PROLIFERATION OF LENTICULAR CELLS IN MOUSE EMBRYOS WITH HEREDITARY MICROPHTHALMIA

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UDC 612.844.1:612.6.05

The duration of the periods of the cell cycle and the proliferative pool in the lenses of 10-day mouse embryos homozygous for the fidget and ocular retardation genes were studied by autoradiography with thymidine-H^3. No difference in the duration of the individual phases of the cell cycle, the proliferative pool, and the localization of the nuclei of cells at different stages of the cell cycle could be found in the lenses of 10-day +/+, fi/fi, and or/or embryos. The results show that mutant fi and or genes do not act in the lenticular cells of embryos and that the appearance of lens defects is unconnected with a disturbance of cell proliferation.

The appearance of certain hereditary anomalies in mammals is connected with changes in the proliferative activity of the tissues. Mutant genes fidget (fi) and ocular retardation (or) in mice determine the appearance of microphthalmia as a result of inhibition of growth of the retinal anlage [1, 4]. In mice homozygous for one of these genes, besides a disturbance of development of the retinal anlage, delayed growth and developmental defects of the lens also are observed. Truslove [6] found that a decrease in size of the lens in homozygous mouse embryos of the mutant fidget line is a primary effect of the fi gene, while a decrease in the size of the eye is the result of the presence of a reduced lens in the optic cup. However, Konyukov and Vakrusheva [3] consider that developmental defects of the lens are secondary defects of the gene and are determined by inhibition of growth of the optic vesicle, leading to inadequate induction of the lens and to a disturbance of its separation from the ectoderm. In mouse embryos homozygous for the ocular retardation gene, the gene acts only in cells of the retinal anlage and developmental anomalies of the lens arise secondarily [4, 5].

Bearing in mind the fact that the fi and or genes prolong the presynthetic period (G_1) of cells in the retinal anlage [2], it was decided to study the rate of cell proliferation in the lenses of 10-day mouse embryos homozygous for these genes.

EXPERIMENTAL METHOD

The duration of the periods of the cell cycle and the proliferative pool were studied by thymidine-H^3 autoradiography in the lenses of 10-day mouse embryos homozygous for the fidget and ocular retardation genes. The control consisted of 10-day embryos of normal (+/+) mice with a similar heterogeneous genetic environment. Details of the method of autoradiography used were described earlier [2]. To determine the periods of the cell cycle an average of 250–300 cells were counted in the lenses of each embryo and the percentage of labeled mitoses calculated. Eight embryos from two mothers were used at each time. To estimate the proliferative pool at least five embryos from mice of each genotype studied were used at each time. The percentage of labeled nuclei in five central sections through the lens was calculated. The duration of periods of the cell cycle was determined graphically [7] by recording changes in the percentage of labeled mitoses at different times after a single injection of thymidine-H^3.

Laboratory of Phenogenetics, Institute of General Genetics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR B. N. Klosovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 7, pp. 107–109, July, 1972. Original article submitted January 6, 1972.

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TABLE 1. Index of Labeled Nuclei (in %) in Lenses of 10-Day +/+, fi/fi, and or/or Embryos after 1, 3, and 6 Injections of Thymidine-H^3 (M ± m)

Genotype of embryos	Index of labeled nuclei (in %) after injections of thymidine-H^3		
	1	3	6
+/+	59,1±1,57	92,4±0,84	100
fi/fi	59,9±1,34	92,1±1,25	100
or/or	60,8±1,85	92,9±0,89	100

EXPERIMENTAL RESULTS

The first labeled mitoses (12%) were observed in the lenses of +/+ embryos 1 h after injection of thymidine-H^3. The number of labeled mitoses reached the first maximum (90%) 4 h after the injection. The number of labeled mitoses 6 h after injection of the isotope was lower, and reached a minimum (15%) in the embryos after 10 h. The second maximum of labeled mitoses (65%) was observed 14 h after injection of thymidine-H^3. The number of labeled mitoses remained at the same level (65%) until 16 h after the injection and then gradually diminished. More than 50% of the lenticular cells in +/+ embryos passed through the stage of DNA synthesis in the period between 2.5 and 9 h.

The first labeled mitoses (12%) were found in the lenses of the fi/fi embryos also 1 h after injection of the isotope. The number of labeled mitoses reached the first maximum (90%) 5 h after injection. The number of labeled mitoses after 6 h was considerably reduced and the minimum (10%) was reached 10 h after injection of thymidine-H^3. The percentage of labeled mitoses rose sharply 14 h after injection of the isotope and reached the second maximum after 15 h (65%). The number of labeled mitoses thereafter fell again. In the fi/fi embryos more than 50% of the lenticular cells passed through the period of DNA synthesis between 2 and 8.5 h.

In the lenses of the or/or embryos the first labeled mitoses (15%) appeared 1 h after injection of thymidine-H^3. The number of labeled mitoses reached its first maximum (90%) 4 h after the injection. During the next 3 h the percentage of labeled mitoses remained at the same level. The number of labeled mitoses reached the minimum (15%) 9 h after injection of the isotope. The number of labeled mitoses reached the second maximum (65%) 14 h after injection. Starting from 18 h after injection of thymidine-H^3 the percentage of labeled mitoses fell gradually. In the or/or embryos more than 50% of the lenticular cells passed through the stage of DNA synthesis during the period between 1.5 and 8 h.

Analysis of the curves of labeled mitoses shows that the duration of the mitotic cycle in individual phases was identical in the lenses of mouse embryos of all the genotypes investigated. The duration of the mitotic cycle (T) for the lenticular cells of 10-day +/+, fi/fi, and or/or embryos was 10 h. The postsynthetic period (G_2) lasted 1 h and the period of DNA synthesis (S) lasted 6 h 30 min. The combined duration of the presynthetic period and of mitosis (G_1 + M) was 2 h 30 min.

In the embryos of all the genotypes investigated the curves of labeled mitoses were symmetrical for both peaks, indicating homogeneity of the cell population of the lenses for the duration of the presynthetic period of the cycle.

Experiments with repeated injection of thymidine-H^3 showed that all lenticular cells of 10-day +/+, fi/fi, and or/or embryos participate in division. The nuclei of all the cells were labeled after 6 injections of thymidine-H^3 (Table 1).

No differences were found in the localization of the nuclei of cells in different phases of the cell cycle in the lenses of the +/+, fi/fi, and or/or embryos. Nuclei of cells in the phase of DNA synthesis were located at the periphery of the lens, nearer to the retina, whereas the nuclei of mitotically dividing cells were located nearer to the cavity of the lens vesicle. This arrangement of the nuclei of the lenticular cells in different phases of the cell cycle is not accidental but is due to migration of the cell nuclei of the retinal anlage. Nuclei of cells of the retinal anlage in the phase of DNA synthesis are arranged as an inner layer facing the lens. In the phase of DNA synthesis the cell nuclei of the retinal anlage and of the lens are nearest together, and this evidently facilitates their interaction.

No differences are thus found in the duration of the individual phases of the cell cycle, the proliferative pool, or the localization of the nuclei in cells in the various stages of the cell cycle in the lenses of 10-day +/+, fi/fi, and or/or embryos. These results show that the mutant fi and or genes do not act in the lenticular cells of the embryos and that the appearance of defects of the lens, which are secondary effects of the genes in nature, is unconnected with a disturbance of cell proliferation.

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