Cholinesterase in Larvae of the Ascidian, Ciona intestinalis, Developing from Fragments Cut from Centrifuged Eggs

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Summary. Unfertilized Ciona eggs were centrifuged, stratifying their mitochondria and some other cytoplasmic components. Each centrifuged egg had a mitochondria-free, centripetal clear layer that was contiguous with centrifugal layers containing mitochondria. By cutting centrifuged eggs in two at various levels along the centripetal-centrifugal axis, it was possible to obtain centripetal fragments including virtually no mitochondria, about one-tenth of the uncut egg's mitochondria or about one-fourth of the uncut egg's mitochondria. Most of these centripetal fragments, when fertilized, developed into larvae. However, only the centripetal fragments that included about one-fourth of the uncut egg's mitochondria developed into larvae giving the cytochemical reaction for cholinesterase, a convenient indicator of muscle cell differentiation in Ciona. Therefore, the inclusion of a minimum number of mitochondria (more than one-tenth but less than one-fourth the number in the uncut egg) is correlated with muscle cell differentiation in larvae developing from the centripetal fragments. The possible influences of mitochondria and of other cytoplasmic components on muscle differentiation are discussed.

In ascidians, the presumptive territories (also called fundamental plasms) of the larval organs become conspicuously localized in portions of the egg cytoplasm shortly after fertilization. Then, during the first few cleavages, each of the presumptive territories becomes separated into a specific group of blastomeres. Each of these blastomere groups, after further division, finally differentiates into the organ for which the territory of the egg cytoplasm was presumptive. The term segregation is often used for both (1) the initial localization of territories and (2) the subsequent separation of territories into groups of blastomeres (Pucci-Minafra and Ortolani, 1968); however, segregation is sometimes used only in its first sense (Costello, 1948) and sometimes only in its second (Mancuso, 1959). The presumptive territories of ascidians have been distinguished from one another by criteria of pigmentation, cytochemistry and fine structure.

The most studied presumptive territory of ascidian development is the so-called myoplasm, which ultimately gives rise to the larval muscle cells. The most conspicuous feature of differentiation in these muscle cells is the synthesis of myofilaments. It is probable that these myofilament proteins, like most other postgastrular proteins of developing ascidians, are synthesized under more or less immediate transcriptional control of the nuclear genome (Smith, 1967). Many, but not all, students of ascidian development have proposed that muscle cell differentiation may be triggered (or at least profoundly influenced) by the appropriate developmental stage of cytoplasmic components inherited from the
myoplasm of the fertilized egg. Of all the myoplasmic components (both molecular and organellar) that might influence muscle differentiation, the abundant mitochondria there have received most of the attention.

The high concentration of mitochondria in the myoplasm reacts intensely with cytochemical tests specific for mitochondria and mitochondrial enzymes (an excellent example is the supravital staining of ascidian eggs and embryos with Janus green by Reverberi in 1957. More recently electron microscopy of ascidian eggs and embryos has demonstrated conspicuous concentrations of mitochondria in the myoplasm (Berg and Humphreys, 1960; Mancuso, 1962; Mancuso, 1964; Pucci-Minafra and Ortolani, 1968). The slightly bilobed shape of mitochondria at the eight-blastomere stage of an ascidian led Mancuso (1962) to suggest that some mitochondrial replication might be taking place during development. However, most students of ascidian embryology presume that the mitochondria inherited from the egg cytoplasm do not replicate appreciably during development, which seems to be the rule in the development of animals in general (Dawid, 1972).

There is no doubt that, during normal development, high concentrations of mitochondria are correlated with ultimate muscle differentiation; however, mere correlation does not establish causality. Reverberi (1961 and 1971) has reviewed a number of experimental studies that have attempted, among other things, to establish a causal relationship between myoplasmic mitochondria and differentiation of the larval muscle cells. These studies, not all of which agree with one another, have included the following methods: separation of uncleaved eggs into fragments by cutting, separation of individual blastomeres or groups of blastomeres during early cleavage, and redistribution of cytoplasmic components (including the mitochondria) of uncleaved eggs by centrifugation.

For the unfertilized egg of the ascidian, *Ciona intestinalis*, appropriate centrifugal forces will not only redistribute cytoplasmic components, but will also separate the dechorionated egg into a small centripetal part and a large centrifugal part (Reverberi and La Spina, 1959; La Spina, 1960, 1965). After fertilization, the centrifugal part develops into a complete larva with muscle cells; by contrast, the centripetal part usually does not develop at all, or, at best, only reaches the four-blastomere stage (La Spina, 1960). According to La Spina (1965), the failure of the centripetal part to develop past the four-blastomere stage (much less develop into a larva with muscle cells) is probably due to the absence of mitochondria. Although the female nucleus is absent from the centripetal fragment, that absence alone is not the cause of developmental failure, since anucleate fragments of comparable size cut from uncentrifuged eggs can develop (La Spina, 1965, p. 286). However, La Spina (1961 and 1963) obtained different results when species other than *Ciona* were used.

In the present investigation, the unfertilized *Ciona* egg has been centrifuged in such a way as to localize the mitochondria and some other organelles without separating the egg. Subsequently, the centrifuged egg has been divided into two parts by cutting. This procedure allowed cutting at any desired level along the centripetal-centrifugal axis of the egg. Fragments cut from these centrifuged eggs usually developed into larvae after fertilization. The differentiation of muscle cells in the developing larvae was assayed by a cytochemical test for cholinesterase. Durante (1956) has demonstrated that cholinesterase can first be