosomes, but are not implicated in evolutionary rearrangements and are not significantly affected by X-irradiation (Table 4).

(2) Five human fragile sites (1p22, 2q33, 5q31.1, 7q32.3, 8q24.1) are found as fragile sites in MFA chromosomes and are also affected significantly by X-irradiation but are not implicated in evolutionary rearrangements (Table 4).

(3) Three human fragile sites are found as fragile sites in MFA chromosomes, are affected significantly by X-irradiation and are implicated in evolutionary rearrangements. These are HSA bands 1p32, 7p22 and 11q13 (Table 4). HSA 11q13 corresponds to a breakpoint involved in inversions in seven species of the family Cercopithecidae and in one hominid (*Pongo pygmaeus*).

(4) Seven human fragile sites are found as fragile sites in MFA chromosomes, are implicated in evolutionary rearrangements but are not significantly affected by X-irradiation. These are HSA bands 2p13, 5p13, 8q22, 10q22, 14q21, 17q23.1 and 18q21.3 (Table 4).

(5) Three human fragile sites (1q21, 2q21.3, 4p16.1) are implicated in evolutionary rearrangements, but are not found as fragile sites in MFA chromosomes and are not significantly affected by X-irradiation. HSA 2q21.3 is considered as an ancestral centromere in eight species of Cercopithecidae and in two species of Hominidae (Table 4).

In conclusion, our data suggest that there is a significant correspondence between human and MFA fragile sites. It is possible that the structural characteristics that are responsible for the susceptibility to breakage of a given region, in response to the effect of chemical agents such as aphidicolin, are conserved during evolution.

Most HSA fragile sites that coincide with bands implicated in evolutionary rearrangements in primates are also fragile sites in the homologous chromosomes of MFA. In MFA, the breakpoints implicated in the evolutionary rearrangements needed to produce HSA chromosomes are preferentially located in fragile sites, in contrast to what happens in chromosome bands preferentially affected by X-irradiation. As a consequence, fragile sites can be considered as 'targets' for evolutionary rearrangements because of their special tendency to break and reorganise. However, a comparison of these data with other primate groups located in different branches of the evolutionary tree of primates will be needed to support or reject such a conclusion.

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Trabajo 3

Distribution of intrachromosomal telomeric sequences (ITS) on Macaca fascicularis chromosomes and their implication for chromosome evolution

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ORIGINAL INVESTIGATION

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Distribution of intrachromosomal telomeric sequences (ITS) on *Macaca fascicularis* (Primates) chromosomes and their implication for chromosome evolution

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Abstract The intrachromosomal location of the telomeric sequence in the crab-eating macaque, *Macaca fascicularis* (F. Cercopithecidae, Catarrhini) has been analysed by fluorescent in situ hybridisation with a long synthetic $(TTAGGG)_n$ probe. A total of 237 metaphases was analysed. As expected, all telomeres hybridised with the probe and 90 intrachromosomal loci with different hybridisation frequencies were also detected. The chromosomal location of interstitial telomeric sequences in *M. fascicularis* and in *Homo sapiens* was then compared, 37 sites (41.11%) being found to be conserved. Some of these sequences can be derived from rearrangements, such as inversions (MFA13q23) or fusions (MFA2p13 and MFA13p12), that have taken place during karyotype evolution.

Introduction

Telomeric DNA in vertebrate chromosomes is a variable tandem-repeated nucleotide sequence $(TTAGGG)_n$ (Meyne et al. 1989). In 1990, Meyne et al. described the presence of non-telomeric sites of the (TTAGGG)*n* sequence, named ITS (interstitial telomeric sequences), in 100 different species by using in situ fluorescence hybridisation. Since then, ITS have been described in various species of mammals (Wurster-Hill et al. 1988; Scherthan 1990; Lee et al. 1993; Vermeesch et al. 1997; Bertoni et al. 1996; Thomsen et al. 1996; Garagna et al. 1997; Metcalfe et al. 1997, 1998; Fagundes and Yonenaga-Yassuda 1998; Silva and Yonenaga-Yassuda 1998; Svartman and Vianna-Morgante 1998; Liu and Fredga 1999; Finato et al. 2000; Pagnozzi et al. 2000; Go et al. 2000; Lear 2001), amphibians (Wiley et al. 1992), reptiles (Schmid et al. 1994; Pellegrino et al. 1999), fish (Abuín et al. 1996) and birds (Nanda and Schmid 1994).

