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Review

Quantum budding and symbiogeny in Hydra

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ABSTRACT

Hydra's buds develop from cellular modules in the budding region through the interaction of quanta of epithelial and interstitial cells. Symbiogeny may have played two constructive and creative roles in the evolution of Cnidaria and budding: Amoeba equipped with an extrusion apparatus may have set in motion the evolution of cnidarian interstitial cells and cnidocytes; modifying the mechanism of cellular rejection may have generated asexual reproduction *via* budding. These conjectures suggest that early in the Neoproterozoic Era, ancestors of cnidocyst-bearing amoebae infected cellular mats resembling the contemporary Placozoa. The primitive epithelium's attempt to reject foreign amoebae failed but led to the formation of permanent symbiogenic relationships and contemporary hydras' ability to reject specific quantities of excess cells in buds. Supporting these conjectures is evidence that hydras fill bud modules as a function of growth rate, while "epithelial animals", hydras deprived of interstitial cells, fail to maintain budding despite being force-fed and growing. "Epithelial animals" resume budding, however, following the reintroduction of interstitial cells suggesting that hydra's epithelia require a quantum of interstitial cells to trigger the eruption of buds from modules.

KEYWORDS: Cnidocytes, cnidocysts, epithelia, "epithelial animals", Hydra, interstitial cells, symbiogeny.

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INTRODUCTION

Under optimal laboratory conditions of feeding and temperature, budding in Hydra proceeds like clockwork with unfailing regularity and morphological consistency. Remarkably, while their cell populations grow constantly well-fed and maintained hydras reach an equilibrium, retaining a constant size and cell density. The hydras achieve this morphological stability by funneling definitive quanta of excess cells into modules that erupt into buds [1-6].

Hydra's precise budding requires the coordination of hydra's two cell types: (1) interstitial (amoeboid or basal) cells that differentiate as cnidocytes (nematocytes) with their stinging or ensnaring organelles, cnidocysts (nematocysts), nerve, gland, and sex cells; (2) epithelial (epithelial-muscular) cells that form the didermic body wall with extensions into tentacles, hypostome, and foot. The question for developmental biologists is, how are cells of both types teamed up in recurring bud morphogenesis in hydras at equilibrium?

A stock answer is that cells of these types originated from a single cellular source and thus share an overriding control system that operates in the coordination of budding. Alternatively, hydra's two cell types might have risen from independent sources and their ability to work together in budding evolved secondarily. Phenomena such as budding, thus, might not have been the result "of the aggregation of a single species of unicellular organisms, but the results of various symbiotic events between different types of protistan organisms" [7].

The notion that cells of different types combined resources and evolved together was coined and

codified in the concept of endosymbiosis by the late Lynn Margulis [8, 9]. Indeed, the evolutionary fallout of endosymbiotic cooperation is abundantly illustrated by the variety of eukaryotes bearing mitochondria and chloroplasts.

The concept of symbiogeny extends endosymbiosis to eukaryotes capturing each other in evolutionarily productive assemblages [10-12]. Conceivably, early in the Neoproterozoic Era, before the imposition of highly species-specific barriers to phyletic association, a variety of eukaryotes combined to lay the foundation for the rise of multicellular phyla with varieties of normal and pathological cells and tissues. Symbiogeny may even provide the explanation for the similarity of pond amoeba and blood-borne megakaryocytes [13].

In the case of Cnidaria, a symbiogenic relationship may have been formed by cellular mats and amoebae. The cellular mat may have tried and failed to reject the amoebae, but, given enough time the system of cell types found a selective advantage in togetherness: The removal of excess cells through budding [3-5, 10-12].

At present, four sorts of evidence support the possibility that Cnidaria evolved from cells of two different types combining resources symbiogenically: (1) Amoeboid cells equipped with an extrusion apparatus are common among protoctistans (to use Lynn Margulis' term [9]). (2) Hydra routinely produces excess cells that move toward and accumulate in the budding region. (3) Excess cells form discrete modules that erupt and are ejected as buds. (4) Interstitial cells play a critical role in initiating bud morphogenesis.

1. Amoeboid cells equipped with an extrusion apparatus are common among protoctistans

My guess is that initially, ancient protoctistans were infected by monerans (bacteria?) already equipped with an extrusion apparatus. The protoctistans absorbed the apparatuses *via* the same mechanisms primitive eukaryotes used for absorbing mitochondria and chloroplasts.

A protoctistan bearing an extrusion apparatus then formed a relationship — whether as colonizer or guest, ingested prey or invader — with a cellular mat — whether host, victim, or predator. The mat, resembling a contemporary Placozoa [14], evolved into Cnidaria's epithelia, while the amoeba evolved into Cnidaria's interstitial cells and the variety of cnidocytes bearing cyndocysts [15].

