DETECTION OF LENTICULAR ANTIGENS IN THE EARLY STAGES OF DEVELOPMENT OF Rana temporaria

S. S. Kirzon, R. F. Averkina, and O. E. Vyazov

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During embryogenesis of Rana temporaria the organ-specific antigen of the lens is clearly demonstrable after the stage of the tail bud, corresponding to the period of laying down of the lenticular placode. The general organ antigen of the lens was detected at all early stages of development of the frog, and also in unfertilized oöcytes. After fertilization there is a rapid decrease in the number of antigens in the oöcyte, with a gradual increase after the gastrula stage.

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Morphological differentiation of the tissues of many animals has been studied in considerable detail in the course of ontogenesis. However, no clear idea has yet been obtained of the differentiation of specific tissue proteins (and, consequently, of tissue antigens), lying at the basis of morphogenetic processes [1, 3, 4], in the course of individual development. In particular, too little is known of the time of appearance of organ-specific antigens.

The object of the present investigation was to determine which lenticular antigens of the frog Rana temporaria are present in the early stages of individual development, and to study the dynamics of their changes in this period.

EXPERIMENTAL METHOD

Unfertilized frog's oöcytes, embryos at the stages of cleavage, blastula, early and late gastrula, early and late neurula, tail bud, and newly hatched tadpoles were used as test object. Homogenates prepared as

TABLE 1. Detection of Lenticular Antigens in Homogenates of Frog (R. temporaria) Embryos in Early Stages of Development

Antiserum	Antiserum No.	Tissue extracts and homogenates									
		Lens	Unfertilized oöcytes	Embryos at stages of: Cleavage	Blastula	Early gastrula	late gastrula	tail bud	late neurula	tail bud	Newly hatched tadpoles
Rabbit	10	6	4	1	1	1	2	3	3	3	3
	9	5	4	1	1	1	2	3	3	3	3
	875	5	3	1	1	1	2	2	3	3	3
	845	4	2	1	1	1	1	2	2	2	2
Guinea Pig	27	2	1	0	0	0	0	1	1	1	1
	28	3	1	0	0	0	1	1	1	1	1

Note. The results of 173 tests are given in this table. In the terminology of Cambar and Marrot early neurula corresponds to stages 16-17, late neurula to stage 19, tail bud stages 20, 21, and 22, and the newly hatched tadpole to stage 23.

Laboratory of Immunology of Embryogenesis, Institute of Experimental Biology, Academy of Medical Sciences of the USSR, Moscow (Presented by Academician of the AMN SSSR N. N. Zhukov-Verezhnikov).
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TABLE 2. Detection of Specific Frog Lenticular Antigens

Antiserum	Antiserum no.	Tissue extracts and homogenates								
		Lens	Unvertilized oöcytes	Embryos at stages of						
				cleavage	blastula	early gastrula	late gastrula	neurula	tail bud	newly hatched tadpoles
Rabbit	10	4	0	0	0	0	0	1	1	1
	9	3	0	0	0	0	0	1	1	1
	875	3	0	0	0	0	0	1	1	1
	845	3	0	0	0	0	0	0	1	1
Guinea pig	27	1	0	0	0	0	0	0	1	1
	28	2	0	0	0	0	0	0	1	1

Note. Results of 181 tests are summarized in this table.

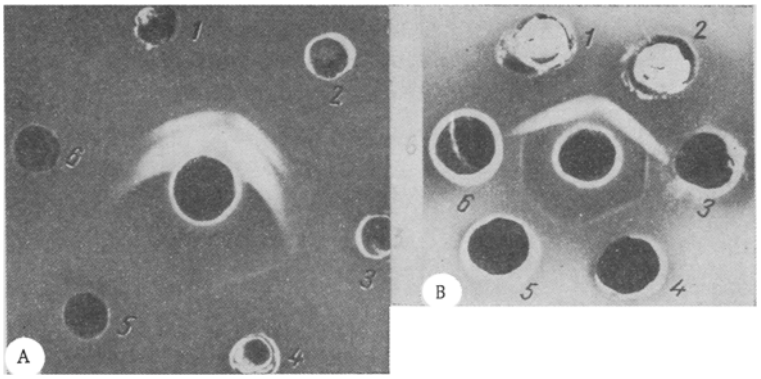

Fig. 1. Agar precipitation test with rabbit lenticular antiserum (A) and with guinea pig serum (B) absorbed with blood serum and extracts from frog kidney, liver, and spleen (in central wells). 1 and 2) extract from frog lens; A: 3 and 4) homogenate of newly hatched tadpoles; 5 and 6) homogenate of embryos at gastrula stage. One precipitation band is visible between central well and 3-4, merging with one of the three precipitation bands formed between the central well and 1-2. B; 3 and 4) homogenate of embryos at tail bud stage; 5 and 6) homogenate of embryos at neurula stage. One precipitation band visible between central well and 3-4 and also 5-6, merging together and also with one of the two bands formed between the central well and 1-2.

follows were used in the experiments: 25 eggs taken at the same stage of development were ground in a mortar with the addition of 0.2 ml physiological saline.