Some hypotheses regarding the origin of intrachromosomal telomeric $(TTAGGG)_n$ sequences have been proposed that are not mutually exclusive: (1) telomerase having a role in the repair of double-strand breaks introducing telomeric arrays (Flint et al. 1994; Azzalin et al. 2001), (2) differential crossing-over or genic amplification (Wiley et al. 1992), (3) chromosome integration of extrachromosomic segments or transposons with telomeric sequences (Cherry and Blackburn 1985), (4) telomeres, which have lost their function, in a non-terminal position after intrachromosomal rearrangements, as described in some human genetic disorders (Park et al. 1992; Rossi et al. 1993; Vermeesch et al. 1997; Devriendt et al. 1997) and (5) remnants of ancestral chromosomal rearrangements (inversions and fusions) produced during karyotype evolution (Ijdo et al. 1991; Lee et al. 1993; Vermeesch et al. 1996; Thomsen et al. 1996; Metcalfe et al. 1997, 1998; Fagundes and Yonenaga-Yassuda 1998; Pellegrino et al. 1999; Go et al. 2000; Finato et al. 2000). This last possibility has been used to explain the presence of ITS in human chromosome 2q13, which is the result of a telomere-telomere fusion of two ancestral chromosomes (Wells et al. 1990; Ijdo et al. 1991). In 1997, Azzalin et al. described interstitial hybridisation signals in all human chromosomes by using a 1–20 kb large synthetic polynucleotide probe $(TTAGGG)_n$.

In *Macaca fascicularis* (MFA; F. Cercopithecidae, Catarrhini), a primate with a stable karyotype, the characterisation of intra- and interchromosomal rearrangements that relate human and macaque chromosomes (Ruiz-Herrera et al. 2002) with other primate species shows that, in this species, the presence of intrachromosomal $(TTAGGG)_n$ sequences might be the consequence of the evolution of

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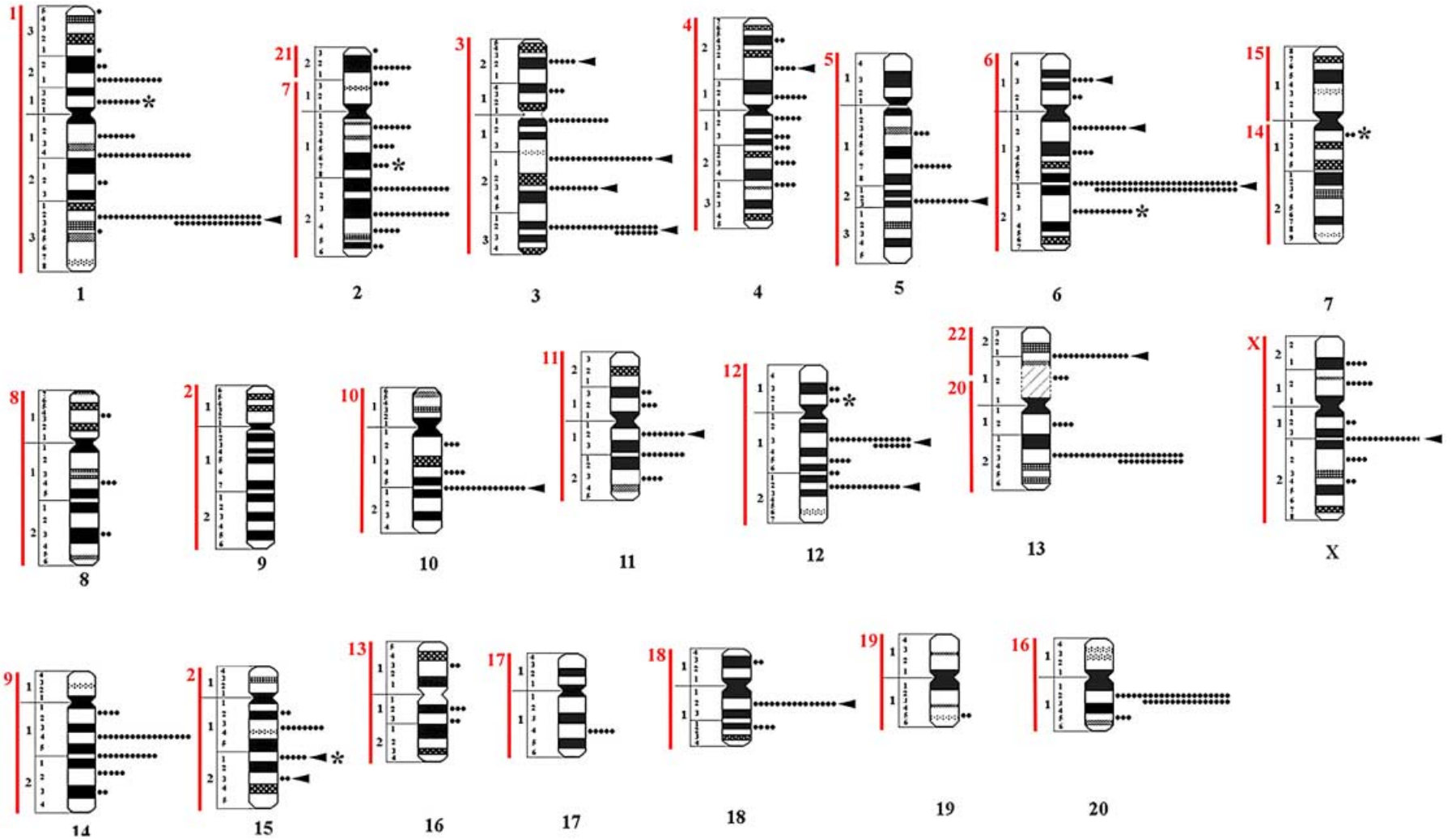

