RECONSTRUCTION OF EMBRYONIC MORPHOGENESIS ON THE BASIS OF SEM AND TEM DATA

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It is quite common to consider the electron microscopy as a method of purely descriptive science. However, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are the most powerful tools allowing to reconstruct the dynamic of developmental events. The investigation of cell movements in the embryonic development is the most striking example of such a reconstruction (1). For an instance, the phenomenon of cell intercalation (coherent movements of embryonic epithelial cells shaping the embryo) has been discovered and characterized by a combination of SEM and light microscopy based time-lapse movies (2). During epithelial – mesenchymal transition (EMT) epithelial cells lose their contacts with neighboring cells and acquire a migratory mesenchymal morphology (3). EMTs occurring in the embryogenesis of sea urchin, Drosophila and amphibians have also been reconstructed at the ultrastructural level by SEM and TEM techniques (4, 5).

Studying of low Metazoa animals is crucial for the understanding of the evolution of early development. However, very little is known about the details of their embryogenesis, in particular, at the ultrastructural level. That is why my main goal is to reconstruct the embryonic morphogenesis‘ of low Metazoa representatives, Cnidaria, using the SEM and TEM techniques.

The main objects of my investigation are the marine hydroid polyp Dynamena pumila and the sea anemone Nematostella vectensis. Embryos of both species at successive developmental stages were fixed in 2.5% glutaraldehyde/0.1 M cacodylate buffer (pH 7.2) overnight at +4°C, and then postfixed in 1% OsO4 in the same buffer for 1 h. Samples for TEM were dehydrated through a graded series of ethanol and acetone and then embedded into the Araldite embedding medium (Fluka). Sections were stained in water solutions of uranyl acetate and lead citrate and examined by microscope JEOL JEM-1000B. Samples for SEM were also dehydrated and then dried from acetone using the critical-point technique. Samples were sputter coated with gold and examined by microscopes S-405A (Hitachi) and CamScan. Some embryos were fractured by a microsurgical scalpel during the 70% ethanol step of dehydration.

Cnidarian’s body plan is evolutionary primitive and very simple. They consist of two epithelial cell layers, the ectoderm and endoderm, and has only one body axis. However, the diversity of their developmental pathways is extremely high. Early development of Dynamena starts with irregular cleavage and at the 64 cell stage the embryo becomes a morula, an aggregate of loosely packed cells (Fig.1) (6). Formation of the continuous superficial epithelial sheet is the main process in Dynamena development. I have revealed the mechanisms of this process analyzing 3D morphology of cells and embryos, shape of cell’s apical surfaces and their protrusion activity (Fig.2,3,4). Analysis of cell morphology and protrusions was also very useful for the reconstruction of the pattern of cell motility during the formation of Dynamena main body axis. Nematostella form epithelial blastula instead of morula, and future endoderm cells get the inner position passing through the blastopore opening (Fig.5). SEM and TEM techniques allowed us to reconstruct the dynamic of blastopore formation and changes in the morphology and ultrastructure of future endoderm cells (Fig.6, 7). Finally, Nematostella gastrulation has been described as a combination of invagination, EMT and immigration of future endoderm cells and involution of blastopore lips (7).
Fig.1. *Dynamena* embryo at the morula stage, scale bar 100 μ. Fig.2. Apical surfaces of moving embryonic cells (LE – cell leading edge, arrows – filopodial protrusions), scale bar 10 μ. Fig.3. Close up of cell leading edge, filopodia of neighboring cells form contacts with each other. Fig.4. Fracturing of *Dynamena* embryo allows to reveal 3D morphology of epithelial cells. Fig.5. *Nematostella* embryo at the gastrula stage (bl – blastopore). Fig.6. Morphology of fractured *Nematostella* embryo at the gastrula stage (en – endoderm, ec – ectoderm cells). Fig.7. Close up of future endoderm cells undergoing EMT.