Antisera against the lenses of R. temporaria were obtained in 4 rabbits and 2 guinea pigs. The rabbit antisera contained antibodies in titers of 1:10,000-1:20,000. The titer of antibodies in the immune sera obtained in guinea pigs was 1:320-1:640. The ring-precipitation test and Ouchterlony's agar precipitation test were used.

EXPERIMENTAL RESULTS

The experimental results obtained with unabsorbed lens antisera are given in Table 1.

As Table 1 shows, from 1 to 4 antigens similar to lenticular antigens of the adult frog were detected by means of rabbit antisera. A definite pattern was observed: with unfertilized frog oöcytes from 2 to 4 precipitation bands were formed, after fertilization one precipitation band, and starting from the gastrula stage an increase in the number of bands from 1 to 3. The same pattern was observed in tests between lenticular antisera obtained in guinea pigs and antigens in the early stages of development.

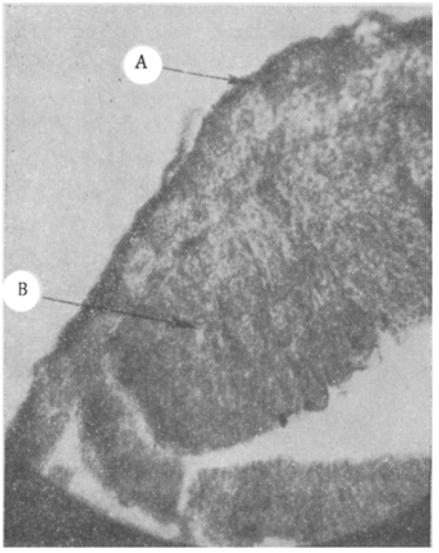

Fig. 2. Formation of the lenticular placode at the tail bud stage of R. temporaria. Contact between optic vesicle (A) and ectoderm (B). Thickening of the ectoderm is seen in the region of contact, with formation of the future lens, 90 ×. Carazzi's hematoxylin.

To determine which lenticular antigens are formed in the early stages of development of the frog, species-specific antibodies were first removed from the antisera by absorption of these sera with normal frog blood serum, after which the same sera were absorbed by a mixture of normal blood serum and extracts from liver, kidney, and spleen.

Lenticular antisera absorbed by normal frog blood serum only continued to react with extracts of liver, kidney, and spleen forming one or two precipitation bands, and with lenticular antigens forming 4 or 5 bands; these sera formed fewer precipitation bands with extracts from frog embryos in the early stages of development compared with the results given in Table 1: one precipitation band with extracts from embryos at the stages of cleavage, blastula, and gastrula; two precipitation bands at the stages of neurula, tail bud, and newly hatched tadpole.

Lenticular antisera absorbed by a mixture of normal serum and extracts of the above organs did not react with any extract of frog tissues except lens extract.

As Table 2 and Fig. 1 show, by means of these rabbit antisera 3 or 4 organ-specific lenticular antigens were detected in lens extracts, while in homogenates of frog embryos starting from the neurula stage, and more clearly from the tail bud stage, one organ-specific lenticular antigen was detected. Specific guinea pig lenticular antisera formed one precipitation band each with embryonic extracts starting from the tail bud stage.

The experiments thus showed that organ-specific lenticular antigen is clearly detectable for the first time at the tail bud stage. The time of discovery of organ-specific lenticular antigen corresponded to the period of laying down of the lens placode (Fig. 2). Formation of the lenticular placode was observed in these experiments at the tail bud stage. Other workers [6, 8] have also found lenticular antigen at the stage of formation of the lenticular placode. The results now obtained thus agree with the observations just cited and do not confirm other results [7] indicating preformation of organ-specific lenticular antigens in the frog oöcytes and in the early stages of individual development.

General organ antigens, i.e., antigens present in the lens and also in certain other frog organs (liver, kidney, spleen) were discovered in unfertilized oöcytes and at all early stages of development of R. temporaria. After fertilization there is a rapid decrease in the number of antigens detected in the oöcytes, and after the gastrula stage their number gradually increases. These results are in agreement with other observations [2, 5] showing that until the late blastula stage protein synthesis takes place through maternal messenger RNA, which is stable throughout this period. Toward the early gastrula stage, intensive formation of ribosomal structures synthesizing protein with the aid of newly formed messenger RNA is observed.

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