



Fig. 3 Six pairs of X chromosomes from metaphase spreads of female *G. gerbillus* primary lung fibroblasts. In each pair the left chromosome is the inactive X chromosome. The first pair (on the left), which show replication bands following 5-BrdUrd treatment, was taken from Fig. 1. The autosomal regions, AI and AII, and the 'original X' chromosome (OX) are marked. The arrow indicates the early replicating band within the original X chromosome. The other five pairs of X chromosomes illustrate representative autoradiographs of nick-translated mitotic cells; in each pair the inactive X chromosome is free of label except for the labelled early replicating segments which include the autosomal regions AI and AII and the small band within the OX. In some of the inactive X chromosomes which have been underlabelled, one or even two of these regions are hardly labelled. However, the inactive X-chromosomal regions are always free of label. The active X chromosome—the right one in each pair—is labelled along both the autosomal stretches and the X-chromosomal segments.

used to identify the activity state of the X chromosomes. Of the 117 labelled metaphase plates, 93 mitotic cells (79%) show differential labelling of the two X chromosomes. In all cases, the active X chromosome was labelled both in the autosomal (AI and AII) and X-chromosomal regions while the inactive X chromosome was labelled in the autosomal regions and in the early replicating band within the 'original X' chromosome. As the inactive X appeared slightly shorter in most of these preparations it was possible to show that the unlabelled chromosome corresponded to the inactive X. Examples of *in situ* nick-translated X chromosome pairs from several individual cell preparations are shown in Fig. 3.

In 24 nick-translated mitotic cells (21%) we observed very heavy labelling and in these cells both X chromosomes were found to be labelled nondifferentially. It is possible that some cells are affected by the fixation procedure so as to cause nonspecific DNase I nicking. Note in this regard that all labelling observed in these experiments was DNase I-dependent. Very few grains (10–15 per cell) were observed in all the screened preparations that were not treated with DNase I (see Fig. 2B and ref. 8).

These results show that in fixed mitotic chromosomes the active X chromosome is more sensitive to DNase I than the inactive X. Whereas most chromosomes contained extensive regions of DNase I sensitivity, the inactive X chromosome appeared to be in a conformation which made it resistant to this enzyme. This lack of sensitivity was in apparently sharp contrast to two internal positive controls, the active X chromosome and the autosomal segments of the inactive X. Thus, the DNase I-sensitive conformation typical of many active genes is also present in active X chromosome genes but absent from the inactive genes in the inactive X chromosome.

It is interesting that the small early replicating region within the "original inactive X chromosome"⁹ shows high sensitivity to DNase I indicating its active DNA conformation. Previously, it was shown that human female inactive X chromosomes include some genes which escape inactivation^{13,14}. One such gene, the steroid sulphatase (STS) gene, has been mapped to an early replicating region of the inactive X chromosome^{15,16}. These results and others led to the assumption that early replicating regions in the inactive X chromosome remain active¹⁷. The use of our technique allows identification of active regions in the inactive X chromosome and the study of any correlation between early replicating regions in this chromosome and gene

activity. This technique should be useful for following the kinetics of X-chromosome inactivation and reactivation in individual cells during animal embryogenesis and germ cell formation. Furthermore, it may allow the identification and mapping of active gene clusters including tissue-specific genes.

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Corrigendum

In the letter 'Direct surface imaging in small metal particles' by L. D. Marks and David J. Smith, *Nature* **303**, 316–317 (1983), in the second sentence of the third paragraph on page 317, the value $\langle 1\bar{1}1 \rangle$ should read $\langle 1\bar{1}0 \rangle$.

Erratum

In the letter 'Characterization of rat hypothalamic growth hormone-releasing factor' by J. Spiess, J. Rivier and W. Vale, *Nature* **303**, 532–535 (1983), in the final sentence of the third paragraph on page 533, 'Ca²⁺-independent' should be 'Ca²⁺-dependent'.

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