Demonstration of lateral asymmetry in G-banded mouse embryo chromosomes*

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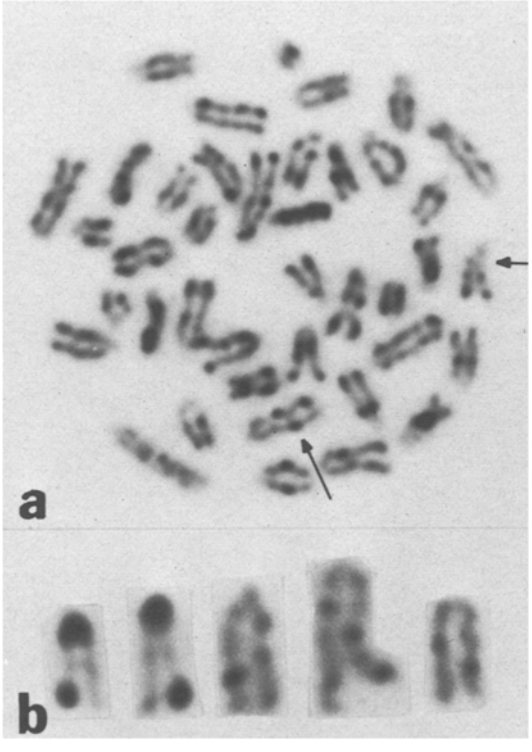
Summary. Cells obtained from 10-day mouse embryos were cultured for 3 h in medium containing colcemid. Chromosome preparations were subjected to G-banding in either calcium-magnesium free Hank's solution or the ASG method. From one to several occurrences of lateral asymmetry (unequal banding of sister chromatids) were observed in the majority of karyotypes analyzed.

When 5-bromodeoxyuridine (BrdU) is incorporated into chromosomal DNA, alterations in staining properties of the chromosomes have been observed. For example, fluorescence of the Hoechst 33258 fluorochrome is partially quenched following binding to the BrdU-substituted chromosomal DNA[2]. In addition, Giemsa stained chromatids, containing BrdU in both strands of DNA, stain lighter than their sister chromatids with only unifilar substitution[3]. If the Hoechst 33258 followed by Giemsa (FPG) technique is used to stain chromosomes, BrdU-substituted regions again stain lighter[4]. The observation that BrdU-containing segments of chromosomes stain lighter has also been implicated in the phenomenon of lateral asymmetry (i.e., the unequal banding of sister chromatids). Differential banding of BrdU-containing chromatids in mouse cells was first described by Lin et al.[5]. Since it had been demonstrated[6] that there is a bias in the thymidine (dT) arrangement in mouse satellite DNA, with one strand containing 45% dT and the other 22%, Lin et al.[5] suggested that unequal incorporation of BrdU into complementary strands of DNA in this region was responsible for the observed lateral asymmetry. Bostock and Christie[7] suggested that a bias in the distribution of incorporated BrdU was not the sole cause of asymmetrical staining since unequal crossing over during sister chromatid exchanges could also be involved. In the present communication, we report our observations that lateral asymmetry occurs when mouse embryo cells, obtained in vivo, are G-banded without prior administration of BrdU.



a ASG-banded karyotype of a 10-day mouse embryo cell. (→) = asymmetrical band regions. *b* Selected chromosomes from karyotypes of 10-day mouse embryo cells demonstrating unequal staining of sister chromatids. Preparations banded in Ca-Mg free Hank's solution at 60 °C.

For this experiment, 10-day pregnant albino mice of the ICR strain (Camm Research Institute, Wayne, New Jersey) were used. The females were killed by cervical dislocation, the uterus removed, and the embryos were dissected free of all extraembryonic membranes. A cell suspension was prepared by mild agitation with a pasteur pipet in a medium containing RPMI 1640, 15% fetal calf serum (GIBCO) and 0.1 μg/ml colcemid (GIBCO). The cells were cultured in the same medium for 3 h at 37 °C. Following the culture period, cells were concentrated by mild centrifugation and the pellet resuspended in 0.075 M KCl for 15 min. The cells were then fixed in ethanol:acetic acid (3:1). Chromosome spreads were made by dropping aliquots of the cell suspension onto cold slides which were then air-dried. Chromosomes were G-banded by either the ASG method[8] or by treatment in Hank's balanced salt solution (without calcium and magnesium) for 1 h at 60 °C. Slides were subsequently stained in Giemsa solution (GIBCO). Cells were photographed with a Zeiss Photomicroscope II on Panatomic X film (Kodak). Diafine development was used to enhance contrast.

