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Review

Hydra's complexity: Budding and cancer

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ABSTRACT

Hydra's well-touted simplicity conceals complexity in the regulation of growth, morphogenesis, body size, and budding rates. Rates of cell division change proportionally with rates of feeding animals, but higher rates of cell division do not lead to increased cell population size or cell density. The results of grafting experiments demonstrate that the size of the hydra's mesoglea (extracellular material: ECM) regulates the animal's body size, while cell loss through sequestering cells in budding primordia regulates cell population size. Buds form when bud primordia (modules) are filled with cells from adjacent gastric and budding regions. The number of primordia filled at a time determines the budding rate. The size of bud primordia and the duration of development vary with temperature (larger and longer at colder temperatures), but the size of tentacle primordia in buds and regenerating animals are comparable. Bud morphogenesis depends on interactions between epithelial (epithelialmuscular and gastrodermal digestive cells) and interstitial cells that may have evolved through cooperation and competition among multicellular and amoeboid ancestors joined in symbiogents. Hydra and its buds may reflect properties resembling tumors and metastases.

KEYWORDS: atavism, bud primordia, Cnidaria, epitheliomuscular, stem cells, interstitial, mesoglea, symbiogeny

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INTRODUCTION

Hydra has made it to *Newsweek*! The article by Jessica Wapner (August 4, 1917) describes Paul Davies' "atavistic theory" according to which "cancer is an evolutionary regression", a throwback to hydra, "one of the earliest organisms to evolve out of single-cell primitive species". The theory assumes that, like hydras' stem cells, tumors' stem cells are "not programmed to die, rendering them effectively immortal". The comparison is not inappropriate or unwarranted but incorrect in its details.

Since the eighteenth century, the notion of hydra's immortality has attracted attention [1], and in the twentieth century cultures of hydras have been maintained indefinitely under controlled laboratory conditions [2, 3]. Indeed, for a long time, hydras have been widely considered paradigms of homeostasis (morphogenic regulation and steady-state dynamics), asexual reproduction, and regeneration.

But Davies' "atavistic theory" is mistaken in its version of hydra's longevity and the immortality of its stem cells. As for hydra's longevity: Hydras belonging to the *Hydra vulgaris* and *H. viridissima* groups raised in laboratory cultures may fail to exhibit evidence of aging, but members of the Oligactis group of hydras (*H. oligactis*, *H. oxycnida*, *H. canadensis*) age and die following the induction of sexuality by lowered temperature [4].

The immortality of hydra's stem-cells raises a different problem. Of course, the stem-cell character of both cnidaria's epithelia and interstitial tissues

is highly celebrated: "[I]t is the stem cellness [*sic*] of the tissue which allows Hydra its unique life cycle" [5]. But stem cells are *not immortal* — not even in hydras. Stem cells divide and, on average, half their progeny become new stem cells and half go on to differentiate [6]. Nevertheless, studies of hydra's cellular dynamics demonstrate other ways hydra may be relevant to tumors and metastases.

Hydras have long been considered primitive metazoans [7] and have provoked considerable evolutionary speculation on metazoan origins from protozoans [8, 9]. Alternatively, an "unorthodox possibility" proposes that "metazoans (and possibly plants) were the results not of the aggregation of a single species of unicellular organisms, but the results of various symbiotic events between different types of protistan organisms" [10].

The notion of "symbiotic events" as a driving force of evolution has begun to be taken seriously [11, 12]. In particular, Cnidaria may have originated from the symbiogenic combination of primitive epithelial organisms and amoeboid cells. These primitive symbiogents then evolved through the competition and cooperation of their symbionts into the great variety of organisms comprising the Radiata and Bilateria [13-15].

Indeed, the recent elevation of the Myxozoa to a branch of Cnidaria [16] became an impetus for rethinking the symbiogenic origins of Metazoa [17, 18]. While epithelia may have originated from biofilms, syncytia, or plasmodia (e.g., resembling contemporary mycetozoans [slime mold]), interstitial cells may have originated from protozoan-like single cells [19].