Any of a number of protoctistans might have supplied the initial stinging cell to Cnidaria: the "peduncle", "rhizoid", and "perforator" cnidocysts of dinoflagellates [16-19], the trichocysts of trypanosomes [20], zooflagellates [21], and mastigophorans [22], the "apicoplasts" (apical complexes) of "Sporozoa", and the "polaroplast", of microsporidians [23]. Indeed, contemporary myxosporidians once thought of as protoctistans equipped with "polar capsules," *are now recognized as cnidarians* [24-30].

Jiři Lom, the late distinguished Czech protozoologist and parasitologist, suggested that "homologies [between protoctistan and cnidarian cnidocysts] are perhaps too close to be considered only a convergency phenomenon" [31], and Pierre Tardent, the late renowned Swiss coelenterologist commented, "The wheel didn't have to reinvent itself" [32], i.e., cnidocysts came ready-made.

What happened next? My guess is that the incipient cnidarian epithelium attempted to reject the foreign amoebae but its effort was unsuccessful. The effort was not unproductive, however. Given time and opportunity, a symbiogenic relationship evolved, and the dual system found a selective advantage in a modified form of cell rejection. A failed mechanism for evicting amoebae evolved into asexual reproduction by budding, an adaptation for removing while not wasting excess cells produced under favorable conditions of feeding and temperature [3-6].

2. Hydra routinely produces excess cells that move toward and accumulate in the budding region

One of Hydra's attraction to biologists is that under optimal laboratory conditions, Hydra cultures expand exponentially. The cell populations also expand exponentially [33-36], but the sizes of animals and their cell density remain constant (except for transient increases in the budding region), since hydras get rid of excess cells through budding. Rather than treating excess cells as waste, they become assets for asexual reproduction through budding (and colony formation in other cnidarians).

The movement of cells toward Hydra's budding region is well documented [33-38]. Richard Campbell [34, 36] calculated that in *H. littoralis*, under optimal laboratory conditions, 85-86% of hydra's structural cells produced throughout the body cylinder (gastric, budding, and upper peduncle regions) migrate to the budding region. Similarly, in *H. viridis*, about 800 of a thousand gastrodermal [digestive] cells move to the budding region per day [37]. Indeed, interstitial cells and cnidocytes make up more than half of all the cells in the adult animal moving to the budding region [39]. The remainder of a hydra's daily cellular production is lost at the animal's extremities, tentacles and foot.

All the excess cells dedicated to budding are produced along the length of hydra's body wall [33-40] in species-specific patterns of cell division. Paul Brien identified the sub-hypostomal growth zone in *Hydra fusca* [41], and Allison Burnett extended Brien's growth zone in *H. viridis* and *H. pseudoligactis* (*H. canadensis*) to the gastric and budding regions [42].

Campbell showed that in *H. littoralis*, a distal zone of elevated mitotic activity appears among epidermal epitheliomuscular cells ("Ectodermal epithelial cells") and gastrodermal gland cells ("Endodermal gland cells"), but cell proliferation peaks in the budding region for interstitial cells ("Ectodermal interstitial cells") and gastrodermal epitheliomuscular cells ("Endodermal epithelial cells") [36, 38, 43-45].

Measured in mitotic figures and in the incorporation of tritiated thymidine, the epidermis supports a higher rate of cell division than the gastrodermis [36], and labeled epidermal epitheliomuscular cells move toward the budding region faster than gastrodermal digestive cells [34, 35]. Whatever the cell type, and wherever along the body column cells are produced (i.e., both above and below the budding region) they converge on the budding region [33, 36-38, 43-46].

The mesoglea situated between the epidermis and gastrodermis is a substratum for this cell movement rather than a glue holding the two epithelial layers together. Epithelial-muscle cells with longitudinal muscle extensions seem to actively crawl on the mesoglea with the help of their muscle processes [47] and may be seen to migrate over experimentally denuded mesoglea [48]. In contrast, the gastrodermal epithelial-muscular digestive cells with circular muscle extensions seem to become compressed and crowded in the budding region dragging connected cells behind them [49].

3. Excess cells form discrete modules that erupt and are ejected as buds

Neither the budding region nor buds themselves are fonts of highly proliferating cells, meristems, or blastemata. In *H. viridis*, the frequencies of mitotic figures in early buds lacking tentacles (stage I) and buds with tentacle rudiments (stage II) "could not be distinguished" [35]. Given the absence of mitotic figures in the hypostome, the average "number of mitotic figures on the bud proper at the later stage" (stage III) [35] is below that on the parent, but cell divisions proceed at the same rate on the body cylinder of freshly detached buds, during their initial growth period, and in budding animals [1, 35, 37, 50-53].

The distinguishing characteristic of the budding region is the local production of new mesogleal components [52-56]. Indeed, at "sites of tissue evagination… the mesoglea was dramatically remodeled and epithelial cells moved relative to the mesoglea" [46]. Thus, "no loss of ECM [i.e., extracellular material, i.e., the mesoglea] occurs before the time of bud emergence. Rather, the ECM is continuous at the sites of bud formation and what occurs is simply an increase in the expression of… [mesogleal components] as evagination of the bud progresses…. [B]efore evagination of the bud occurs… upregulation of at least… [one mesogleal components] has already occurred. High expression of both basement membrane and interstitial matrix components occurs throughout all stages of bud formation" [56].

