<Further Development 11_06>

The Search for the Myogenic Factor

The search for the yellow crescent myogenic determinant is one of the long-standing quests of developmental biology. From Conklin's first observations that the determinant was located in a particularly colored cytoplasmic region, numerous investigators have tried to isolate it and determine how it functions. Two steps along the way were the demonstration that the factor operated to activate particular genes and that the factor may be an RNA localized to the cytoskeleton.

Richard Whittaker's Experiments on Muscle-Specific Acetylcholinesterase

In 1973, J. R. Whittaker provided dramatic biochemical confirmation of the cytoplasmic segregation of tissue determinants. Whittaker (1973) stained cells for the presence or absence of the enzyme acetylcholinesterase. This enzyme is found only in the larval muscle tissue and is involved in enabling muscles to respond to repeated nerve impulses. From the cell lineage studies of Conklin and others, it was known that only one pair of blastomeres (posterior vegetal; B4.1) in the 8-cell embryo is capable of producing tail muscle tissue. When Whittaker removed these two cells and placed them in isolation, they produced muscle tissue that stained positively for the presence of acetylcholinesterase. No other cell was able to form muscles when separated. (The b4.2 and A4.1 blastomeres can generate muscles, but through interaction with other cells, so they are not autonomous; Meedel et al., 1987; Nishida, 1987, 1990.)

Whittaker (1973) took tunicate embryos of various stages and arrested further cleavage by treating them with cytochalasin B. This drug binds to microfilaments, thus preventing cytokinesis (cell division) while allowing nuclear division to occur normally. In this manner, all further development occurs within the population of cells present at the time when cytochalasin was added. After the embryos had their further cleavages blocked, they were allowed to develop and then were stained for the presence of acetylcholinesterase. A comparison of these results with the cell lineage chart shows a striking concordance. The ability to produce muscle cells was originally present in both of the 2-cell blastomeres. However, by the 4-cell stage, the ability to produce acetylcholinesterase-synthesizing cells is limited to the vegetal blastomeres. In the 8-cell embryo, only the two posterior vegetal blastomeres can give rise to such cells. These are the cells known to form most of the tail muscles of the tunicate larva (Meedel et al., 1987; Nishida, 1987). The cells whose descendants were shown to synthesize acetylcholinesterase are precisely those cells destined to produce muscle autonomously. The production of that enzyme in the isolated cells occurs at exactly the time when it appears in normal embryos (Satoh, 1979).

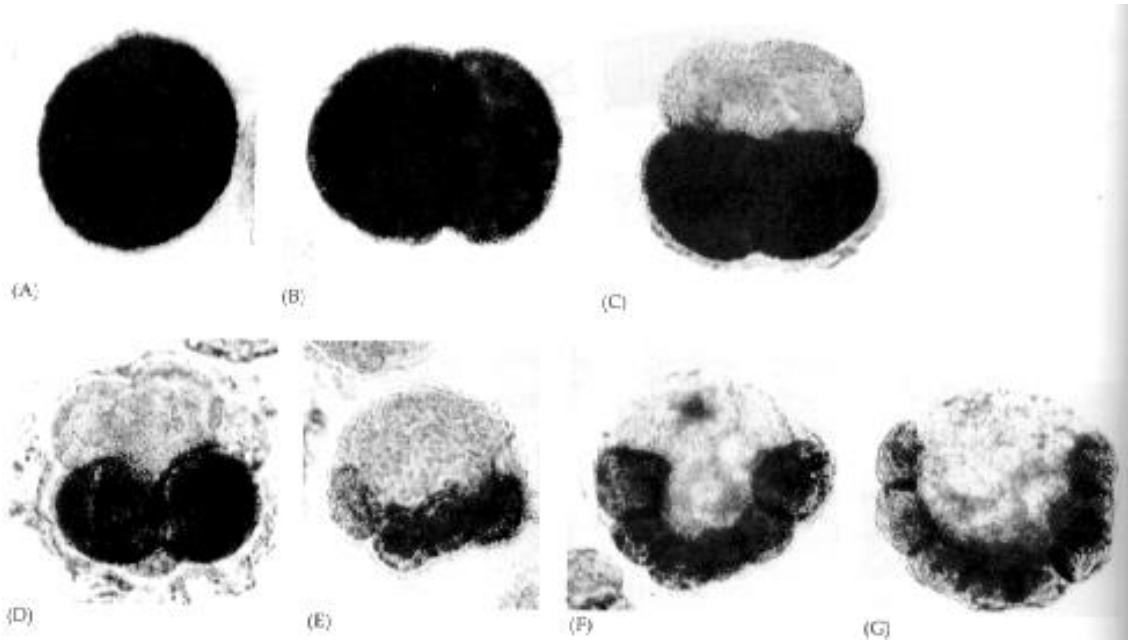

Figure 1 (From Whittaker, 1973a, courtesy of J. R. Whittaker.)

