ACTIVATION OF GENES OF γ- AND β-CRYSTALLINS IN MORPHOGENESIS OF THE MOUSE LENS

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The anlage of the lens (the placode) arises from cells of the cranial ectoderm as a result of the inductive action of the entomesoderm and optic vesicle on them, and the influence of the retina is necessary for the formation of fibers of the developing lens [3, 5]. In chick embryos the RNA for the δ-crystallins appear for the first time in the lens ectoderm 8-9 h after the beginning of induction by the optic vesicle [10]. However, the connection of induction with activation of the genes of the crystallins has not been studied in mammals. In the developing mouse lens, α-crystallins are synthesized first in cells of the proximal wall of the newly formed lenticular vesicle, whereas synthesis of γ- and β-crystallins begins later — in the primary lenticular fibers [2, 4, 8]. The discovery of synthesis α-crystallins in mouse embryos of the mutant eyeless line, in which development of the lens ceases as a rule at the placode stage, indicates derepression of the genes of these proteins at the stage of formation of the lens placode [2]. One approach to determination of gene activation time is by studying the effect of inhibition of transcription by actinomycin D on the development of a certain trait [6]. The manifestation of gene effect after treatment with actinomycin D indicates that activation of the gene (genes) took place before the inhibitor was used.

The object of this investigation was to study expression of the genes of γ- and β-crystallins in anlagen of the mouse lens cultured after treatment with actinomycin D.

EXPERIMENTAL METHOD

Mouse embryos of inbred line CC57BR at the age of 10.5 days were divided into three groups, depending on the stage of development of the lens, defined on the basis of the degree of pigmentation of the outer layer (POL) of the optic cup. During this period of embryogenesis POL, starting on the dorsal aspect of the optic cup, gradually spreads to the ventral part. As a preliminary histological investigation showed, POL corresponded to the following stages of morphogenesis of the lens: the lens vesicle with walls of equal thickness (1/3 POL), the proximal wall of the lens vesicle 1.5 times thicker than the distal wall (1/2 POL), the proximal wall twice as thick as the distal wall (2/3 POL). The lenses were isolated from anlagen of the eyes treated with 0.02% versene solution, and cultured in medium consisting of 60% Eagle's medium with glutamine, 20% homologous embryonic extract, and 20% inactivated embryonic calf serum, in an atmosphere containing 5% CO_2. Analgen of the lenses were treated for 1 h with actinomycin D in a dose of 1 μg/ml which, as was shown by autoradiography, completely inhibits RNA synthesis. The nutrient medium was then changed and the lenses were cultured for 15, 18, or 20 h. After culture of the lenses, crystallins were determined in them by the indirect immunofluorescence method, using antibodies against γ- and β-crystallins obtained by the methods described previously [2, 4]. In control experiments the lenses were cultured without treatment with actinomycin D.

EXPERIMENTAL RESULTS

Cytological analysis of the 10.5-day embryos showed that dividing cells were present in the proximal wall of the lens vesicle in all cases. At the stage of development when the

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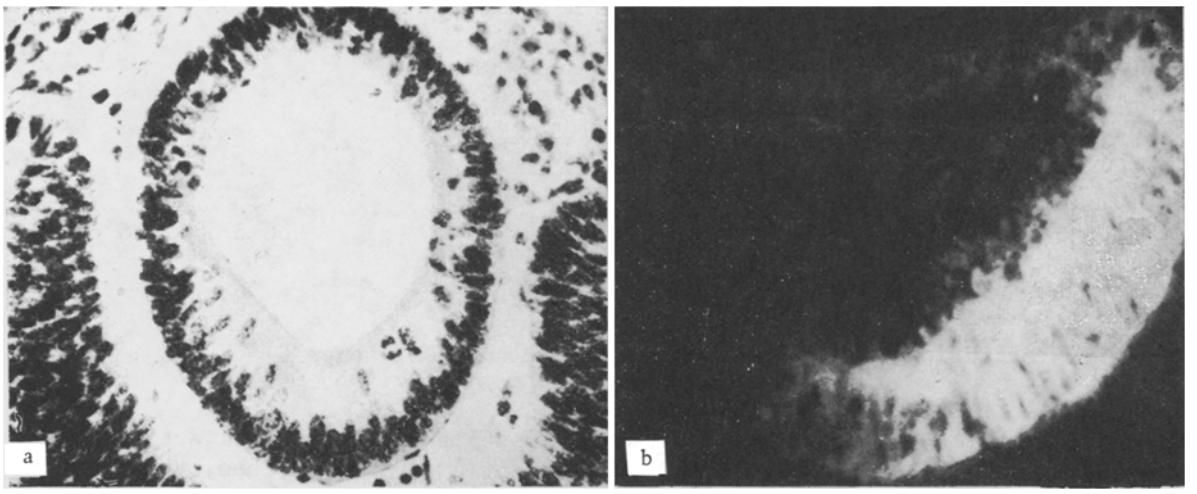

