Inhibition of DNA Synthesis in Embryonic Mouse Retina as a Result of Gene Interaction  

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It has been shown by autoradiography using 3H-thymidine that 11-day mouse embryos doubly homozygous for the autosomal recessive genes fidget (gene symbol fi) and ocular retardation (or), have three to five times fewer labelled nuclei in their retina anlages as do normal (genotype +/+ +/+) embryos singly homozygous for fidget (+/+ fi/fi) or ocular retardation (+/+ or/or). In 11-day embryos of +/+ +/+ , +/+ fi/fi and +/+ or/or genotypes the labelled nuclei are localized mainly in the inner zone of the retina anlage. However, in double homozygotes the indices of labelled nuclei were not significantly different in the inner and outer zones of the retina anlage. The retina anlage of 12-day double homozygote, fi/ff or/or, has practically no nuclei synthesizing DNA. Consequently, the mutant genes fi and or which prolong the G1 period of the cell cycle in single homozygotes, act synergetically to stop DNA synthesis in the retina anlage cells of 12-day fi/ff or/or embryos.

INTRODUCTION

The mutant genes fidget and ocular retardation have similar phenotypical effects in that either of these genes in a singly homozygous state causes microphthalmia. The autosomal recessive gene fidget (gene symbol fi) was described in mice as a "behavior gene" associated with eye defects and polydactylism (Grüneberg, 1943). Truslove (1956) studied the phenotypic expression of this gene and showed that fi/ff mice had both anomalies of the eye and the inner ear (labyrinth). The gene fi begins to act on the ninth day of embryonic development and results in an inhibition of mitotic activity on the cells of the brain and eye-vesicle. The retarded growth of the eye-vesicle leads to its delayed contact with surface ectoderm, thus preventing normal lens induction. In these cases, the lens does not develop at all or often does not bud off from the surface ectoderm (Konyukhov and Vakhrusheva, 1969). The inhibition of the proliferative activity of the retinal anlage and brain cells (the ventral part of diencephalon and the ventral and dorsal halves of myelen-
of embryos which are double homozygotes for the fi and or genes.

MATERIALS AND METHODS

 Autoradiographs labelled with $^3$H-thymidine were used to study DNA synthesis in the retina anlagen of 10, 11 and 12-day old embryos doubly homozygous for ocular retardation (or) and fidget (fi) genes. DNA synthesis was also studied in the retina anlagen of normal (+/+ +/+) and mutant embryos which were single homozygotes for either the fidget or ocular retardation gene.

To obtain mice of the fi/fi or/or genotype, +/+ or/or females were mated with +/+ fi/fi males. Heterozygous females of the +/fi +/or genotype were crossed with single homozygous +/+ or/or males and mice genotypically +/fi or/or were mated “inter se”. Double homozygotes, fi/fi or/or, had microphthalmia and abnormal behavior typical of mice +/+ fi/fi. Double homozygotes were also produced by mating females +/fi or/or with males fi/fi or/or. Mouse embryos of the fi/fi or/or genotype were obtained by crossing double homozygotes “inter se”. The day on which a vaginal plug was found was considered zero day of pregnancy.

A single intraperitoneal injection of $^3$H-thymidine (1.4 Ci/m mole) was given to pregnant females at a dose of 5 $\mu$Ci/g of body wt in order to determine the index of labelled nuclei. The animals were sacrificed 1 hr after the isotope injection and embryos were fixed in Carnoy’s solution and embedded. Deparaffinated serial cross sections 5 $\mu$m thick were coated with liquid emulsion and exposed for 2 wk at 4°C. The autoradiographs were developed and sections were stained through the emulsion with hematoxylin. Nuclei were considered labelled if they contained at least four silver grains. Using an ocular micrometer, the retina was arbitrarily divided into two equal inner and outer zones, the outer zone attaching to the pigment epithelium. The index of labelled nuclei was determined for a whole transverse central section, through the retina for the inner and outer zones, and for the central and peripheral parts of the retina (Fig. 1). An index of labelled nuclei in the retina anlage was determined from five central serial sections through the eye. Each index represents the mean value of indices from 15 embryos. In each 10-day old embryo of any genotype the percent of labelled nuclei was determined by evaluating 1200 nuclei. For each 11-day old embryo, 2000 nuclei were examined while for each 12-day old embryo, 3000 nuclei were scored. However in each 12-day old fi/fi or/or embryo, the percent of labeled nuclei was determined by evaluating 1000 nuclei only.

In order to determine the proliferating fraction (proliferation pool) of the cells of the developing retina anlage, 10 repeated $^3$H-thymidine injections (3 $\mu$Ci/g of body wt) were given to mice at 3-hr intervals. The animals were sacrificed 1 hr after the tenth isotope injection. Deparaffined

![Fig. 1. Diagram of a section through the eye of a 12-day old normal mouse embryo showing the regions where the indices of labelled nuclei in the retina anlage were determined. (a) Peripheral part (an area up to 56 $\mu$m from the retina edge); (b) central part (an area up to 56 $\mu$m from the optic nerve); (c) inner zone; (d) outer zone; P.E., pigment epithelium; N.O., optic nerve.](image-url)
transverse sections of embryos were coated with emulsion and exposed as described above for 3 wk. Quantitative data were evaluated statistically according to the Fisher–Student method.

