Insect Embryogenesis: Macromolecular Syntheses during Early Development

Abstract. *A new technique permits the injection of aqueous solutions into the eggs of certain Coleopteran insects. DNA and protein are synthesized from the outset of development, but the synthesis of RNA is not detectable until the migrating cleavage nuclei arrive at the cortex of the egg.*

Insect embryogenesis presents an excellent opportunity to analyze nuclear-cytoplasmic interaction. Apparently equivalent cleaving nuclei migrate into specialized and spatially restricted regions of cortical cytoplasm, whereupon they undertake new, distinct synthetic activities related to the formation of the embryo. At the same time, the formation of cell membranes isolates the nuclei from one another, and the animal loses much of its capacity to repair major injury (*1*). The morphological and functional, but not the biochemical, aspects of this type of development have been thoroughly studied (*2, 3*). I have developed a technique which permits the injection of reproducible amounts of materials into certain insect eggs, making it possible to apply techniques using radiotracers, antimetabolites, and other substances in the study of these eggs.

The principal hinderance to this approach has been the high turgor and impermeability of eggs of most insect species. When the eggs are punctured, cytoplasm flows from them, and as a result they frequently die. In the eggs of certain Coleoptera, the pressure may be overcome reversibly, and, ordinarily, the procedure itself causes no significant abnormalities during further development.

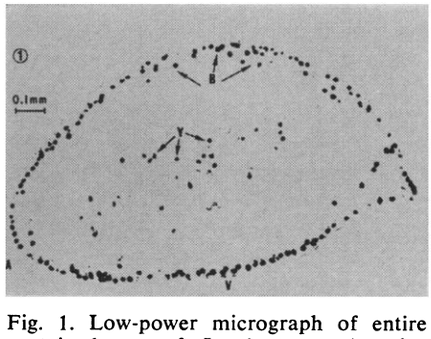

Fig. 1. Low-power micrograph of entire unstained egg of *Leptinotarsa*. Anterior (*A*) and ventral (*V*) sides are indicated. The egg was injected during late cleavage with .01 μc of H^3-thymidine and allowed to develop for 4 hours, at which time it was an early blastema. Blastoderm (*B*) and yolk (*Y*) nuclei are heavily labeled. Exposure time, 14 days.

In eggs of several species of Coleoptera, the tension is maintained by the vitelline membrane, a combination of the chorion and adhering "cement", and by the contractility of the yolk cytoplasm (*4*). The cement surrounding the eggs of *Leptinotarsa decimlineata* and *Tenebrio molitor* may be removed by treatment with mild alkali (5 minutes in 10 percent Na_2CO_3) followed by thorough washing in distilled water. This treatment is not necessary for eggs of *Dermestes maculata*. Both the cement and the rather tough chorion of *Leptinotarsa* eggs may be removed by treatment with sodium hypochlorite (*5*), which renders the eggs extremely fragile and is usually necessary only when the eggs are in the later stages of development. The chorions of *Tenebrio* and *Dermestes* eggs offer little resistance to glass needles as large as 20 μ in outside diameter.

In eggs of all three species, the taut vitelline membrane tends to relax in an atmosphere saturated with water or on damp filter paper. Placing the egg on a surface chilled to between 6° and 8°C is a more satisfactory technique; the volume and contractility of the yolk are markedly reduced, and the eggs may be injected without loss of protoplasm. Under these conditions, aqueous solutions may be injected into the eggs in quantities up to a maximum of 1 percent by volume of the eggs. The eggs are allowed to return slowly to room temperature, during which time the wound seals, and they are then either submerged in liquid paraffin (*3*) or, preferably, maintained in petri dishes on damp, sterile filter paper. One of these precautions must be taken to prevent desiccation of the injected egg.

The mortality following the injection of either distilled water or saline is normally less than 10 percent. Development of dechorionated eggs lags behind that of untreated controls by 4 to 6 hours, but otherwise no abnormalities are seen. Diffusion throughout the egg of injected dyes or radioisotopes is generally complete in less than one hour.

With these techniques, .001 to .01 μc/ml of tritiated thymidine, uridine, leucine, or phenylalanine (1 to 10 nl of solutions containing 1 or 10 μc/ml) could be injected into eggs of *Leptinotarsa* and *Dermestes*. Two to 6 hours later, the eggs were fixed in glutaraldehyde or acrolein, processed according to standard procedures, and sectioned at 1 to 5 μ. The sections were washed for 10 minutes in cold 10 percent trichloroacetic acid, rinsed, and finally coated for autoradiography with Kodak AR-10 stripping film. Control preparations included sections treated for 4 hours with solutions containing 1 mg of trypsin, ribonuclease, or deoxyribonuclease per milliliter, eggs injected with unlabelled substances, and eggs fixed within 1 minute after being injected with radioactive isotopes.

Thymidine is incorporated specifically into nuclei at all stages of early development (Fig. 1). Uridine incorporation is undetectable during cleavage (Fig. 2); it is first seen, after the nuclei have reached the cortex of the

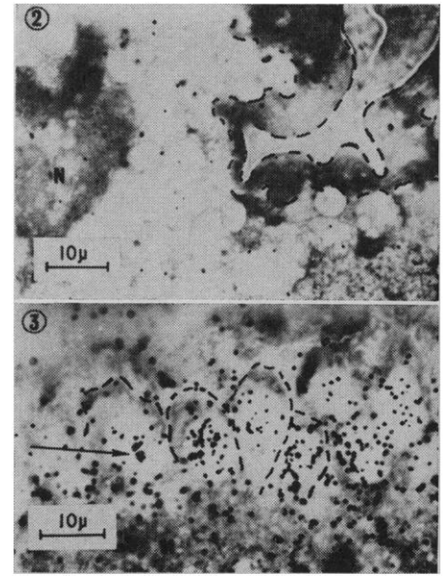

Fig. 2. Periphery of cleaving *Dermestes* egg injected with .004 μc of H^3-uridine and fixed 2 hours later. No incorporation above control level is detectable, either in cortex (outlined) or in island (*N*) surrounding cleavage nucleus. Fold in cortex is an artifact of dehydration. Exposure time, 20 days. Fig. 3. Late blastema cells (outlined) of *Dermestes* egg injected with .004 μc of H^3-uridine and fixed 2 hours later. Uridine is incorporated into basophilic regions of cytoplasm and into forming nucleoli (arrow). Exposure time, 20 days.