Fig. 1. Synthesis of γ-crystallins in mouse lens anlage *in situ*: a) lens anlage of 10.5-day mouse embryo before culture; proximal wall of lens vesicle is twice as thick as distal wall (outer layer of optic cup pigmented by $^2/_3$). Hematoxylin and eosin; b) culture for 20 h after treatment with actinomycin D (1 μg/ml) for 1 h: positive immunohistochemical reaction for γ-crystallins can be seen in cells of proximal wall. 300 ×.

TABLE 1. Expression of Genes of γ- and β-Crystallins in Cells of Lens Analgen Treated with Actinomycin D in a Dose of 1 μg/ml for 1 h and Then Cultured

Stage of morphogenesis of lens	No. of lenses cultured	Duration of culture, h	No. of lenses in which crystallins were found	
			γ	β
Walls of lens vesicle equal in thickness	10	15	0	0
	10	18	0	0
Lens vesicle whose proximal wall is is 1.5 times thicker than the distal wall	10	15	2	0
	5	18	0	0
Lens vesicle whose wall is twice as thick as the distal wall	10	15	7	0
	10	18	5	5
	9	20	9	9

proximal wall of the lens vesicle was twice as thick as the distal wall, only single dividing cells were found in it (Fig. 1a).

After culture for 1 h synthesis of γ- and β-crystallins was not present in the anlagen of either the control or the experimental lenses. This indicates that treatment with actinomycin D had been carried out before the appearance of these proteins in morphogenesis of the lens. Immunofluorescence analysis of lens anlagen cultured for 15 or 18 h after treatment with actinomycin D at the stage of equal thickness of the walls of the lens vesicle likewise showed no synthesis of γ- and β-crystallins (Table 1). These results are evidence that activation of genes of the γ- and β-crystallins has not yet taken place at this stage of morphogenesis of the lens. After treatment with actinomycin D and subsequent culture of the lens anlagen whose proximal wall was 1.5 times thicker than the distal wall for 15 and 18 h, in two of 15 cases γ-crystallins were found in the cells of the proximal wall of the lens vesicle. However, β-crystallins were absent in all the lenses cultured. Treatment with actinomycin D inhibited morphogenesis of the lenses, and after 15 or 18 h of culture

they were still at the lens vesicle stage. Meanwhile, in lens anlagen not treated with antibiotics, the lenticular epithelium and primary lenticular fibers were formed and partially (by 1/3 or 1/2) filled the cavity of the lens vesicle. In all cases γ- and β-crystallins were found in these lenses, and were synthesized, just as *in situ*, only in the lenticular fibers.

Lens anlagen in which the proximal wall was twice as thick as the distal did not develop after treatment with actinomycin D and culture for 15, 18, or 20 h but remained at the lens vesicle stage. However, despite the absence of morphological signs of fiber formation, γ- and β-crystallins were discovered in the cells of the proximal wall of these lenses (Fig. 1b). After culture for 20 h the number of anlagen in serial sections of which both γ- and β-crystallins were found reached 100% (Table 1). Consequently, in these lens anlagen activation of genes of γ- and β-crystallins took place before treatment with actinomycin D.

One of the side effects of actinomycin D is its toxicity for cells, which is not due, as has been suggested, to inhibition of RNA synthesis [6]. In the present investigation lengthening the duration of culture of the lens anlagen treated with actinomycin D to 20 h led to the appearance of destructive changes in some cells of the lens vesicle. The appearance of such changes in the lenses did not allow longer periods of culture to be used.

According to our own observations and data of other workers [1, 9], in the initial stages of formation of primary lens fibers both mitotically dividing cells and cells not synthesizing DNA but on the road to differentiation were present in the proximal wall of the lens vesicle. During this period of morphogenesis of the lens when the cells of the proximal wall are passing through the last mitotic cycle, derepression of genes of the γ- and β-crystallins takes place. This is also shown by the fact that γ- and β-crystallins were synthesized during culture of lens anlagen not treated with actinomycin D, whose proximal wall was 1.5 times thicker than their distal wall.

Consequently, activation of genes of γ- and β-crystallins in the morphogenesis of the mammalian lens takes place at the lens vesicle stage, i.e., much sooner than in amphibians [7, 11].

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