RESULTS

In 10-day embryos of the same genotype the indices of labelled nuclei in the central after a single ³H-thymidine injection, the were not significantly different. An hour after a single H³-thymidine injection, the labelled nuclei were localized mainly in the inner zone of the retina anlage in embryos of all studied genotypes. In +/+ fi/fi and fi/fi or/or embryos the indices of labelled nuclei in the inner and outer zones of the retina anlage are values intermediate between these for +/+ +/+ and +/+ or/or embryos. In +/+ +/- embryos the index of labelled nuclei in the retina anlage was 81.6% ± 1.81, and in +/- or/or embryos, 54.6% ± 2.62. The indices of labelled nuclei in the retina anlage of +/- fi/fi and fi/fi or/or embryos were not significantly different from each other, but their mean value of 69.5% ± 1.82 was significantly different from the values obtained for +/- +/- and +/+ or/or embryos (Fig. 2).

In 11-day old embryos +/- +/-, +/- fi/fi and fi/fi or/or as well as in 10-day old embryos, the indices of labelled nuclei in the central and peripheral parts of the retina anlage were not significantly different within the same genotype. However, in +/- or/or embryos the index of labelled nuclei in the peripheral part of the retina anlage was 2 times lower as compared to its central part. In embryos of the +/- +/-, +/- fi/fi and +/- or/or genotypes an hour after a single ³H-thymidine injection, the labelled nuclei are localized mainly in the inner zone of the retina anlage. However, in the inner and outer zones of the retina anlage of double homozygotes the indices of labelled nuclei were not significantly different. In +/- +/- embryos the index of labelled nuclei in the retina anlage has a mean value of 68.2% ± 1.01, while in +/- fi/fi embryos it is 61.3% ± 0.27, and in +/- or/or embryos, it is 54.5% ± 0.70. In double homozygotes, the indices of labelled nuclei in the retina anlage are 15.9% ± 1.94, three to five times lower than the indices in retina anlagles of embryos of other genotypes (Fig. 2).

In 12-day +/- +/- and +/- fi/fi embryos the indices of labelled nuclei in the central and peripheral parts of the retina anlage were not significantly different from each other. However, in +/- or/or embryos the index of labelled nuclei in the central

![Fig. 2. Histograms of indices of labelled nuclei in the retina anlagles of 10, 11 and 12-day old embryos +/- +/-, +/- fi/fi, +/- or/or and fi/fi or/or after an injection of ³H-thymidine. 11-day old embryos fi/fi or/or have considerably fewer labelled nuclei as compared to normal embryos ( +/- +/-) and embryos homozygous for fi or or genes (single homozygotes). 12-day old double homozygotes have practically no nuclei synthesizing DNA.](image-url)
part of the retina anlage was almost three times as high as that in its peripheral part. In +/+ +/+ and +/+ filfi embryos the labelled nuclei are mainly localized in the inner zone of the retina anlage but in +/+ or/or embryos the indices of labelled nuclei in the inner and outer zones of the retina anlage were not significantly different from each other. In +/+ +/+, +/+ filfi and +/+ or/or embryos the indices of labelled nuclei of the retina anlage were 71.4% ± 2.65, 62.4% ± 0.11 and 49.9% ± 2.57 respectively. In 12-day old double homozygotes the retina anlage consists generally of a single layer of cells with a mean-index value of 0.30% (Fig. 2). Half of the filfi or/or embryos analyzed did not have labelled nuclei in the retina anlage. These data indicate that DNA synthesis in the retina anlage cells of 12-day old double homozygotes is nearly completely blocked.

As seen in Fig. 3, the eye-cup of most 12-day old embryos filfi or/or was small and there were many mesenchymal cells between the lens and retina anlage. The inner cell layer of the eye-cup (the retina anlage) was very thin and cells were infiltrated with pigment granules, especially in the peripheral part. The uniformly thick walled lens vesicle was situated out of the eye-cup cavity (Fig. 3). In embryos with a complete inhibition of DNA synthesis in the retina anlage cells, the eye-cup was very small and flat, and the lens vesicle, as a rule, had not budded off from the surface ectoderm. The inner layer of the eye-cup was, in fact, was indistinguishable from the outer layer which forms the pigment epithelium. Such abnormal eye-cups were situated far beneath the surface ectoderm.