Rather than atavisms and immortal stem cells, hydras' body, its morphogenesis and regulation may be products of the integration of two originally separate tissues in the molding of one organism. Hydras' multicellular epithelia and single-cellular interstitial cells might even provide prototypes of carcinomas and sarcomas, and hydra's buds suggest models for tumors' metastases [20-24].

The hydra you haven't seen before: A review

The problems with Davies' "atavistic theory" and concept of immortal stem cells should not cloud hydra's usefulness as a model for cancers and metastases. Indeed, the complexity of hydra's body wall and its cellular dynamic make the comparisons all the more apt.

Hydra's body wall

Hydra's body wall consists of two tissues: its structural epithelium (epidermis of epitheliomuscular cells and gastrodermis of digestive and gland cells) mounted on either side of an acellular matrix (mesoglea or extracellular material (ECM)) [25, 26], and its interstitial cells (a.k.a. amoeboid, basal cells, neoblast) with their differentiating products (nerve, gland, cnidoblasts, and varieties of cnidocytes containing cnidocysts) [27, 28]. The epidermis provides nests for interstitial cells, and the mesoglea offers pathways for clutches of cnidoblasts migrating to tentacles.

This simplicity has placed cnidarians generally at the hub of metazoan evolution, although not comfortably [8, 29-32]. The assessment is warranted, however, since "what appear to be modern cnidarian developmental stages, including both anthozoan planula larvae and hydrozoan embryos" are found in Precambrian phosphorite rocks of the Doushantuo Formation in Southwest China some 570 ± 20 million years old [33]. Divergence among metazoans may actually have begun a half billion year before the Cambrian explosion — as early as a billion years ago [34, but see 35].

Whether ancient or not, hydra's simplicity conceals a vast complexity of tissue interactions and the devices hydra's tissues employ for working together. Remarkably, hydra's cells can perform many of their normal functions independently of each other. For example, hydras' epithelia can regenerate tentacles in the absence of interstitial cells, and the differentiated products of interstitial cells, cnidocysts and nerves, can occupy tentacles lacking these cells [36-40].

Hydra's cellular dynamics

Paul Brien discovered "La zone de croissance sous hypostomiale" (Brien's sub-hypostomal growth zone) in *Hydra fusca*. "L'Hydre est en perpétuelle croissance par la région antérieure de sa colonne. Cette croissance est presque totalement absorbée par l'édification continue des

bourgeons qui se détachment successivement. L'excédent de cette croissance est employée à l'allongement du pédoncule que neutralise l'usure pédieuse" [41, pg. 18].

Supporting the concept of a sub-hypostomal growth zone, in *Hydra pseudoligactis* (*Hydra canadensis*) mitotic figures are virtually absent in the head (i.e., tentacles and hypostome surrounding the mouth) and foot (basal adhesive disk) while appearing most frequently beneath the head and dropping off by about half in the gastric and budding regions [42].

In *Hydra littoralis*, however, a distal zone of elevated mitotic activity appears among structural epidermal cells (a.k.a. "Ectodermal epithelial cells," epitheliomuscular cells, ectoderm) and gastrodermal gland cells (a.k.a. "Endodermal gland cells"), but cell proliferation peaks in the budding region for interstitial cells ("Ectodermal interstitial cells," basal cells, amoeboid cells) and gastrodermal cells ("Endodermal epithelial cells," digestive cells, gastrodermal epithelial cells) [36, 43].

These high frequencies of mitotic figures in the budding region raise the possibility that budding is promoted by localized cell division. But budding rates are proportional to overall rates of cell division [22, 44-46], and in *Hydra viridis* (a.k.a. *Chlorohydra viridissima*), the rate at which gastrodermal cells accumulate in forming buds is no greater than the intrinsic rate of growth in freshly detached buds [47, 48]. Buds are, therefore, depositories for cells independently of where mitosis is concentrated (or not) on parents.

Indeed, rates of cell division are not governed by the animal's size. Rather, these rates are in equilibrium with the rate of cell loss through budding. In animals cultured under steady-state conditions of feeding and temperature, equilibrium dimensions (length, width, surface area) are reached about two weeks after a bud has detached when the gastrodermal cell density and cell number level off, and budding (number of buds per parent) becomes approximately constant [47, 49]. Moreover, hydras lengthened by grafting additional gastric regions form extra budding regions "at a rate of about one-half additional budding region for each additional [gastric] region" [50].