Fig. 1 Idiogram of *Macaca fascicularis* (MFA) chromosomes illustrating the location of ITS (intrachromosomal telomere-like sequences). Each *dot* shows a hybridisation signal in both chromatids. *Arrowheads* Coincidence with FSs (fragile sites) in MFA chromosomes described by Ruiz-Herrera et al. (2002), *asterisks M. fascicularis* chromosome bands with a significantly high number of breaks after X-irradiation (Borrell et al. 1998). Homology with human chromosomes is shown *left*

chromosomal rearrangements that had occurred in the early evolution of primates. We have therefore analysed the intrachromosomal position of telomere-like repeats in *M. fascicularis* by using fluorescent in situ hybridisation techniques; the conservation of the location of ITS in human and macaque chromosomes and the possible origin of these sequences from the point of view of chromosomal evolution is discussed. The relationship between ITS, fragile sites and bands significantly affected by X-irradiation has also been studied.

Materials and methods

Cell culture and chromosome preparations

Heparinised peripheral blood samples were taken from two unrelated *M. fascicularis* females, (2*n*=42; Centro de Investigación y Desarrollo Aplicado, Barcelona, Spain).

RPMI-1640 medium (Gibco) supplemented with phytohaemagglutinin, pokeweed, 25% fetal bovine serum, L-glutamine, penicillin, streptomycin, heparin and HEPES buffer was used for blood cultures. A volume of 0.5 ml of each blood sample was cultured in 5 ml medium for 72 h at 37°C. Colcemid (10 μg/ml) was added to the cultures for the last 20 min. Cells were harvested and chromosomal preparations obtained by using a standard protocol.

All specimens were chromosomally characterised and an ideogram was constructed according to the standardised karyotype for *M. fascicularis* (Borrell et al. 1998). Homologies between *Macaca* and humans have been established by ZOO-FISH (Wienberg et al. 1992; Ruiz-Herrera et al. 2002) and G-banding comparison (Ruiz-Herrera et al. 2002).

Fluorescent in situ hybridisation

Fluorescent in situ hybridisation (FISH) experiments were performed as previously described (Azzalin et al. 1997). Chromosomes were hybridised with a non-commercial biotin-labelled telomeric probe, i.e. a mixture of synthetic $(TTAGGG)_n$ polynucleotides. Slides were treated with RNase (100 μg/ml) in 2×SSC (1×SSC= 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at 37°C, pepsin-digested (0.005% in 10 mM HCl at 37°C), post-fixed (4% paraformaldehyde in phosphate-buffered saline plus 50 mM $MgCl_2$) and denatured in 70% formamide/2×SSC at 75°C. In situ hybridisation with the probe was carried out overnight at 37°C and the slides were washed three times in 25% formamide/4×SSC at 37°C and three times in 2×SSC at 37°C. The probe and methodology applied were the same as those in Azzalin et al. (1997). Detection was performed and results were interpreted based on the same criteria and in the same laboratory as Azzalin et al. (1997). Metaphases were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 100 ng/ml) and observed with a Zeiss Axioplan microscope equipped with a cooled charge-coupled device camera system. The G-banding pattern was generated by using the DAPI DNA counterstain.

ITS have been classified into three groups according to the frequency of hybridisation (Azzalin et al. 1997): (1) very frequent (19 times or more), (2) frequent (5–18 times) and (3) rare (less than 5 times). Only double spots (hybridisation signals on both chromatids) have been taken into account; since single spots cannot be distinguished from the background, they have been discarded.

Results

A total of 237 metaphases, 59 from one specimen and 178 from the other, has been analysed. All telomeres exhibited hybridisation signals with the telomeric $(TTAGGG)_n$ probe. A total of 90 ITS was found in almost all chromosomes (with the exception of chromosome MFA9) with various hybridisation frequencies, 40 of them being frequent or very frequent. The different hybridisation frequencies were probably related to the different number of tandem repeats of the $(TTAGGG)_n$ sequence present in each locus. The distribution of double spots observed is shown in Fig. 1 and examples of the images obtained can be seen in Fig. 2.

