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14 July 1967; revised 15 October 1967

Protein-Synthesizing Activity of the Anucleate Polar Lobe of the Mud Snail Ilyanassa obsoleta

Abstract. *Polar lobes of eggs of the mud snail* Ilyanassa obsoleta, *detached at the "trefoil" stage of the first cleavage, are capable of incorporating labeled amino acid into protein. The rate of incorporation per unit volume is about half that of the whole egg. The ability to incorporate at a similar rate persists for at least 24 hours after isolation. The sum of the incorporation of isolated lobes and lobeless fragments approximates that of the whole egg. The results extend to this material (the anucleate polar lobe) evidence for long-lived messenger RNA. They suggest also that the demonstrated morphogenetic influence of the lobe, which is exerted primarily during cleavage, may be correlated with its ability to synthesize protein.*

The eggs of annelids, mollusks, platyhelminths, and ascidians have commonly been designated as "mosaics," in that the blastomeres of the early cleavage stages have been thought to be developmentally fully specified, the regional specification applying also to the uncleaved egg (*1*).

The supposition of "mosaicism" is, however, not quite justified. For example, in the oligochaet annelid *Tubifex* (*2*), in the polychaet annelids *Chaetopterus* (*3*), *Nereis* (*3*), and *Sabellaria* (*4*), and in the mollusk *Cumingia* (*3*) double monsters can be obtained from a single egg by simple procedures that equalize the normally unequal first cleavage. Also, whole embryos can be obtained from eggs in which one or more blastomeres have been removed (*3*). Further, in ascidians (*5*) two whole eggs can be fused to give a single embryo. Even in the snail *Ilyanassa,* whose eggs had been thought to be fairly strict mosaics on the basis of early experiments (*6*), more recent analyses (*7*) of developmental effects of deletion of various blastomeres or localized regions show interactive influences rather than full regional specification. Although these interactions may result often in the appearance of early determination, as in experiments with the annelid *Nereis* (*8*), basically the situation is not different from that of "regulative" eggs such as those of sea urchins and amphibia. In these, there are interactions between parts, as revealed in the initial experiments of Lewis (*9*) and Spemann (*10*) with amphibia and of Hörstadius (*11*) with sea urchins. The influences may be predominantly unidirectional as in amphibia, in which they are termed inductions, or more reciprocal, as in the sea urchins, in which they are termed gradient-system interactions.

Although so-called "mosaic" and "regulative" eggs are basically similar in developmental properties, the former often do exhibit some special features that seem useful for the exploration of regional cytoplasmic properties in relation to gene-activation and the determination of embryonic parts. In this connection there have been a number of recent investigations (*12–14*) of protein and RNA synthesis of eggs belonging to the so-called "mosaic" group.

One special feature of eggs of certain annelids and mollusks is the presence of a temporary cytoplasmic protuberance called the polar lobe at specific times during early development. In the mud snail *Ilyanassa obsoleta,* the lobe is particularly prominent at the first cleavage, having approximately one-quarter to one-third the volume of the egg and being almost fully constricted from the two blastomeres during the progress of the first division (Fig. 1). At this time it is readily removed from the egg, and this feature has permitted the earlier experimental embryological investigations (see *6, 7*) with this species.

Experiments (*13*) on the rate of incorporation of labeled leucine into protein of lobeless eggs of this species have shown that it is somewhat lower than that of the whole egg, and especially so around the 4th day, shortly before the most active period of organ formation. Determinations (*14*) of incorporation of labeled uridine into RNA by lobeless embryos showed a decrease in rate, relative to the controls, starting at the gastrula stage (about 1 day).

From these investigations and from studies on anucleate egg fragments in other species (*15*), one might infer that the isolated lobe would be capable

Table 1. Incorporation of C^{14}-labeled amino acids into protein by whole eggs, lobeless eggs, and isolated polar lobes of *Ilyanassa obsoleta.* Incubation for 2 hours at 20°C with three amino acids (2.5 μc of each per milliliter). Experiments 1 to 3: aspartic acid (164 c/mole), arginine (9.23 c/mole), and valine (195 c/mole). Experiments 4 to 9; glutamic acid 195 (c/mole), arginine (234 c/mole), and valine (195 c/mole). The a and b refer to separate tests on eggs from the same lots. Abbreviation: cpm, counts per minute.

Experiment	Time after first division (hr)	Whole eggs		Lobeless eggs			Lobes		
		Number	cpm per egg	Number	cpm per egg	Percentage of whole egg (average)	Number	cpm per lobe	Percentage of whole egg
1*	1½	90	66.4	100	60.7	92	100	7.5	11
2a*	1¼	28	80.1	28	52.1	72	28	11.7	12
b	1¼	21	82.2	21	64.6		21	7.7	
3a	1½	17	61.4	17	65.8	98	17	8.6	15
b	1½	17	73.6	17	66.1		17	12.0	
4a	2¼	14	98.9	14	75.2	85	14	13.3	14
b	2¼	14	86.7	14	82.8				
5a	4¾	23	110.1	23	104.3	105	23	13.4	14
b	4¾	23	87.0	23	103.3		23	13.2	
6	1⅔	7	59.4	7	43.1	73	14	10.7	18
7a	1½	16	78.4	20	98.7	109	19	9.2	11
b	1½	19	94.8	20	89.9		14	10.3	
8	27	16	117.2	16	113.6	97	16	8.1	7
9a†	24	10	86.5	10	106.8	104	10	9.9	14
b	20	8	105.9	8	93.0		8	17.4	

* Eggs, lobeless eggs, and lobes pooled equally from three lots. † The parallel a and b groups were from different lots of eggs.

