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High-resolution R-banding and localization of fragile sites in *Oryctolagus cuniculus*

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INTRODUCTION

Fragile sites on chromosomes are points where chromosomes are liable to break. They are divided into two major groups, common and rare, according to their population frequency and their mode of unmasking (Sutherland and Hecht, 1985; Hecht *et al*, 1990). In humans, fragile sites have been correlated to mental retardation (Lubs, 1968), to cancer breakpoints (De Braekeleer, 1987), to targets for mutagens and carcinogens (Yunis *et al*, 1987) and to breakpoints involved in the chromosomal evolution of primates (Miró *et al*, 1987). Yunis and Soreng (1984) found several fragile sites shared by man, chimpanzee and gorilla demonstrating that fragile sites are conserved among closely related species. Data on fragile sites in chromosomes of domesticated animals are limited, but they have been demonstrated in the pig (Riggs and Chrisman, 1989) and the horse (Rønne and Poulsen, unpublished). This paper reports on 8 putative fragile sites located in the rabbit karyotype.

MATERIALS AND METHODS

Four animals, 2 males and 2 females, were randomly selected from the stock of laboratory rabbits at the Institute of Biomedicine, Odense University. R-band induction and fragile site-unmasking were performed simultaneously as follows: peripheral blood was allowed to sediment for 1 h at room temperature and 0.5 ml from the buffy/plasma layer was added to 9.5 ml of RPMI-1640 (Gibco) supplemented with 10% fetal calf serum (Gibco), 5 IU/ml heparin (Sigma), 50 μg/ml gentamycin (Sigma) and phytohemagglutinin (PHA-M) (Gibco). Cultures were synchronized with fluorouracil (FU; Sigma; 5×10^{-7} M) and processed as previously described (Rønne, 1984). For each animal, 4 cultures (1 control and 3 test cultures) were

exposed to different chemicals as listed in table I. Subsequent harvesting, staining and band induction were performed as described by Rønne (1983, 1984).

Table I. Time (h) for chemical addition.

Culture	*FU* 0.065μg/ml	*BrdU* 30μg/ml	*Hoechst* 60μg/ml	*APC* 0.5μg/ml	*AZA* 0.6μg/ml	*CAF* 430μg/ml	*COL* 0.2μg/ml	*Harvest*
1	48	66	66				70	72
2	48	66	66	48 (x)	48 (y)	48 (z)	70	72

FU: fluorouracil; BrdU: bromodeoxyuridine; Hoechst: Hoechst 33258; **ACP**: aphidicolin; **AZA**: 5′-cytidine; **CAF**: caffeine; **COL**: colcemid; **1**: control culture; **2**: test cultures x, y, z.

RESULTS AND DISCUSSION

For each animal, 4 cultures (1 control and 3 test cultures) were examined for the presence of fragile sites. From each culture, 50 metaphases were selected at random, photographed and analyzed on a screen at a magnification of × 5 000. If chromosomes were difficult to identify directly, photographic copies were made and karyotyping performed.

For all 4 animals, the putative fragile sites were not expressed in the control cultures (control), while the test cultures (table I: x, y, z) yielded metaphases with non-randomly distributed chromosomal breaks. The localization of chromosomal breaks is shown in figure 1. The mode of action of the 3 agents employed is similar, but APC seems to be the most potent one. Only two sites, 1p32 and Xp14, were sensitive to all 3 agents. Fragility of these sites was expressed in all animals at high frequencies. In the females, Xp14 was expressed both on the active and the inactive X-chromosome at approximately the same frequencies.

Chromosomal breaks were also found in 1q26, 4p14, 4q12, 4q14, 15q12, Xq24, but only at low frequencies (range: 2–8%). Breakpoints at 4q12 and 4q14 were only observed in one animal (A) after APC exposure. Both females displayed a breakpoint at Xq24. This specific break was only unmasked at a relatively low frequency after APC exposure. It was only detected on the active X chromosome, but this may be due to the staining technique used or to a sampling error. In rare cases, the X chromosome displayed both breakpoints (fig 1). It is interesting that the laboratory rabbit has a breakpoint at Xq24 similar to that observed for horses and humans. The Y chromosome showed no breaks either in the controls or in the test cultures. The most important findings are summarized in table II.

According to Hecht *et al* (1990), the status of a fragile site may be either tentative, provisional or confirmed. Judged from the frequencies and the distribution of the breakpoints observed in the rabbit karyotype, these fall into two separate groups. The breakpoints 1p32 and Xp14 may represent APC-sensitive common fragile sites with a provisional status, while the other breakpoints may be either rare or common fragile sites with a tentative status.

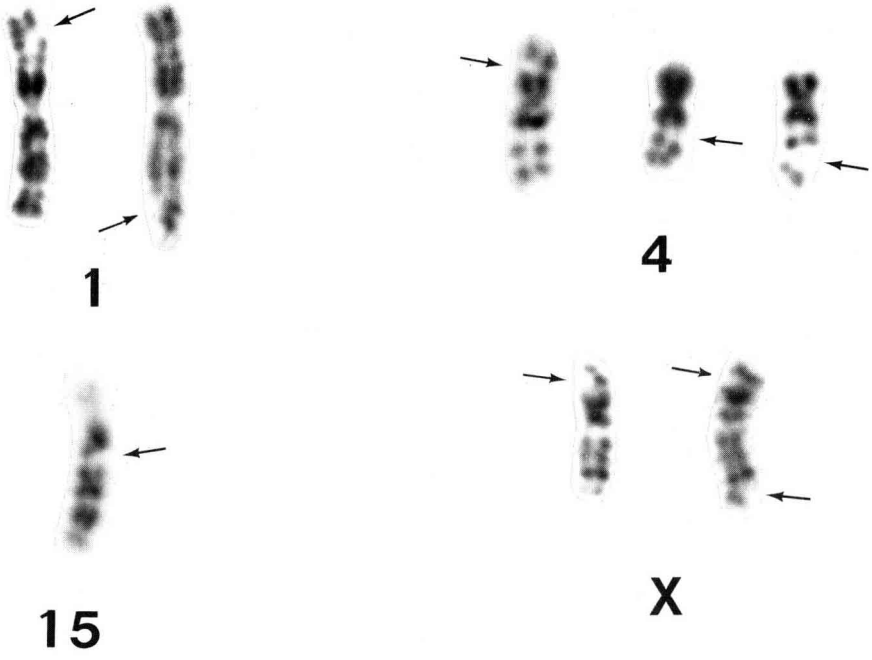

Fig 1. Localization of chromosomal breaks in *Oryctolagus cuniculus* L. Arrows indicate fragile sites localized to: 1p32, 1q26, 4p14, 4q12, 4q14, 15q12, Xp14, Xp14 and Xq24.

Table II. Location and frequencies (%) of putative fragile sites.

Animal/sex/ treatment[a]	*Putative fragile sites*					
	1p32	*1q26*	*4p14*	*15q12*	*Xp14*	*Xq24*
A/F/APC	28		4	4	74	8
A/F/AZA	8	4	4		24	
B/F/APC	18		2		16	2
B/F/CAF	8			6	20	
C/M/APC	16				4	
C/M/CAF	10		2	2	4	
D/M/APC	8				26	
D/M/CAF	4			28		

A, B, C, D: animal number; **F**: female; **M**: male; **APC**: aphidicolin; **AZA**: 5′-cytidine; **CAF**: caffeine.

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