Very frequent signals, i.e. those that hybridised 19 or more times with the telomeric probe, were located in seven different chromosome bands: 1q32 (42 times), 3q21 (19 times), 3q32 (28 times), 6q21 (52 times), 12q13 (22 times), 13q23 (32 times) and 20q12 (33 times). Frequent signals, which hybridised 5–18 times, were located in 33 different chromosome bands: 1p21, 1p12, 1q12, 1q14, 2p22, 2q12, 2q21, 2q23, 2q24, 3p22, 3q11, 3q23, 4p11, 4q12, 5q17, 5q23, 6q12, 6q21, 6q23, 10q21, 11q12, 11q21, 12q22, 13p21, 14q14, 14q21, 14q22, 15q13, 15q21, 17q14, 18q12, Xp12 and Xq21. Rare signals, which hybridised fewer than 5 times with the telomeric probe, were found at 50 different sites (Fig. 1).

As seen in Fig. 1, the number of ITS varies between chromosomes. Some chromosomes have a high number of ITS, such as chromosomes MFA1 and MFA2 (both with 10 ITS), and others lack ITS, such as MFA9, or have a low number (1 to 3) of ITS, such as MFA7, MFA8 and MFA17.

The distribution of ITS according to chromosome position appeared to be random: 33.33% were located in the central region, 42.22% in the mid-region of the chromosomes and 24.44% in the distal region. According to G-banding, 60% of ITS were present in G+ bands and 40% were present in G– bands.

When the position of MFA ITS was compared with the localisation of chromosome bands significantly affected by X-irradiation (described by Borrell et al. 1998), six out of 19 MFA bands significantly affected by X-irradiation (31.57%) coincided with ITS in MFA (MFA1p12, MFA2q17, MFA6q23, MFA7q12, MFA12p12 and MFA15q21; Fig. 1).

Cytogenetic studies on aphidicolin-induced fragile sites in MFA have also been performed in our laboratory (Ruiz-Herrera et al. 2002). Of 95 common fragile sites mapped in the MFA chromosomes, 45 (47.37%) coincide with ITS located in the same band in MFA (Fig. 1).

The location of ITS in MFA was also compared with ITS previously described in the human karyotype (Azzalin et al. 1997) to study whether these sequences had been conserved during evolution. The karyotype of *Homo sapiens* (HSA) has 103 ITS sites distributed in all chromosomes, with a different hybridisation frequency: two very frequent ITS, 50 frequent ITS and 51 rare ITS. The proportion of frequent and very frequent ITS in relation to rare ITS is the same in MFA and in HSA (Chi-square= 0.3995, P=0.5273). Of 90 ITS located in the chromosomes of MFA, 37 (41.11%) coincide with ITS located in the equivalent band in their HSA homologue (Table 1). Human chromosome 3 has been previously studied in Old