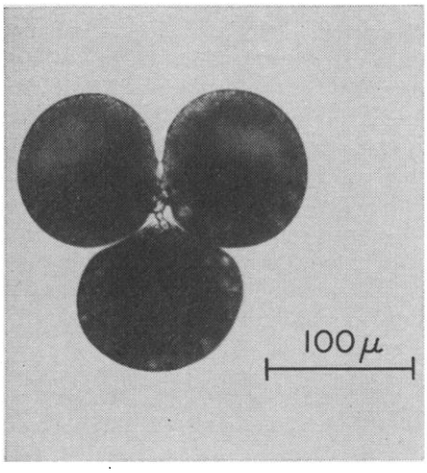

Fig. 1. The egg of *Ilyanassa* at the "trefoil" stage of first cleavage. The upper spheres are blastomeres, the lower one the polar lobe.

of protein synthesis. Nevertheless, it seemed to us desirable that this be examined directly inasmuch as the information would be of importance in any attempts that may be made to ascertain the biochemical mechanism of the lobe's morphogenetic action.

Measurements of the incorporation of C^{14}-labeled amino acid into protein were made on isolated lobes, lobeless eggs, and whole eggs of *Ilyanassa* in nine sets of experiments. In two of these the measurements were made 1 day after the lobes were isolated. Handling of the eggs was done in artificial seawater (*16*) by previously described methods (*7*); lobes were detached by gently agitating dividing eggs in seawater free of calcium. All cultures were checked to insure that they contained only the isolated lobes, lobeless eggs, or whole eggs as designated, and that it was, in fact, the lobe and not a blastomere that had been detached in each case. This is readily ascertained by inspection, at the time of isolation and transfer, since the lobe differs in appearance from a blastomere, the latter showing a clear area where the nucleus or spindle is located (Fig. 1). It is further checked, during incubation, by noting the occurrence of cleavage in the lobeless eggs and its absence in the isolated lobes. The numbers of eggs, lobes, or embryos in the individual cultures ranged from 7 to 100 (Table 1). Lobes and lobeless fragments were derived from the same eggs in each experiment, and the whole eggs came from the same capsule except as noted. For zero-time control (t_0) equivalent numbers of whole eggs were employed. The t_0 values were generally close to background, indicating no significant binding of the labeled amino acids to egg material precipitable with hot acid, or carry-over and binding to the filter papers employed in processing the material.

The material was processed on filter paper, and the radioactivity of the protein was assayed in a scintillation counter as described elsewhere (*17*). Since it could be assumed that in eggs of *Ilyanassa*, as in those of sea urchins (*18*) and in many other kinds of cells, there is competition among amino acids of similar chemical type for entrance into the cell, "noncompeting" mixtures of three C^{14}-labeled amino acids were employed so as to provide for more efficient labeling of the resulting protein.

The isolated lobes in all cases showed considerable ability for amino acid incorporation (Table 1). In the seven sets of experiments in which the measurements were made soon after isolation, the lobes exhibited about one-eighth the incorporating activity of the whole eggs. Measurements of the diameters of lobes and blastomeres from photographs indicated that the relative size of the polar lobe varies somewhat in different lots of eggs; data from five lots (on the equivalent of about 100 eggs) gave an average value of about 29 percent of the volume of the whole egg, with the average ranging from 24 to 34 percent in different lots. The lobes therefore have about half as much incorporating ability per unit volume as the whole egg. The sum of the activities of the lobe and the lobeless egg is not significantly different from that of the whole egg; or, stated differently, our data do not indicate that any marked change in activity has occurred in the lobeless fragment after removal of the lobe.

In the two sets of experiments in which the measurements of amino acid incorporation were made 1 day after isolation, the lobes maintained approximately the same activity as is exhibited soon after isolation. At this time, the corresponding whole eggs and lobeless embryos had increased in activity by a third or more compared with the average of the results of the other experiments.

The lower activity, per unit volume, of the isolated lobe relative to that of the blastomeres, may be correlated with its relatively higher content of yolk, or conversely, with its relatively lower content of clear cytoplasm. The activity of the lobe obtained in our experiments (about 13 percent of the whole egg) is fairly close to a previously recorded estimate (*19*) of the amount of clear cytoplasm content in the lobe (16.5 percent that of the whole egg).

From our results, it is clear that the isolated lobe is active in protein synthesis although its activity per unit volume, if it is assumed that there are no differences in amino acid pool or in permeability to amino acids, is about half that of the whole egg, or one-third that of the lobeless blastomeres. Also, the activity is maintained for at least a day. This, then, along with experiments on anucleate egg fragments in other species (*15*) provides another example of the occurrence of long-lived messenger RNA in animal cells. Since, also, the lobe exerts its morphogenetic influence during the 1st day (*7*), our results indicate that hypotheses concerning the mechanism of such action can include the possibility that the effective agents are synthesized locally in the lobe during this early period, without immediate gene control.

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20. Supported by NSF grants GB-28 to A.T. and GB-5331X to A.C.C. and PHS grants GM-12777 and 8 RO1 HD 03476 to A.T.
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18 October 1967