Experiments with repeated isotope injections starting at the 11th day of pregnancy show that in the retina anlage of double homozygotes, the fraction of proliferating cells is only to 15.0%, six times less than that for embryos of other genotypes (Table 1). The data obtained in experiments with repeated ³H-thymidine injections administered on the 12th day of pregnancy indicate that DNA synthesis is absent in all retina anlage nuclei of double homozygotes. On the other hand, the proliferation pool in the retina anlage of normal embryos (+/+ +/+ ) is less as compared to that of +/+ filfi and +/+ or/or embryos (Table 1), and is the result of the differentiation of a larger number of retina anlage cells in normal embryos than in mutant ones. After repeated isotope injections in +/+ +/+ embryos, unlabelled nuclei were localized in the inner zone of the retina anlage, mainly in the central part. These cells appear to be

![Fig. 3. Sections through the eyes of 12-day old mouse embryos: (a) Normal embryo genotype +/+ +/+ (x 120); (b) double homozygote, genotype filfi or/or (x 200).](image)
TABLE 1

INDICES OF LABELED NUCLEI (in %) IN THE RETINA ANLAGES OF MOUSE EMBRYOS OF DIFFERENT GENOTYPES AFTER 10 REPEATED INJECTIONS OF tH-ThYMIDINE

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Beginning of tH-thymidine injections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11th day of embryogenesis</td>
</tr>
<tr>
<td>++/++</td>
<td>91.8 ± 0.88</td>
</tr>
<tr>
<td>+/- or/or</td>
<td>91.2 ± 1.86</td>
</tr>
<tr>
<td>++ fi/fi</td>
<td>98.8 ± 0.30</td>
</tr>
<tr>
<td>fi/fi, or/or</td>
<td>15.0 ± 0.05</td>
</tr>
</tbody>
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*Index of labeled nuclei in the retina anlage was determined from five central serial sections through the eye. Each value is expressed as the mean of indices for seven embryos + the standard error about the mean.

The retina anlage consists generally of a single, heavily pigmented layer of cells.

differentiating ganglion and Muller glial cells. In ++/++ fi/fi embryos unlabelled nuclei are, as a rule, localized in the inner zone of the retina anlage, although a small number of unlabelled nuclei are also situated in the anlage outer zone. In +/- or/or embryos unlabelled nuclei are observed both in the inner and outer zones with most of the unlabelled nuclei localized in the peripheral part of the retina. It should be noted that in the peripheral part of the retina anlage of +/- or/or embryos, some cells contain melanin granules. Embryos of this genotype at later stages of embryogenesis have heavily pigmented cells in the peripheral part of the retina. Abnormal regional distribution of labelled nuclei in mutant embryos is evidently due to a disturbance in retinal histodifferentiation.

DISCUSSION

Most of the data on gene interactions during the development of higher organisms comes from studies of the effects of mutant genes. Nonallelic mutant genes with similar phenotypical effects can interact sinergetically, having additive effects upon the defective development of certain organs. Many cases of such interaction of mutant genes are described in hemopoiesis (Russell et al., 1968), skeletal development (Forsthoefel, 1962) and hair pigmentation in mice (Silvers, 1963), and also pigmentation and hair structure in guinea pigs (Wright, 1963). Konyukhov et al. (1970) found that in mice homozygous for mutant genes fi and or, double homozygotes, eye development is more disturbed than in single homozygotes (+/+ fi/fi or ++/+ or/or). In double homozygotes these mutant genes act sinergetically and eye development stops at the eye-cup stage. In the late stages of embryogenesis and in newborn fi/fi or/or mice, the development of the eye does not progress and does not differ greatly from that achieved by the 11- or 12-day old embryos. In double homozygotes the beginning of the action of or gene occurs one day later than it does in mouse embryos singly homozygous for gene or. The gene fi begins to act in the retina anlage cells earlier than the gene or, resulting in a delay in retina anlage differentiation.

The data presented here also show that in double homozygotes the effects of gene or are manifested later than in embryos which are single homozygote (+/+ or/or). In 10-day old fi/fi or/or embryos the index of labelled nuclei in the retina anlage is the same as that for +/- fi/fi embryos, but it is much higher than the index of labelled nuclei in the retina anlage of +/- or/or embryos. This fact shows that the or gene is inactive in the retina anlage cells of 10-day double homozygotes. The gene or begins its activity in the retina anlage cells of 11-day old embryos fi/fi or/or. The 11-day old double homozygotes have a significantly smaller number of nuclei synthesizing DNA in the retina anlage than do normal embryos (+/+ ++/+ or/+ fi/fi) or single homozygotes (+/+ fi/fi and +/+ or/or). The or gene is activated later in the retina anlage of double homozygotes because the
retina anlage cells divide fewer number of times due to the action of the \( fi \) gene. Initially the \( or \) gene does not seem to act in all retina anlage cells of 11-day old embryos \( fi/\text{or} \), but later, this gene is activated causing a complete cessation of DNA synthesis in the rest of the cells of the retinal anlage.

Molecular events that are responsible for DNA synthesis take place in the presynthetic period of interphase and emphasize the extreme importance of this period in the cell cycle. Prescott (1968) has demonstrated that the regulatory mechanisms which set the rate of the cell renewal act during the \( G_1 \) period. In his opinion there is "a control point" of cell reproduction at the end of the presynthetic period. In mouse embryos of the \( fi/\text{or} \) genotype, the mutant genes \( fi \) and \( or \) seem to act synergistically in the presynthetic period, causing the disturbance of some event(s) for the preparation of DNA synthesis in retina anlage cells.

REFERENCES