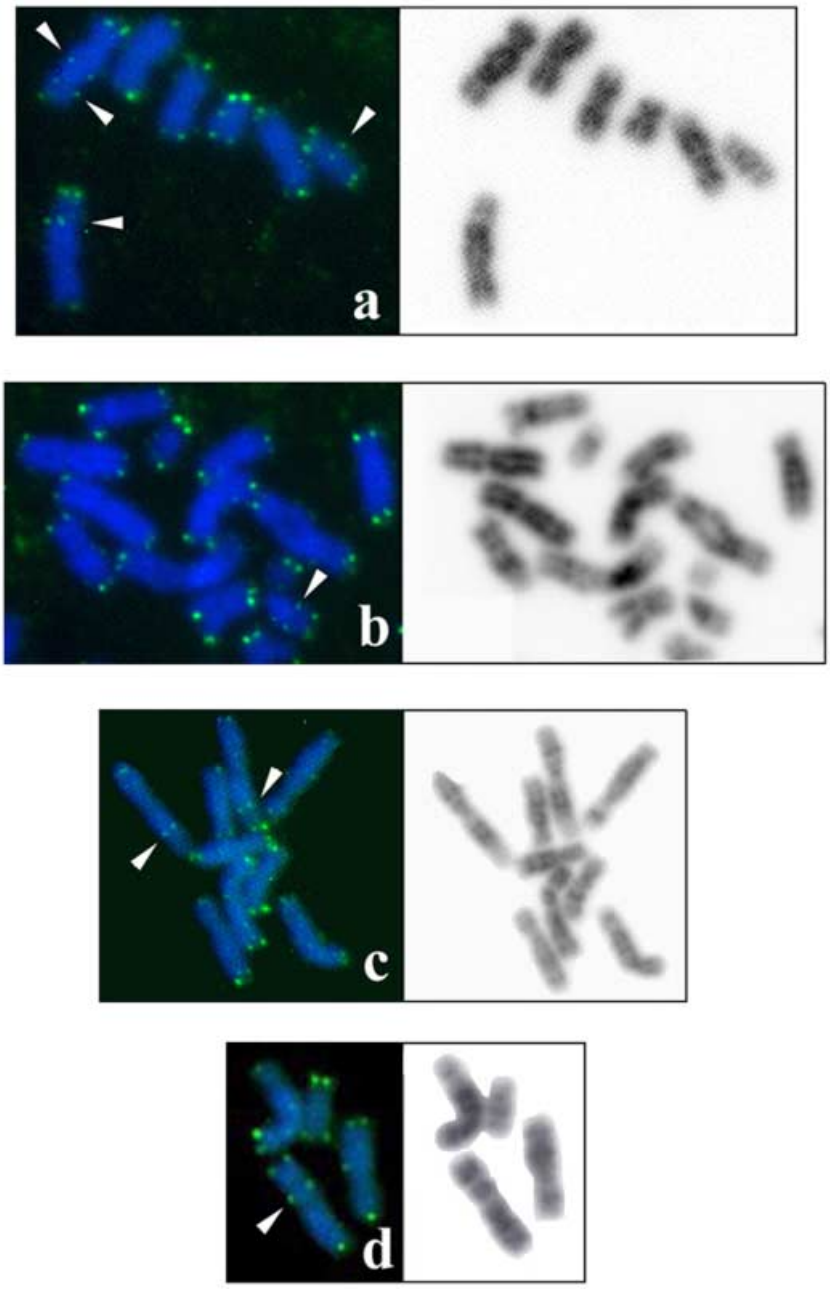

Fig. 2a–d Partial metaphase images of *M. fascicularis* chromosomes showing ITS FISH hybridisation signals with the $(TTAGGG)_n$ probe (images *left*) and DAPI bands (images *right*). *Arrowheads* Double spots in: **a** 6q13, 6q21, 6q21 and 20q15, **b** 13q22, **c** 1q32 and 1q32, **d** 3q34

World monkeys by molecular cytogenetics with specific probes for chromosome sub-regions (Müller et al. 2000; Müller and Weinberg 2001). Because of the complex structural rearrangements needed to explain the homology between MFA3 and HSA3 (at least, three inversions), this chromosome has been excluded from the comparison with human ITS.

Discussion

The distribution of ITS in MFA chromosomes, as revealed by using a $(TTAGGG)_n$ probe, is described for the first time in this report. Ninety ITS with various hibridisation frequencies have been detected, 7 loci being very frequent, 33 loci being frequent and 50 loci being rare; of these, 41.11% are located in human homologous bands. After a comparative study, it can be seen that 31.57% of MFA bands are significantly affected by X-irradiation and 47.37% of common fragile sites mapped in the MFA karyotype coincide with MFA ITS.

ITS versus primate ancestral karyotype

As has been previously presented herein, many hypotheses regarding the origin of ITS have been proposed over the past few years. The oldest proposal is that, where ITS

Table 1 Correspondence between intrachromosomal telomere-like sequences (ITS) on human (HSA) and *M. fascicularis* (MFA) chromosomes

ITS-HSA (Azzalin et al. 1997)	ITS-MFA
1p35[a]	1q34[a]
1p32[b]	1q32[c]
1p22[b]	1q22[a]
1q21[c]	1q12[b]
1q23[a]	1q14[b]
1q25[b]	1p22[a]
1q32[b]	1p31[a]
2q23[a]	15q13[a]
2q33[a]	15q21[b]
2q35[a]	15q23[a]
4p15[a]	4p24[a]
4q25[b]	4q21[a]
5q23[b]	5q23[b]
6p21[a]	6q23[b]
6cen[b]	6q21[c]
6q21[b]	6q12[b]
6q25[a]	6p13[a]
7p15[a]	2q14[a]
7q21[a]	2q21[b]
7q32[b]	2q24[a]
8p21[b]	8p13[a]
8q13[a]	8q14[a]
9p21[a]	14q23[a]
9q11[a]	14q12[a]
9q22[a]	14q14[b]
9q31[b]	14q21[b]
10q21[a]	10q21[b]
10q22[a]	10q14[a]
11q13[b]	11q12[b]
11q23[b]	11q23[a]
12q13[b]	12q13[c]
12q21[a]	12q21[a]
12q23[b]	12q23[b]
13q14[a]	16p13[a]
13q21[a]	16q12[a]
18q21[b]	18q12[b]
Xq21[b]	Xq21[b]

