Nonrandom X-Chromosome Inactivation—an Artifact of Cell Selection
(mouse chimera)

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ABSTRACT The present study shows that a high degree of variability in the distribution of XX and XY cells exists among various tissues of artificially assembled or spontaneously occurring mouse chimeras. This variation from the expected equal distribution in various tissues of an animal may have resulted from random distribution, unequal segregation, or selective differences of such cells during development. This variation may also explain the observed intertissue and interanimal variations in the distribution of inactive paternal and maternal X-chromosomes in some mammalian females.

Clear experimental evidence in support of the random X-chromosome (X) inactivation hypothesis (1) was provided by Mukherjee and Sinha (2) who studied the DNA replication patterns of the X-chromosomes in cultured leukocytes from a female mule. The female mule complement was chosen because it is unique in that its paternal (donkey X = Xp) and maternal (horse X = Xm) X-chromosomes can be clearly distinguished morphologically (3). About one half of the metaphase figures studied showed a late-replicating Xp and the other half contained a late Xm. Since the inactive X-chromosome completes replication later than all other chromosomes in a mammalian female complement, this experiment provided clear cytological evidence for the random inactivation hypothesis (1). Hamerton et al. (4) repeated this study and also examined the type of glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49) expression in cultured cells from female mules, taking advantage of the fact that this enzyme is controlled by an X-linked gene, and has different electrophoretic mobilities in donkey and horse. They observed an excess of late Xp-chromosomes and a preponderance of horse G-6-PD activity in cells from all three mules studied. These observations were interpreted as evidence for "nonrandom X-inactivation in the female mule". It should be pointed out, however, that in this study the relative frequency of figures with a late Xp or Xm varied between fibroblasts and lymphocytes of the same animal and in no case did all cells from every tissue of the same animal contain a late Xp. Furthermore, donkey G-6-PD was not totally absent from any of the tissues examined. Since the cells used by Mukherjee and Sinha (2), and Hamerton et al. (4) were those at the end of the line of differentiation (skin fibroblasts and lymphocytes), far removed from their cells of origin in which random inactivation of one X-chromosome occurred, Mukherjee et al. (5) argued that the observed deviation from equal representation of cells with inactive Xp and Xm in any given tissue (4) may result from the random distribution or selection of these two cell types during differentiation of such tissue. Although this explanation has now been generally accepted (6, 7), experimental evidence was still lacking. Recent studies on the G-6-PD isozyme patterns of tissues from female mules by Hook and Brustman (14), and the present study on the frequency of distribution of XX and XY cells in various tissues of spontaneously produced and artificially assembled male–female chimeric mice provide such evidence.

MATERIALS AND METHODS

Artificially assembled chimeras, produced by the fusion of two eight-cell mouse embryos in vitro, develop as single mice, even when the two embryos have different sex-chromosome constitutions. Details of the techniques for embryo collection, fusion, culture, and implantation have been published (8, 9). Eight artificially assembled mice developed by the fusion of embryos obtained from F1 females, from a cross between mice of the C57BL/10Wt and SJL/J inbred strains backcrossed to males of one or the other of the strains, and three intersex mice selected from the BALB/cDg albino stock were kindly donated to us by Dr. W. K. Whitten (Jackson Laboratory, Bar Harbor, Maine, USA). Karyological identification of XX and XY cells was based on the criteria described by Stich and Hsu (10), and Ford (11). Skin, spleen, and kidney tissues were obtained from each mouse by biopsy; the metaphase cells were obtained either from outgrowths of the primary cultures or from their subcultures. Only well-spread metaphase figures with all 40 chromosomes were photographed and used for this experiment. After the chromosome analyses of the biopsy materials were done, the mice were killed and direct karyological observations were made on corneal epithelium, according to the method described by Fredga (12) and on bone marrow cells, by Lee's method (13). Four of the eight artificially assembled chimeras were found to be XX/XY sex-chromosome chimeras—two phenotypic males, one female, and one intersex. Two of the three spontaneously produced intersex mice were XX/XY mosaics. The results of the karyological analysis of all six mice are given in Table 1.

RESULTS AND DISCUSSION

The data presented in Table 1 clearly suggests that a high degree of variability in the distribution of XX and XY cells exists among various tissues of an artificially assembled or a spontaneous mouse chimera. The ratio of distribution of these
two-cell types deviated significantly from the expected 1:1 ratio in some tissues, but in other tissues of the same mouse, they were represented more or less equally. Since, at least in the case of the artificially assembled chimeras, the number of XX and XY cells was equal at the time of fusion (two eight-cell embryos were fused) and they formed a single blastocyst before implantation (X-inactivation appears to take place about the time of implantation), any variation from their equal distribution in various tissues may have resulted from random distribution, unequal segregation, or selective differences during differentiation of such tissues. The findings in spleen are particularly instructive. In one case all the cells had a paternal origin, in another all cells had a maternal origin, and in a third cells had about half of each. If such processes also operate during post-implantation development of a female mule embryo (after random X-inactivation has occurred), some of her organs or tissues may be expected to be composed entirely of cells with an X<sup>p</sup> or an X<sup>m</sup>, whereas others may show an admixture of both cell types in various proportions. This may then result in the observed interfascial and interanimal variations in the distribution of X<sup>p</sup> and X<sup>m</sup> (2, 4, 6, 7, 14).

Hook and Brustman (14) recently studied the G-6-PD isozyme patterns of tissues from several organs of 54 female mules and showed very elegantly that "there were two organ types: those in which the horse phenotype was usually predominant, and those in which expression was not significantly different from what would be expected on a random basis". These observations agree well with our findings in sex-chromosome chimeric mice and support very strongly the view presented by Mukherjee et al. (5).

Studies on sex-chromosome replication patterns (15) and expression of G-6-PD (16) and phosphoglycerate kinase (17) isozymes in cells from female kangaroos have also been reported recently; it has been concluded "that the mode of dosage compensation in kangaroos is paternal X inactivation". However, the sex-chromosome replication patterns were studied only in leukocytes and in at least 4% of such cells from one animal and in 8% of the other, the maternal X-chromosome was indeed found to be late-replicating. G-6-PD in the kangaroo was also analyzed only in erythrocytes. Results from the present experiment and that of Hook and Brustman (14) suggest very strongly that the conclusion that there is preferential paternal X-inactivation in marsupials should not be accepted until similar studies are extended to more than one tissue of the same animal. Finally, whether or not the gene for phosphoglycerate kinase is X-linked in the kangaroo has not been definitely established.

Excess of cells with an inactive X<sup>p</sup> in some tissues of mules or of any other mammalian females may well result from in vivo or in vitro selection in favor of cells with an active X<sup>m</sup>, but such selection must take place after the random inactivation of one X-chromosome has occurred, otherwise none of the tissues of the same animal could show the expected 1:1 distribution of cells having X<sup>p</sup> and X<sup>m</sup> (2, 5, 14). It is unfortunate that some investigators (4, 7) are invoking unequal
representation of \(X^P\) and \(X^M\) chromosomes in some tissues of female mules and hinnies as evidence for "nonrandom X-inactivation" and "nonrandom late-replication of X-chromosomes", when such variation may simply represent random distribution or selection of such cell types during development. Their conclusion ignores the fact that in some female mules, at least in some of their (2, 5, 14) tissues, cells with an \(X^P\) and \(X^M\) are equally represented, and that some other tissues may be composed entirely of \(X^P, X^M\), or both.

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