Metaphase chromosomes obtained from 10-day mouse embryo cells, banded with Hank's or the ASG method, exhibited from one to several occurrences of lateral asymmetry in the majority of karyotypes examined (figure). It should be noted that previous studies[5,7,9] have shown that lateral asymmetry occurs in C-band regions. Since the preparations used in this study were subjected to G-band techniques, asymmetrical bands were revealed in all chromosomal regions.

Since BrdU was not employed in our study, the suggestion of Lin et al.[5] that bias in BrdU incorporation into AT regions is responsible for lateral asymmetry does not appear to be an essential element in the occurrences of

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lateral asymmetry in mouse embryo chromosomes. A possible explanation for our observations, which is consistent with current theories, is that lateral asymmetry may occur through the process of sister chromatid exchange which may involve unequal crossing over. Such an event would occur whenever an unequal number of base pairs are exchanged between sister chromatids during a crossing over event. Indeed, a theory proposed by Smith[10] suggests that the evolution of repeated DNA sequences could have developed by the mechanism of unequal crossing over while Bostock and Christie[8] have suggested that unequal sister chromatid exchange could also be implicated in the occurrence of asymmetric banding in mitotic chromosomes. The developmental significance of our observation remains to be determined.

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Inheritable precocious opening of the vagina in laboratory rats exposed to 300 R and 200 R of X-rays on day 6 of their intrauterine life

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Summary. A highly significant inheritable advancement in opening of the vagina in laboratory rats has been induced by the X-ray application to pregnant females of different filial generations originating from the X-irradiated F_0 embryos. The reaction to X-rays has so far been followed through 9 filial generations.

As far as we know there are no data to show how X-irradiation acts on the factors controlling the onset of puberty in filial generations of laboratory animals, as measured by opening of the vaginal orifice. Mandel and Griesewood[1] have claimed that the vagina of rat opened precociously after irradiation of the ovarian region, but Beaumont[2] has failed to confirm this finding. On the other hand, we know that advanced opening of the vagina can be effected by more than one experimental approach, but the phenomenon is not transmitted to filial generations[3-6].

Material and methods. The animals used in this experiment belonged to a strain of close-bred Wistar rats, in our possession since 1929. All matings were carried out between animals belonging to the same litter. The X-irradiation was performed on etherized animals with the use of a Siemens set under the following conditions: 200 kV, 14 mA, Cu 0.5 mm filter, 42 cm FSD, dose rate 100 R/min. The region of the uterus of normal pregnant females was exposed to 300 R of X-rays on day 6 of pregnancy. The young of these animals represented F_0 generation. All members of the F_2 generation and a number of F_5 offspring were also exposed to X-rays at day 6 of their intrauterine life, using this time dose of 200 R. The opening of the vaginal orifice was followed in all animals through 9 filial generations, starting from F_0. Only those

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Opening of the vagina in experimental rats through a number of filial generations

	F_0 E_1	F_1	F_2 E_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
	1*	5*	16*	17*	50*	91*	114*	109*	129*	60*
I		42.0 ± 1.3	35.5 ± 0.9	34.3 ± 1.1	34.0 ± 0.9	40.4 ± 0.6	34.7 ± 0.5	31.6 ± 0.4	31.4 ± 0.5	31.0 ± 0.6
II		38–46	30–40	28–41	22–53	27–59	24–52	23–46	22–48	21–39
III		0.00	18.75	41.18	40.00	6.59	36.84	63.30	59.69	60.00
						E_3				
						13*	19*	41*	37*	20*
					I	35.9 ± 1.4	31.5 ± 1.2	31.9 ± 0.7	28.9 ± 1.0	27.2 ± 1.1
					II	30–45	23–42	24–40	19–37	20–37
					III	38.46	57.89	63.41	72.97	80.00

F_{0-9}, Filial generations; ● E_1, E_2, E_3, exposure to X-rays. *No. of rats; I, vaginal opening – days (mean ± SE); II, range; III, percent of rats with advanced opening of the vagina.