"Two opposing views" on the regulation of hydra's dimensions

Hydra may very well be a paragon of stability, but it is a stability achieved while cells are dividing and disappearing. The cells poured into buds are not dead, like cells differentiating in hair follicles or at the apexes of intestinal villi. Rather, buds are very much living products of asexual reproduction. A close parallel, therefore, might be drawn to tumors and metastases. The question is, what can the regulation of size in hydra tell us about the "asexual reproduction" of cancers.

While acknowledging the stability of the mesoglea and hydra's habit of funneling cells into buds, Aufschnaiter *et al*. [51] claim to have identified "[t]wo opposing views" on the regulation of hydra's dimensions: "(1) the mesoglea is a stationary structure that serves as a substratum for active epithelial motility" [42, 52], "or (2) tissue movements are the result of continuous tissue expansion that includes both epithelia and the mesoglea" [36, 53]. Thus, "the general nature of *Hydra* tissue movements [to tentacles, foot, and budding region] represents active epithelial migration relative to a stationary mesoglea" [42, 52, 54] or "the apparent 'movement' of epithelial cells relative to the morphology of the animal can be understood as continuous 'outward' expansion of the whole tissue" [36, 53].

The results of experiments with patches of labeled mesoglea indicated to Aufschnaiter *et al*. [51] that, "not only epithelial sheets but also the intervening mesoglea is subject to a constant centrifugal tissue renewal." These authors, therefore, reject "the assumption that the general nature of *Hydra* tissue movements represents active epithelial migration relative to a stationary mesoglea… [and] support the view… that the apparent 'movement' of epithelial cells relative to the morphology of the animal [in the budding region] can be understood as continuous 'outward' expansion of the whole tissue".

Other results of experiments on *Hydra viridis* with body regions lengthened by grafting cast doubt on the regulation of hydra's length by funneling cells into buds [20, 21, 55-58]. As expected, these results support long held views of inhibitory gradients [59], dominance [60], and determination [61] in hydra's regeneration and budding. Thus,

virtually all the animals with excess peduncles developed secondary feet on peduncles farthest from the original foot, while animals with excess gastric regions formed secondary heads with increasing frequency at graft borders farthest from the original head [55]. Many grafted animals also formed waists at graft borders after regenerating feet and heads, and these animals separate into diminutive individual hydras [62].

Remarkably, animals with additional gastric regions that remained intact formed secondary budding regions within a day after grafting on most of the gastric regions [62]. Surprisingly, budding regions farther from the head supported more budding than budding regions closer to the head suggesting that the diffusion gradient of head inhibitor also acted on budding regions or that the greater volume of cells distal to the most proximal budding region provided more cells for budding.

Similarly, intact animals with multiple gastric + budding regions (as many as five) budded at higher rates in budding regions farthest from the head. In fact, in both intact grafted animals with multiple gastric regions and multiple gastric + budding regions budding slowed and ceased in budding regions closer to the head [20]. But none of these multiply-grafted animals were seen to shrink as long as they remained intact.

Thus, lengthened animals that remained intact returned to a single budding region without shrinking and ultimately resumed hydra's usual shape if lengthened. The failure of these lengthened animals to shrink as a result of budding indicates that budding does not determine hydra's length. Alternatively, the mesogleas that healed into place in grafted animals provided a stable lengthened mesoglea that determined hydra's new dimensions [20, 55].

A stable mesoglea may, therefore, determine the animal's dimensions. Abundant evidence points to a structurally stable mesoglea upon which epithelial and interstitial cells migrate both down and up toward the budding region [44, 45, 54, 63, 64]. Indeed, ever since mesogleas were first freed of their epidermis [65] their toughness and stability have been abundantly demonstrated [66] as well as the ability of epidermal cells to adhere to them and migrate over them [52]. What is more, the fact that tentacle number is set if not fixed prior to bud detachment suggests that the position of tentacles as well as budding region and foot are built into stable mesogleas [67].