Bud modules formed by hydra's excess cells funneled into the budding region break with parental symmetry, project outward, form a hypostome, tentacles, body cylinder, and feet, and ultimately detach as buds [33-38]. In transgenic *H. vulgaris* [46] and grafted *H. viridis* [57], cells are definitely seen moving from parent onto buds. Cells "near the evaginating centre will end up in the oral/distal part of the bud; those located more distantly will move to a more aboral/proximal part of the bud" [56]. The further elongation "of the early bud is driven by recruitment of epithelial tissue from the mother polyp into the newly forming protrusion" [56].

4. Interstitial cells play a special role in bud modules

Interstitial cells seem to play a special role in bud modules since the so-called "epithelial animals" partially or fully deprived of interstitial cells have difficulty budding. Hydras' interstitial cell population is reduced or eliminated in a variety of ways: treatment with colchicine, nitrogen mustard (NM), hydroxyurea, urethane, and lowered temperature [58-64]. Treated hydras do not restore the missing cells and suffer additional losses of nerve and gland cells. "Epithelial animals" do not move, capture prey, or ingest food.

Like starved animals [65-67], "epithelial animals" may bud initially [58]. Seen in photographs, "epithelial animals" are bloated with stubby tentacles [58, 60]. Even if force fed and evacuated "epithelial animals" surviving the initial treatment frequently die from bacterial infections of slowly healing wounds inadvertently inflicted during maintenance. Surviving, nonbudding "epithelial animals" (one in twenty) may enlarge, especially in their peduncle and add thin supernumerary tentacles [58].

Interstitial cells can be restored in "epithelial animals" [67] and to clones of reaggregated cells from nitrogen mustard-treated hydras [68-70] by the addition of normal tissue. Along with the missing interstitial cells and their cell linages [71], including sperm [72] and eggs [73], the hydras resume budding along with re-acquiring normal morphology, and behavior. Interstitial cells thus seem to provide a necessary component to bud modules and possibly a trigger for budding.

DISCUSSION

The question, "How are cells of both types [epithelial and interstitial] teamed up in recurring bud morphogenesis in hydras at equilibrium?" has led to a host of additional questions: "Are cnidarians composite metazoans… metazoan chimeras" [33]? Did a protoctistan equipped with a cnidocyst evolve into Cnidaria's interstitial cells and cnidocytes while a cellular mat evolved into cnidarian epithelia? Did an amoeba's descendants evolve into cnidarian nerves and a cellular mat into epidermal battery cells hosting cnidocytes and a gastrodermis containing gland cells? Did symbiogeny fashion Hydra's body plan with tentacles, hypostome, gastric and budding regions, peduncle and adhesive foot?

Answers may ultimately emerge from molecular and kinetic evidence, but for the present some indirect if tangential facts are available. Under optimal laboratory conditions, hydras reach an equilibrium or a steady state at which body size is constant and the rate of cell production is balanced by the rate of cell loss mainly through budding. At equilibrium, excess cells move to the budding region, consolidate in bud modules, and move off the parental body column into developing buds [33-44]. In contrast, starved hydras and "epithelial animals" bereft of interstitial cells and their products may produce buds initially but shrink and cease budding [58, 60].

Normal well-fed and maintained hydras at equilibrium and "epithelial animals" exposed to agents that destroy interstitial cells [65-67] have a residue of filled bud modules that erupt at the initiation of starvation. These modules would seem to be fully determined, although foot cells involved in detachment may be defective in "epithelial animals" accounting for the occasional retention of buds.

The absence of excess cells would seem to be inhibiting budding in normal starved animals since they recommence budding when feeding is resumed. On the other hand, surviving force-fed and evacuated "epithelial animals" sequester excess epithelial cells in their expanding body cylinder while failing to bud, although budding resumes when interstitial cells are reintroduced [68-73]. Thus, a quantum of interstitial cells in a bud module seems necessary for the eruption of a bud.

CONCLUSION

In conclusion, given Lynn Margulis' justified claims for the creative consequences of endosymbiosis in eukaryotes [8], the present claims for symbiogeny's role in the evolution of Cnidaria are not surprising. An epithelial mat having adopted amoebae equipped with an extrusion apparatus may well have fallen into symbiogeny's constructive and creative grip and taken off in the evolution of cnidocysts and budding [3-6, 10-11].

Symbiogeny's ingenuity would have led to the adoption of amoeboid cells bearing an extrusion apparatus, and they would subsequently have evolved into the variety of cnidarian cnidocysts functioning in capturing prey and protection [15].

Symbiogeny's resourcefulness would then have transformed the early cnidarian epithelial mat's failure to reject amoeba into asexual reproduction through budding. Indeed, a precise quantum of interstitial cells in bud modulus may provide the signal for the eruption of buds. The modification of cellular rejection into budding may not fit evolution's prototypical formula, but "Natural selection… is not [after all] the only force governing evolution, nor had Darwin ever suggested that it was" [74].

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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