egg but prior to cell membrane formation, over these "blastema" nuclei (Fig. 3). Synthetic activity in the future germ cells (pole cells) is either lower than that of other cells, or is asynchronous with other synthetic activities.

In contrast, leucine and phenylalanine are incorporated into all basophilic regions of the egg, including the cortex, from early cleavage onward—that is, 8 to 24 hours before the arrival of the nuclei in the cortex (Fig. 4). Amino acid incorporation increases two- to three-fold after the nuclei reach the cortex (Fig. 5). No local differentiation of this activity has as yet been detected.

Results of administration of antimetabolites, followed by histological comparison of the injected eggs to uninjected eggs from the same clutch, have confirmed these findings, in that puromycin (7 ng/mg live weight of the egg) stops development within minutes of being injected. Neither actinomycin D (0.02 to 0.2 ng/mg) nor tetracycline (8.4 ng/mg) appear to interfere with development prior to the late blastema stage. Thus, the evidence suggests that the Coleopteran egg early in development is capable of synthesizing protein in the absence of concomitant release of new information in the form of newly synthesized RNA. New information does not appear to be released until the migrating nuclei establish functional and physical contact with the highly differentiated regions of the peripheral cytoplasm. The techniques now available permit analysis of the interactions which occur at this time.

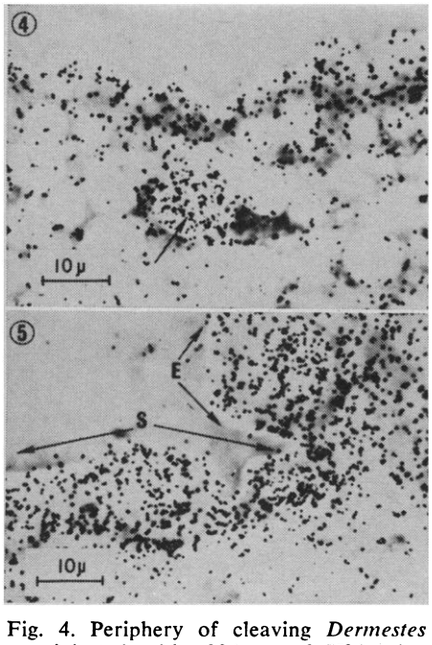

Fig. 4. Periphery of cleaving *Dermestes* egg injected with .004 μc of H^3-leucine and fixed 2 hours later. All basophilic regions of the cytoplasm incorporate the amino acid, even though nuclei (arrow) have not reached the periphery. Incorporation may also be seen during very early cleavage stages. Exposure time, 20 days. Fig. 5. Differentiating embryonic (*E*) and serosal (*S*) regions of *Dermestes* egg treated as described in Fig. 4. No spatial differentiation in the pattern of incorporation is distinguishable.

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Rate of Movement and Redistribution of Stainable Neurosecretory Granules in Hypothalamic Neurons

Abstract. *Electrical stimulation of the olfactory tract of goldfish for one minute can deplete completely the stainable neurosecretory granules from cells of the preoptic nucleus as well as from their axons. Thus, in stimulated neurons secretory granules appear to move toward the neurohemal point of discharge at a rate of about 2 millimeters per minute. Reaccumulation of neurosecretory granules in depleted neurons to approximately normal numbers requires about 1 to 1.5 hours. Histological evidence indicates that, during the period of reaccumulation, granules move out of the perikaryon until normal granulation in the axons is achieved; finally, granulation of the perikaryon is restored.*

Neurosecretory cells, defined as a class, are neurons which end at a vascular space rather than at a synapse or upon an effector, as is more usual. Their secretory substance (or its "carrier") is stainable and usually appears to be in granular or droplet form (*1*); it is thought to be transported by axoplasmic flow toward the axonal terminals (*2*).

It is important to know whether the intracellular rate of movement of the neurosecretory substance is consistent with the known rates characteristic of physiological neuroendocrine responses, yet such information is difficult to obtain. Calculations based on indirect evidence (*3*) are tentative; observations of granular movements in living neurons (*4*) remain open to question since only limited areas of the axons can be studied and there is no certainty that what is being observed is, in fact, the neurosecretory substance or even the neurosecretory axon.

An opportunity to estimate rates of movement of neurosecretory granules developed from experiments in which the olfactory tract of goldfish was stimulated electrically. Sixty seconds of such stimulation evoked a complete "emptying" of neurosecretory granules from cells in the preoptic nucleus and from their axons. Because of the brevity and precision of the event it offered a means for relatively close approximation of rates of passage of these granules. Furthermore, with a planned delay between effective stimulation of the olfactory tract and death of the fish, the rate and pattern of reaccumulation could apparently be studied.

Thirty-eight goldfish immobilized with gallamine triethiodide (4 mg/kg) were wrapped in wet cloths and placed in a specimen-shaped holder of aluminum after dorsal exposure of their brains. Dechlorinated water was kept continuously flowing through their mouths and over their gills. Electrical stimuli (Grass S4 stimulator, 2 volt, 3 msec, 10 cy/sec) were delivered to the