Budding as a modular event

Hydras deposit as much as 86% of their daily structural cell output in buds [43, 47]. Budding is not, however, achieved by cells gradually becoming buds' cells. Budding is a "cusp catastrophe" [68], more like an overhanging cliff caving in than the accretion of sand on a hillock [50]. Indeed, "tentacle and bud formation… [are] 'modular' events… triggered by the accumulation of a precise number of cells" prior to differentiation [22].

The notion of cellular primordia underlying the induction of tentacles and buds is not new, of course: "[P]hysiological isolation" liberating buds from "parental dominance" was suggested by Rulon and Child [61], and the possibility of tentacle induction was examined by Li and Yao [69]. But bud induction is complicated by the requirement to put different parts of the bud together (i.e., does not seem to be a single event). Indeed, hydras treated with Colcimid prior to the appearance of a bud fail to form a foot and become double-headed instead [24, 70]. Thus, the bud's foot formation would seem to depend on the differentiation of peduncle cells rather than the bud's distal structure.

Feeding schedule

Numerous efforts have been made to estimate the size of the bud's primordium giving rise to a bud (distal and proximal parts) by altering the animal's feeding schedule. In *Hydra attenuata*, about 10,000 structural cells are present on a newly detached bud. Since it takes 2.5 to 3 days to produce a bud and 0.33 cells divide a day, it would seem that the bud's primordium consists of something between 5000 and 5600 structural cells occupying about twenty percent of the parent's budding region [71].

The sizes of budding primordia have also been estimated for *Hydra viridis* [48]. Animals fed brine shrimp one to four days a week had between 4500 and 12,000 digestive cells on freshly

detached buds and an intrinsic growth rate of 0.33 digestive cells per feeding day. Regression analysis shows that these buds in animals cultured under different feeding schedules were produced from initial growing masses of 6,000 total structural cells (3600 digestive cells plus 2400 epitheliomuscular cells) [72]. The size of bud primordia in *H. viridis* is comparable, therefore, to that in *H. attenuata*.

Strikingly, the duration of bud development (2.46 ± 0.80 days) and the duration of each stage of bud development [49] are not significantly different in *H. viridis* raised on different feeding schedules. As expected, the number of buds detaching per day differed significantly with feeding schedule (between 0.44 and 1.06) [48, 49]. Likewise, the number of cells present in detached buds increased significantly with feeding schedule [48].

The number of tentacles per bud (6.00 to 6.92) did not differ significantly with feeding schedule either, albeit the slope (linear regression) of tentacle number as a function of days fed (from one to four days a week) was significant [48]. Possibly, buds growing on animals fed on different feeding schedules started out with the same number of tentacles, but buds on animals fed more frequently and hence growing faster added an additional tentacle before detaching.

In general, animals fed more often and growing at faster rates produced buds with more cells. But the size of the initial bud primordium seems to be the same in animals growing at different rates, since the duration of bud development, of stages, and the number of tentacles are the same despite different feeding schedules. Animals growing at faster rates, therefore, have greater numbers of bud primordia accounting for the production of more buds, but the primordia would be the same size and absorb the same number of parental cells accounting for the uniform duration of bud development, stages, and initial tentacle number.

Temperature

Dynamics have been examined in *Hydra viridis* incubated at different temperatures — 18 °C, 23 °C, and 28 °C [49, 72-74]. Significant differences in the number of buds in budding regions (2.97, 2.78, and 1.88 buds) of animals incubated at these temperatures would seem at least partially explained by significant differences in the duration of bud development (4.73, 2.04, and 1.91 days), but significant differences in the number of tentacles per bud (6.68, 6.50, and 6.02 tentacles) suggests differences in the size of initial bud primordia [49, 73]. Tentacle numbers on 200 buds developing 2 to 6 days at 18 °C did not differ significantly, however, suggesting that the number of tentacles on buds reflects differences in the initial size of bud primordia and not growth rate.

Regression equations indicate that, at detachment, buds growing at 17-18 °C with seven tentacles would have arisen from primordia of 7-10,000 structural cells; those at 22-23 °C with six tentacles would have arisen from primordia of about 5000 structural cells; buds at 27-28 °C with five tentacles would have arisen from primordia of 3-4,000 structural cells [72]. Thus, the size of bud primordia would seem to be physiologically regulated and not constant.

