Preferential late replication of one of the two morphologically distinguishable X-chromosomes in a female muntjac[1]

T. Sharma[2] and Rajiva Raman

Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi-221005 (India), 3 March 1977

Summary. In a female barking deer, Muntiacus muntjak, whose 2 X-chromosomes are mutually distinguishable from each other, one X has been found to be late replicating in 57.8% cells compared to the other which is late replicating in 42.2% cells. These data are suggestive of preferential inactivation of one X-chromosome. These findings have been discussed in the light of Lyon's hypothesis of random X-inactivation in eutherian mammals.

Muntiacus muntjak, the barking deer, is an extremely favourable animal for cytogenetic study because of its low diploid number (2n = 6♀/7♂) and easy identification of each chromosome of the complement[3]. In a breeding colony of muntjacs, a female individual has been encountered whose X-chromosomes are unambiguously distinguishable from each other. It has been ensured by the C- and G-band studies that the variation between the 2 X's is due to the difference in the disposition of the C-band positive heterochromatin of the centromeric 'neck', and not to any loss or gain in the size of the other chromosomal material (figures 1a, b). This variation of length between the necks renders the 2 X's unequivocally distinguishable from each other, and thus provides an excellent opportunity to examine the randomness of their late replication to serve as a model system to evaluate the randomness of X-inactivation in mammalian females as envisaged by Lyon's hypothesis of X-dosage compensation[4]. The same was attempted in the lymphocyte cultures of this female individual. The results are reported below.

Phytohaemagglutinin-stimulated whole blood cultures were grown for 48 h to obtain the first wave of mitosis. Since the DNA replication pattern of the muntjac lymphocytes has already been studied[5], the cultures were treated with ^{3}H-TdR (1 μCi/ml culture; sp. activity 8000 mCi/mM; BARC, India) only during the terminal end of the S for $3^1/_2$ and $2^1/_2$ h prior to fixing the cells. In both cases, duplicate cultures were set up. The so-called X-chromosome is submetacentric, formed by the translocation of the X to an acrocentric autosome. Therefore, the actual X is only the short arm; the long arm com-

1 The authors are grateful to the University Grants Commission, India, for financial assistance.

2 Present address: Director, Life Science Centre, The University of North Bengal, P. O. North Bengal University, Distt. Darjeeling (W. B.), Pin-734430, India.

3 D. H. Wurster and K. Benirschke, Science *168*, 1364 (1970).

4 M. F. Lyon, Nature *190*, 372 (1961).

5 T. Sharma and M. K. Dhaliwal, Exp. Cell Res. *87*, 394 (1974)

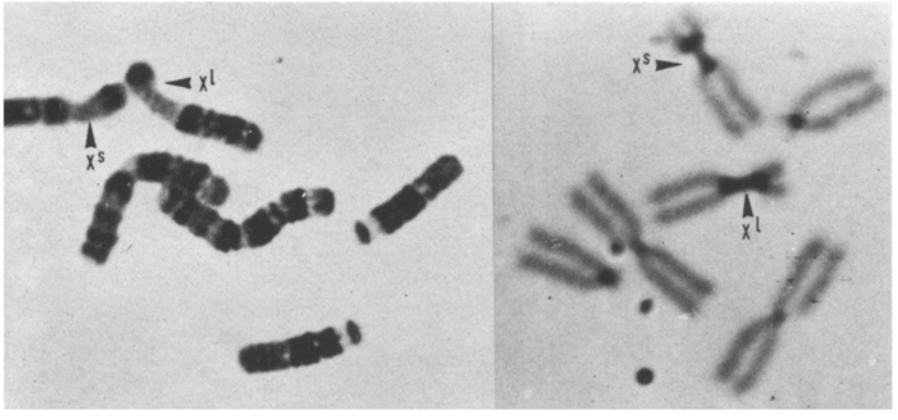

Fig. 1. *a* G-band metaphase of the Muntjac female. Arrows indicate X^1 and X^s. ×1800. *b* C-band metaphase of the Muntjac. ×1800.

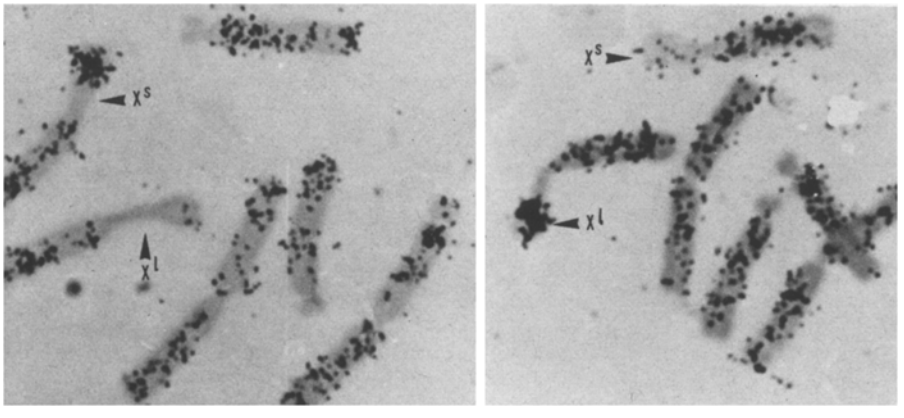

Fig. 2. *a* Autoradiograph of the DNA labelled metaphase showing 'hot' X^s. ×1800. *b* Autoradiograph showing 'hot' X^1. ×1800.

prises the autosome to which the X is translocated. Replication of only the short arm, the actual X, was taken into account.