[a]Rare ITS
[b]Frequent ITS
[c]Very frequent ITS

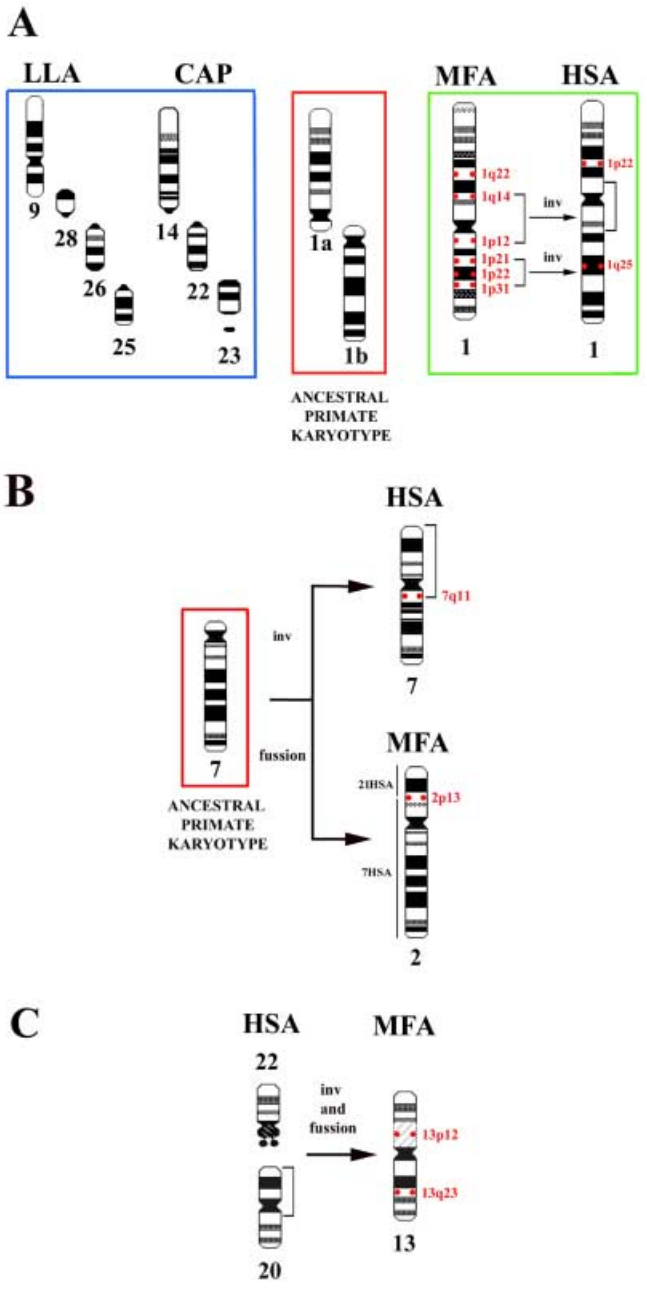

Fig. 3a–c Idiograms showing chromosome rearrangements explaining the presence of ITS sites found in *Homo sapiens* (*HSA*) and *Macaca fascicularis* (*MFA*). **a** Ideogram showing the ancestral primate karyotype and the homologies among *Homo sapiens* (*HSA*), *Macaca fascicularis* (*MFA*), *Lagothrix lagothricha* (*LLA*) and *Cebus apella* (*CAP*). **b** Idiogram showing the homologies between HSA7 and MFA2 from the ancestral primate karyotype. **c** Idiogram showing homologies among MFA13 and HSA20 and HSA22. *Red dots* in MFA chromosomes show the position of ITS found in this work and, in HSA, the position of ITS found by Azzalin et al. (1997), which might be the consequence of evolutive chromosomal rearrangements

are considered as being remnants of ancestral chromosome reorganisations and irrespective of mechanisms of origin, the presence of arrays of telomeric repeats could provide alternative sites for telomere formation within chromosomes (Meyne et al. 1990). This indicates the presence of a high degree of flexibility for karyotype rearrangements providing preferential sites for chromosome reorganisations.

If we consider that the ancestral primate karyotype is indeed that recently published by various authors (O'Brien and Stanyon 1999; Müller et al. 1999; Müller and Wienberg 2001; Murphy et al. 2001) and if we take the chromosome homologies between human, MFA and some Plathyrrini species, viz. *Cebus apella* (CAP) and *Lagothrix lagothricha* (LLA), into account, we postulate that some of the ITS present on MFA and HSA chromosomes can be considered as being: (1) the result of ancestral chromosome rearrangements (such as fusions), (2) unstable regions where fissions and inversion might occur during karyotype evolution; in other words, ITS are the storage for new telomeres, the fission points at which chromo-

some reorganisations can be "fixed" during the evolutionary process. Here, the most representative cases are presented.