These primordia suggest that tentacles arose from primordia of 600 structural cells in hydras incubated at 18 °C and 300 cells in those incubated at 23 °C and 28 °C. Surprisingly, the number of cells in regenerating tentacle primordia in animals kept at 23 °C is similar, namely, 200-600 structural cells per tentacle [74]. It would seem, therefore, that tentacles are induced in tentacle primordia of more or less the same size.

Further research on hydra

A new round of research might begin by estimating the size of different tissue components (epithelial and interstitial) in bud's and tentacle's primordia. Since hydras can be separated into cells and these can reaggregate to form new hydras [39, 75-78], one approach would be to reaggregate cells in different proportions.

Research on separate morphogenic roles for hydra's different tissues might have wide implications for theories of evolution and pathology. Indeed, ever since symbiogeny was proposed as the source of hydras' separate tissues [14, 15, 17-19, 28] and budding was conceived of as a model for metastasis [24] hydras among other invertebrates have loomed at the edge of cancer research [20, 22, 23, 58].

Today, symbiogeny would seem relevant to understanding the integration of multicellular epithelia and amoeboid cells (e.g., a variety of blood cells in vertebrates). Moreover, opportunities for studying these different types of cell would seem vastly expanded by new techniques for identifying and labeling cellular constituents and through genomic techniques.

For example, straightforward physiological differences between extant unicellular (amoeboid) organisms and multicellular organisms appear in strategies for acquiring nutrients. Thus, unicellular eukaryotes grow and proliferate directly upon taking up nutrients available in their environment, whereas cells in multicellular tissues such as epithelia are restricted by growth factor signaling pathways. "Indeed, unicellular amoeboid eukaryotes such as *Dictyosstelium* can satisfy their dietary requirements through macropinocytosis… Mammalian cells [in contrast] have little intrinsic macropinocytic activity without external stimulation." What is more, unlike normal mammalian cells, "cancer cell lines harbouring Ras mutations commonly display high macropinocytic activity" [79].

Presently, genomic studies on differences between epithelial and amoeboid cells are booming [80, 81]. Consequently, "ancestral forms of myc and max [onco]genes … [are now known to appear in] the early diploblastic cnidarian *Hydra*" [82]. Moreover, a "National Human Genome Research Institute-funded Hydra genome project at the J. Craig Venter Institute currently provides 6x coverage of the *Hydra magnipapillata* genome with an assembled draft genome sequence appearing later this year" [5].

Gratifyingly, recent analyses of results suggest that an "ancient mutational response to stress that evolved among prokaryotes was co-opted to maintain diversity in the germline and immune system, while the original phenotype is restored in cancer". Indeed, the "non-mutated genes in these pathways are orthologous to those underlying stress-induced mutation in bacteria, which results in the clustering of single nucleotide variations" [83].

CONCLUSION

Hydra's structural cells move up the body column to tentacles and down the body column to the foot, while excess cells move both up and down toward the budding region where they are deposited in buds. The body of Hydra is thus a storage organ for structural cells serving in the maintenance of tentacles and foot while excess cells are collected in primordia and rejected in buds. Budding is not a mechanism for the regulation of organismic length but an adaptation to cellular homeostasis commandeered for asexual reproduction.

Hydra's structural cells move over the mesoglea (the acellular extracellular material between epithelial layers), and bud primordia form at the top of the budding region and soak up cells while moving down the budding region. The size of a bud's primordium is a function of physiological conditions, i.e., larger in colder than warmer environments, but not growth rates, i.e., faster when fed more often. The size of a tentacle's primordium does not alter with temperature, however, and may be the same in buds and regenerating heads.

The next stage of research on hydra's morphogenesis will undoubtedly utilize the burgeoning resources of cell labeling and genomics. If, indeed, Cnidaria in general and Hydra in particular evolved *via* the symbiogenic combination of syncytial multicellular and amoeboid organisms then competition and cooperation may have left their legacy in the manifold tissues of the radiates and bilaterians. The failure of tissue integration manifest as tumors and metastases may yet be understood as the legacy of budding in Hydra.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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