In both the lots of continuously labelled cells ($3^1/_2$ and $2^1/_2$ h ^{3}H-TdR), one X was hot in most of the plates. A total of 483 metaphases with one late replicating X were scored. Of these, the long-necked X (X^l) was late replicating in 57.8% (279 cells) and the short-necked X (X^s) displayed the same property in the rest of the 42.2% (204 cells) metaphases (figures 2a, b). Assuming that the X-chromosome inactivation takes place at random, the percentage value of each type of the late replicating X deviates considerably from the expected value of 50%. A simple χ^2 test reveals that this difference between the 2 X's is statistically very significant ($\chi^2 = 11.64$; $p > 0.001$).

The prerequisite for the cytological evaluation of randomness of X-inactivation in mammalian females[4] is the occurrence of 2 morphologically distinguishable but normal X-chromosomes in a female. Therefore the two female systems mostly used for this purpose are mules as well as mice heterozygous for Cattanach's X-autosome translocation. In both the instances, however, contradictory results have been obtained[6–10]. Recently, in Cattanach mice, Takagi and Sasaki[11] as well as Wake et al.[12] have shown that whereas in advanced stages of embryonic development the frequency of late replicating X was random, in early embryonic development paternal X was preferentially late replicating. These results are in accordance with earlier findings of Chandley[9], who observed selective inactivation of paternal X in Cattanach mice. The present report on the Muntjac X's also illustrates preferential late replication of the X^l chromosome. We do not know the pattern of inheritance of these 2 X's and hence cannot derive their parentage. Moreover, although the results are clear-cut, it may be premature to draw decisive evidence in favour of non-randomness of X-inactivation. The present data could as well be an exaggerated expression of what might have been a chance deflection at the time of differentiation of the X-inactivation in the primordial cells. Alternatively preferential distribution (or selection) of one type of X in a highly differentiated tissue cannot be ruled out as a possibility.

6 B. B. Mukherjee, A. B. Mukherjee and A. B. Mukherjee, Nature *228*, 1321 (1970).
7 B. B. Mukherjee and A. K. Sinha, Proc. Nat. Acad. Sci. *51*, 252 (1964).
8 J. L. Hamerton, F. Giannelli, F. Collins, J. Hallett, A. Fryer, V. M. McGuire and R. V. Short, Nature *222*, 1277 (1969).
9 A. C. Chandley, Nature *221*, 70 (1969).
10 H. J. Evans, C. E. Ford, M. F. Lyon and J. Gray, Nature *206*, 900 (1965).
11 N. Takagi and M. Sasaki, Nature *256*, 640 (1975).
12 N. Wake, N. Takagi and M. Sasaki, Nature *262*, 580 (1976).

No association between Gilbert's syndrome, the AB0 blood groups and the haptoglobin phenotypes[1,2]

R. Platzer and J. Bircher

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Bern (Switzerland),

9 February 1977

Summary. The different AB0 blood groups and haptoglobin phenotypes are as frequent in a well-defined group of subjects with Gilbert's syndrome as in appropriate samples of the general population.

Gilbert's syndrome is generally characterized by mild unconjugated hyperbilirubinemia in the absence of evidence for hepatic or hemolytic disease. Although a familial incidence with autosomal dominant transmission and variable expressivity has been noted[3–8], the mode of inheritance has not been satisfactorily established. Since genetic markers may be useful in such situations, the recently reported association between slow acetylator phenotypes and Gilbert's syndrome appears noteworthy[9]. In a search for further genetic markers, the association between Gilbert's syndrome, the AB0 blood group system and the haptoglobin phenotypes was investigated in the subjects previously published[9].

Material and methods. The records of the 27 patients with Gilbert's syndrome investigated for the acetylator phenotype[9] were examined for blood group determinations. In 22 cases serum (stored at -20 °C) was available for determinations of serum haptoglobin phenotypes. Determinations were done by starch gel electrophoresis. The diagnosis of Gilbert's syndrome was based on a) long-

AB0 blood groups and haptoglobin phenotypes in patients with Gilbert's syndrome

Analyzed proportions	Gilbert's syndrome	Controls	χ^{2*}
0 : A	13:11	1067:1274	0.703
0 : (B+AB)	13: 3	1067: 366	0.410
A : (B+AB)	11: 3	1274: 366	0.006
A : (0+B+AB)	11:16	1274:1433	0.432
Hp 2-1 : Hp 2-2	12: 7	1376: 970	0.160
Hp 2-1 : Hp 1-1	12: 3	1376: 431	0.127
Hp 2-2 : Hp 1-1	7: 3	970: 431	0.163
Hp 2-1 : (Hp 1-1+Hp 2-2)	12:10	1376:1401	0.218

*χ^2 corresponding to $p < 0.05$ is 3.9.

1 Acknowledgments. The haptoglobin phenotype determinations were kindly performed by the Control Laboratory, Transfusion Service of the Swiss Red Cross, Wankdorfstrasse 10, CH-3014 Berne.
2 Supported by the Swiss National Science Foundation.

3 W. T. Foulk, H. R. Butt, C. A. Owen, F. F. Whitcomb and H. L. Mason, Medicine *38*, 25 (1959).
4 E. Meulengracht, Klin. Wschr. *18*, 118 (1939).
5 N. Alwall, Acta med. scand. *123*, 560 (1946).
6 W. Damasluk and K. Singer, Arch. intern. Med. *67*, 259 (1941).
7 L. W. Powell, E. Hemingway, B. H. Billing and S. Sherlock, New Engl. J. Med. *277*, 1108 (1967).
8 E. Penner, W. R. Mayr, S. Djawan, H. Seyfried and M. Pacher, Schweiz. med. Wschr. *106*, 860 (1976).
9 J. Bircher, R. Platzer, A. Küpfer and R. Preisig, Gastroenterology *71*, 896 (1976).