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Annual Sea Slug Population's Life Cycle is the Result of Apoptosis

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The opisthobranch mollusc, *Elysia chlorotica*, has an intracellular, symbiotic relationship with functional chloroplasts it sequesters from the filamentous alga *Vaucheria litorea.* The slug slits open the algal filaments with its specialized radula and ingests the contents. During digestion, specific cell types of the digestive diverticulum sequester the chloroplasts, which retain photosynthetic function while in the host cell cytoplasm [1]. In starved animals kept in aquaria, this association can last upwards of nine months with the chloroplasts retaining their photosynthetic function throughout [2]. The Slugs all begin to die in the late spring each year at which time numerous virus particles have been reported in the slug's cells [2].

The aim of this study was to determine the cellular events leading up to the slug's annual death. A progressive amount of morphological degeneration occurred in dying slugs through the last months of their life cycle. These changes were recognizable in the gross morphology and at both the light and electron microscopy levels. The digestive diverticula of slugs in early Spring were filled with chloroplast-containing cells. Slugs begin showing signs of auto-lysosome formation in mid spring. In aging *E. chlorotica,* consistent with the characteristics of apoptosis, chloroplasts were degenerated in lysosome forming cells (Fig. 1.). Aut o-lysosomes appear to degenerate nuclei of digestive cells and merged to form large centrally located virus-containing autolysosome. Large lipid droplets were plentiful in digestive cells of dark green slugs. When the chloroplast-containing cells become apoptotic, the entire digestive diverticulum of *E. chlorotica* lost its structure. The degenerating chloroplasts are surrounded by lysosomal vacuoles, which digest the chloroplasts. Healthy digestive epithelial cells phagocytose degenerating apoptotic cell, only to become apoptotic. Slugs die within a few weeks of the onset of apoptosis. Hoechst stain reactivity and morphological changes in the digestive and the ectoderm cells indicate the coordinated demise of the *E. chlorotica* population is due to apoptosis. Aged, starved slugs in late Spring had numerous digestive cells full of residual bodies, whereas in early Spring, slugs rarely had these structures present in cells. Near-death slugs, which were light green and brown, begin to show a decrease in the number of chloroplasts in digestive epithelial cells and an increase in crypt cells containing liesegang-like ringed residual bodies as animals die. Near death slugs fed *V. litorea* for three weeks showed a reestablishment of its digestive diverticula's fi ne structure. Decreases in the number of residual bodies and the number of virus -containing autolysosomes occurred, as well as the sequestering of large numbers of chloroplasts and large lipid bodies within the digestive cells. Lipid filled vacuoles were present in starved dark green colored slugs, but these lipids were not as plentiful, nor were the vacuoles as large as those in dark green slugs that had not been starved. There were few, if any, lipid bodies in starved brown slugs. In brown slugs that had been fed *V. litoria*, lipid bodies re-appeared in the lumen of digestive tubules and in digestive cell vacuoles.

Its not clear what signals slug's cells to undergo apoptosis, but it is clear that each year apoptosis causes a large amount of tissue dam age which results in the cyclical death of the entire population of *E. chlorotica*.

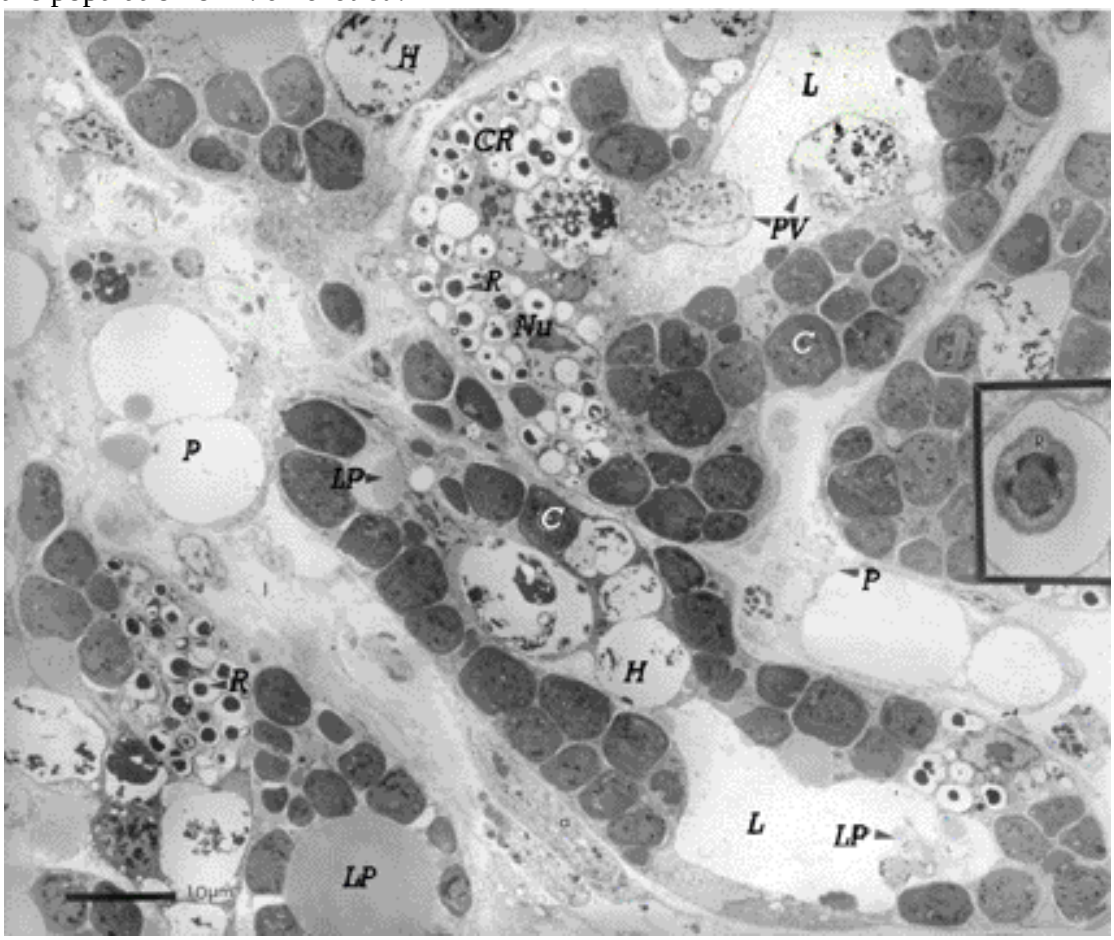

Figure 1. Digestive tubules of slugs collected in the fall and starved for seven months. Fewer smaller lipid droplets are present than in the digestive epithelium of type-1 slugs. Debris from degenerated apoptotic cells is being phagocytized by healthy digestive epithelial cells. Many large heterolysosomes are present and crypt cells filled with residual bodies are a prominent feature. Blood sinuses contain many large pore cells, and amoebocytes are present along basal lamina of digestive tubules. Inset shows a high maginification image of residual body with the typical number of concentric rings found in type-II slugs. (R, residual body; P, pore cell; LP, lipid droplet; a, amoebocyte; H, Autolysosome; CR, crypt cell; Nu, nucleus; PV, phagosomal vacuole.)
(Scale bar = 1μm)

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