MFA chromosome 1

Ten ITS loci have been detected (Fig. 1), seven of which are also present in the homologous chromosome of HSA (Table 1). In the ancestral primate karyotype, HSA1 is present in two different chromosomes (Clemente et al. 1990a; Müller et al. 1999; Murphy et al. 2001). In *Papio, Macaca* and *Cercocebus* species (T. Papionini), as in other Cercopithecidae, HSA1 is homologous to one chromosome because of the fusion of the two ancestral chromosomes and a pericentric inversion (Dutrillaux et al. 1979; Ponsà et al. 1986; Wienberg and Stanyon 1998; Ruiz-Herrera et al. 2002; Fig. 3a). In addition to this reorganisation and in agreement with Müller and Wienberg (2001), *Macaque* chromosome 1 needs a paracentric inversion to be homologous to HSA1. In some New World monkey species, such as *Cebus* and *Lagothrix*, HSA1 is homologous to three and four chromosomes, respectively. In CAP, the chromosomes homologous to HSA1 are CAP14, CAP22 and CAP23 (García et al. 2000) and, in LLA, the four chromosomes homologous to HSA1 are LLA9, LLA25, LLA26 and LLA28 (Stanyon et al. 2001) because of fissions from the ancestral primate karyotype.

Although the hypothesis in which the homologue to human chromosome 1 is represented as two chromosomes in the ancestral primate karyotype (Clemente et al. 1990a; Müller et al. 1999), another possible situation can be taken into account when comparing the ZOO-FISH results published recently. It should be noted that there are 2–5 chromosomes homologous to human chromosome 1 in most mammalian species (Wienberg et al. 2000) and so the possibility that the ancestral primate karyotype can be represented by three or four chromosomes, as is the case with CAP and LLA, can be considered. However, this last hypothesis must be demonstrated by further comparative mapping in more placental species.

In any case, after comparing HSA, MFA, CAP and LLA karyotypes and if the currently accepted ancestral primate karyotype is considered, we note the following. (1) The ITS located in MFA1p12 could be the result of the fusion of the two ancestral chromosomes homologous to HSA1. The direction of the change of the inversion necessary for the homology probably extends from the morphology present in MFA to the morphology present in HSA because the human chromosome 1 is the only chromosome that presents the pericentric inversion within the Hominidae and Cercopithecidae. (2) The ITS located in MFA1p22 corresponds to HSA1q25 (Table 1). If the ZOO-FISH results from CAP (García et al. 2000) and from LLA (Stanyon et al. 2001) are considered with some modifications, HSA1q25 may be the fission point for chromosomes CAP22/CAP23 and LLA25/LLA26, respectively. Thus, this site could be a potential new telomere and could be present in the ancestral primate karyotype as a latent telomere. The difference in the hybridisation frequency (higher in HSA than in MFA) could be attributable to a variation in the number of repeats of the telomeric sequence originating during the divergence of these species. (3) For the same reason, we consider that the ITS located in MFA1q22, which corresponds to HSA1p22 (Table 1), could be another potential telomere and a site for the fission of chromosomes LLA9 and LLA28. (4) The chromosome bands involved in the pericentric inversion (MFA1p12 and MFA1q14) have ITS but only one (MFA1q14) is conserved in the homologous chromosome of HSA1 (Table 1). In the same way, the chromosome bands involved in the paracentric inversion (MFA1p31 and MFA1p21; data in preparation) have ITS and one of them (MFA1p31) is also conserved in HSA (Table 1). In these two cases, the inversion points are located within ITS.

MFA chromosome 2

This chromosome has 10 ITS loci (Fig. 1), three of which (MFA2q14, MFA2q21 and MFA2q24) are conserved in the homologous HSA chromosome (Table 1). MFA2 results from a telomere-telomere fusion of ancestral chromosomes homologous to HSA7 and HSA21 (Wienberg et al. 1992; Ruiz-Herrera et al. 2002) and the ITS located in MFA2p13 could be the remnant of the fusion (Fig. 3b).

In the ancestral primate karyotype, the chromosome homologous to HSA7 is present as a single chromosome (Müller et al. 1999; O'Brien and Stanyon 1999). CAP and MFA chromosomes homologous to HSA7 have an equivalent banding pattern and, as a result, both species can be considered to have a chromosomal form similar to the ancestral chromosome 7 in agreement with the data published by O'Brien and Stanyon (1999). As a consequence, the ITS present in HSA7q11 (Azzalin et al. 1997) could be interpreted as the internalisation of the 7p telomere from the submetacentric ancestral form by pericentric inversion. The results obtained by Azzalin et al. (2001) also suggest that this telomeric site could be the result of an ancestral rearrangement. MFA chromosome 2 has no ITS homologous to ITS HSA7q11 because it has probably conserved the ancestral form (Fig. 3b).