Cytoskeletal Clues

Whereas the various inclusions of the fertilized egg (including yolk, pigment, and soluble proteins) can easily be displaced by centrifugation, such displacement does not usually affect embryogenesis (reviewed in Morgan, 1927). It appears, then, that either the determinants are too small to be moved by centrifugation or they are somehow anchored within the egg. The lack of diffusion manifest in the cytoplasmic localization of these determinants weighs against the first possibility. Most likely, the determinants are attached to insoluble material, probably the cytoskeletal framework of the cell. This infrastructure of filaments and tubules is particularly prominent in the oocyte cortex, but it extends throughout the cell. Cervera et al. (1981) have reported that most cellular RNA in cultured cells is associated with the cytoskeletal framework. Thus, the cytoskeleton might be a means of specifically localizing cytoplasmic determinants.

The cytoskeleton can be isolated by extracting cells with nonionic detergents such as Triton X-100. The detergent solubilizes lipids, tRNA, and monoribosomes. The remaining cytoskeleton contains microtubules, microfilaments, intermediate filaments, and roughly 200 proteins, including one that is capable of binding the 5' cap of mRNAs (Zumbe et al., 1982; Moon et al., 1983). In the tunicates *Styela*and *Boltenia*, the muscle-forming yellow crescent is characterized by an actin-containing cytoskeletal domain. This domain is originally coextensive with the unfertilized egg. After fertilization, however, the actin microfilaments contract and become segregated into those blastomeres fated to form muscle cells, taking with them the yellow pigment granules and a set of mRNAs (Jeffery and Meier, 1983; Jeffery, 1984). Figure 2 shows that the cytoskeletal framework contains the yellow pigment granules and that these granules are given their intracellular localization by the movements of the oocyte cytoplasm during fertilization.

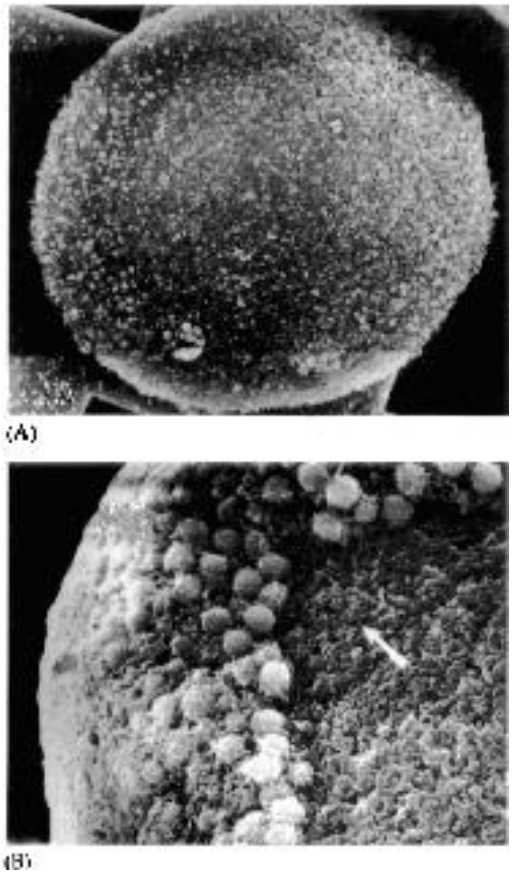

Figure 2 Scanning electron micrographs of tunicate eggs undergoing the segregation of morphogenetic determinants. The eggs have been extracted with Triton X;nb100 detergent to solubilize the membrane and to allow the observation of cytoskeletal components. (A) Unfertilized *Styela* egg; yellow pigment granules and the cytoskeleton can be seen around the entire surface. (B) A newly fertilized *Boltenia* zygote segregating its yellow cytoplasm. The region containing the yellow pigment granules is elevated and consists of a plasma membrane lamina and a deeper network of filaments. The presumed direction of the pigment migration is shown by the arrow. (From Jeffery and Meier, 1983, courtesy of S. Meier.)

The proteins of this yellow crescent region differ significantly from the proteins of the rest of the egg, whereas the mRNAs in this region are not seen to be specific to the yellow crescent (Jeffery, 1985). One of these cytoskeletal proteins is a 58-kDa peptide that is localized in the periphery of the unfertilized egg, segregates to the yellow crescent during fertilization, and enters the tail muscle cells during subsequent development (Swalla et al., 1991). Thus, this cytoskeletal protein appears to follow the same route as the myoplasm during development. Moreover, in those species of ascidians that have direct development and lack a tailed tadpole stage, this protein is absent. The 58-kDa cytoskeletal protein is likely to be involved in myogenic determination in tunicates, perhaps as the protein that binds and moves the muscle cell determinant of ascidians.

In 1995, Swalla and Jefferey screened a one-cell zygote RNA library with probes made from the yellow crescent cytoplasm. This identified a 1.2 kilobase polyadenylated RNA that segregated with the yelow crescent. Moreover, as predicted, it could not be washed away by detergents which extracted everything but cytoskeletal-bound RNAs. This became the primary candidate for the yellow crescent myogenic determinant.

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