MFA chromosome 4

Of the eight ITS detected (Fig. 1), two are conserved in the homologous HSA chromosome. MFA chromosome 4 is homologous to HSA chromosome 4 by pericentric inversion (Marzella et al. 2000) and the two bands involved in this rearrangement (MFA4p12 and MFA4q12) have ITS.

MFA chromosome 11

Five ITS have been detected (Fig. 1). Two of them, MFA11q12 and MFA11q23, are conserved in the homolo-

gous HSA chromosome (Table 1). MFA11 is homologous to HSA11 by pericentric inversion (Ruiz-Herrera et al. 2002) and the same homology has been described in Papionini and Cercopithecini (Ponsà et al. 1986; Clemente et al. 1990b). One of the bands involved in the inversion (MFA 11q12) has an ITS.

MFA chromosome 13

Four ITS have been detected, none of which is conserved in HSA. MFA13 is homologous to HSA20 and HSA22 through two reorganisations: one pericentric inversion in HSA20 followed by a telomere-telomere fusion (Ruiz-Herrera et al. 2002; Fig. 3c). The ITS located in MFA13q23 could be the result of an internalisation of the p-arm telomere of the ancestral HSA20 form (Clemente et al. 1990a), whereas the ITS located in MFA13p12 could be the remnant of the fusion of chromosomes HSA20 and HSA22 (Fig. 3c).

Another possibility in consideration is that the ancestral HSA20 form is an acrocentric chromosome equivalent to *C. nigrivittatus* chromosome 10 (data in preparation). In this case, the ITS located in MFA13q23 would be an inversion point.

MFA chromosome 14

Five ITS have been detected (Fig. 1). Four of them (MFA14q21, MFA14q14, MFA14q12, MFA14q23) are conserved in the homologous HSA chromosome (Table 1). The ITS located in MFA14q22 (Fig. 2) is not found in HSA; its origin could be related to the internalisation of the 14p-arm telomere by pericentric inversion (Ruiz-Herrera et al. 2002).

MFA chromosome 18

Three ITS have been detected (Fig. 1). MFA18q12 is conserved in the homologous HSA chromosome and corresponds to one of the bands involved in the inversion necessary for the morphological conversion of HSA18 into MFA18 (Ruiz-Herrera et al. 2002).

Overview of the studied ITS

In summary, we can conclude that some of the studied ITS can be considered as (1) the result of evolutionary reorganisations, such as fusions and intrachromosomal reorganisations (internalisations of telomeres) and/or (2) unstable loci, because these telomeric arrays are located within fission points, and loci involved in inversions. Some of the remaining ITS found in MFA and HSA could have originated from chromosomal rearrangements that have occurred after the divergence of the HSA and MFA species. However, the possible implication of other mechanisms in the origin of these ITS, such as gene amplification, unequal crossing-over or the insertion of telomeric repeats at sites of double-strand breaks during the repair by telomerase, cannot be discarded. A study including a larger number of primate species will probably elucidate the evolutionary contribution of ITS.

ITS versus fragile sites and chromosome instability

Some authors have related ITS to spontaneous chromosome rearrangements (Bertoni et al. 1994) and to induced chromosomal rearrangements (Álvarez et al. 1993; Fernández et al. 1995; Slijepcevic et al. 1996). Other authors have related ITS to recombination and chromosomal unstable events (Hastie and Allshire 1989; Pluta and Zakian 1989; Katinka and Bourgain 1992; Mondello et al. 2000; Kilburn et al. 2001). Moreover, in view of the coincidence between ITS and fragile sites suggested by Farr et al. (1991), Musio et al. (1996) and Musio and Mariani (1999), the cytogenetic studies on aphidicolin-induced fragile sites in MFA chromosomes performed in our laboratory (Ruiz-Herrera et al. 2002) show a relationship between ITS and fragile sites. If the position of MFA ITS is compared with the localisation of common fragile sites, we observe that 45 MFA ITS (51.14%) are located in chromosome bands in which fragile sites have been detected. These results support the previous suggestions from cytogenetic studies showing that ITS are sites for preferential chromosomal breakage and from molecular studies in which the instability of ITS in the human genome have been reported (Mondello et al. 2000). In our comparative study, some ITS coincide with inversion points (MFA1p12, MFA1p21, MFA1p31, MFA1q14, MFA4p21, MFA4q12, MFA11q12 and MFA18q12) and with fissions (MFA1p22 and MFA1q22), when MFA and HSA are compared by taking the ancestral primate karyotype into account.

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