

A Review of “The Will to Bud”: A Three Rivers Evolution Lecture Presented September 22, 2018

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ABSTRACT

Hydra viridis is cultured under different temperatures and feeding schedules bud continuously with clockwork precision. At different temperatures, buds are initiated at different rates, and hydras support different numbers of developing buds for different periods, but freshly detached buds have the same six to seven tentacles (statistically identical). Similarly, hydras fed on different schedules support different number of buds having different numbers of digestive cells but, within statistical limits, the same number of tentacles (six-seven).

Since buds develop from parental gastric region and peduncle cells converging on the budding region, modules containing a minimum number of parental cells would seem to initiate bud development, determine tentacle number, break with parental symmetry, and grow outward. Additional cells provided to buds by the movement of parental cells and intrinsic growth do not alter tentacle number.

Keywords: *Hydra viridis*, Statistical limits, Alter tentacle number

INTRODUCTION

Budding in hydras

Under laboratory conditions, hydras achieve a steady state, neither elongating nor shrinking, while, at the same time their cell populations grow, buds sprout, elongate, develop a head of tentacles and hypostome, a body column of gastric region and peduncle, and a terminal adhesive pad before detaching [1-9].

Hydra's two epithelial tissues, an outer epidermis (“Ectodermal epithelial cells,” epitheliomuscular cells, ectoderm) and an inner gastrodermis (“Endodermal gland cells”) lie on either side of the mesoglea (matrix or extracellular material [ECM]). In addition, interstitial (basal cells, neoblast) is concentrated in intercellular spaces between *hydra*'s epidermal cells.

Hydra's epithelial and interstitial cells are self-sustaining cell populations that differentiate locally into non-dividing cells of the head (hypostome and tentacles) and foot. Interstitial cells have stem-cell properties, dividing and giving rise to cells that become nerve, gland cells, and cnidoblasts that subsequently differentiate into various cnidocytes [10]. Cnidocytes migrate from the body column to tentacles where they are large among epithelial battery cell and function in predation and defense. Other interstitial cells become sperm, egg and probably, adhesive gland cells of the foot [11-13].

Bud dynamics

Buds form in the budding region at the juncture of the gastric region and peduncle. The reorientation of circular gastrodermal musculature may be disturbed at this juncture, hence, encircling a cellular module and thrusting it outward upon contraction [14].

New mesogleal components are added as buds develop while moving downward (**Figure 1**). The budding region is the site of local production of new mesogleal components [15-17]. 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” [18].

Beyond epithelia, interstitial cells play an essential role in budding. Indeed, they are required for the eruption of a developing bud, since hydras deprived of their interstitial

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cells (known as “epithelial hydras”: [7,19] do not sustain budding. Epithelial hydras may enlarge and form supernumerary tentacles when force-fed, but epithelial cells

alone do not restore interstitial cells [20] or the products of interstitial cell differentiation.

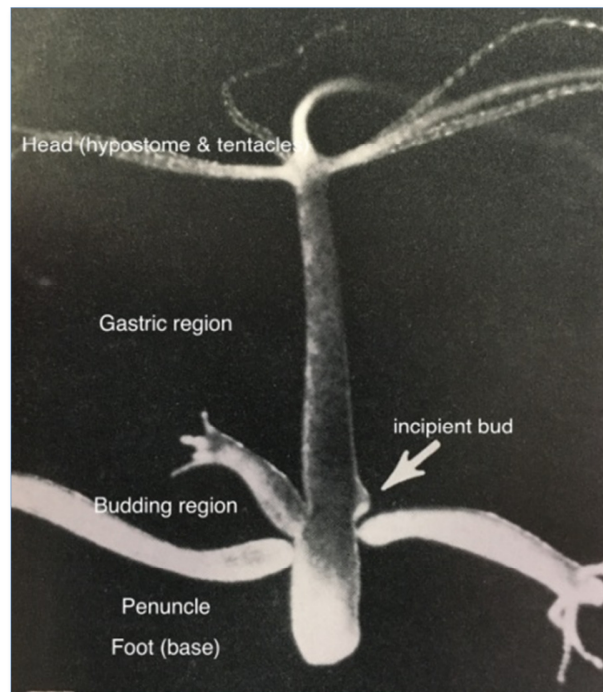


Figure 1. *Hydra viridis* with four buds developing its budding region between its gastric region and peduncle.

Bud dynamics

Estimates of the size of the initial bud module are made from estimates of the number of digestive cells. Digestive cells

were counted (**Figure 2**) because they can be distinguished unambiguously from the other cells comprising hydras.

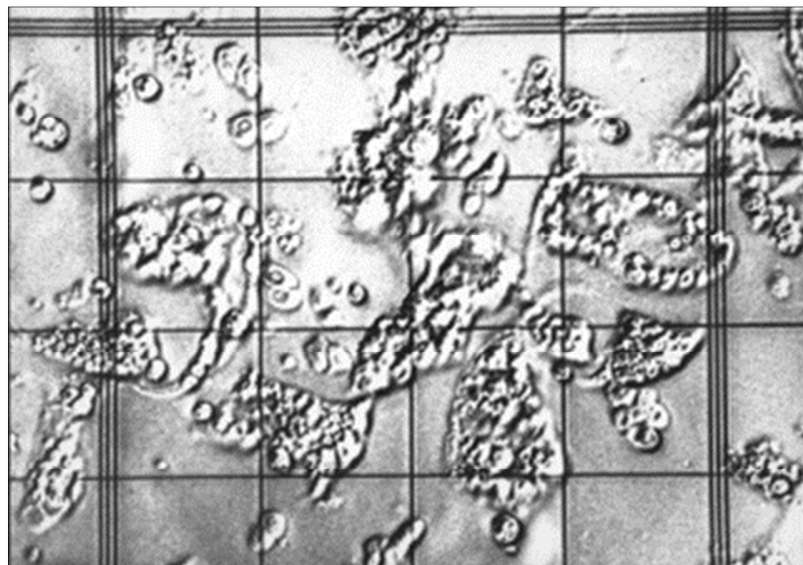


Figure 2. Phase-contrast micrograph of macerated *Hydra viridis* cells spread on a hemocytometer grid. Gastrodermal cells are distinguished by their large size, refractive endosymbiotic algae, and prominent basal muscle fiber. Epidermal cells are smaller, and cnidoblasts occur in nests of two to eight cells frequently containing differentiating cnidocysts.

Hydras fed three days a week were cultured at different temperatures (**Table 1**), 18°, 21°, and 28° C or fed one to

Table 1. Variable budding vs. constant tentacle number as functions of temperature and feeding schedule.

Temperature*	18°C	23°C	28°C	Average
Buds initiated per day	0.68±0.04	1.28±0.06	1.02±0.04	1.01±0.02
Buds per parent	2.97±0.06	2.78±0.06	1.88±0.14	2.54±0.01
Duration bud development	4.73±0.22	2.04±0.12	1.91±0.14	2.89±1/12
Tentacles per bud	6.68±0.16	6.50±0.16	6.02±0.17	6.40±0.06
Number days fed four days/week**	1	2	3	4
Number tentacles per bud	6.32±0.26	6.42±0.19	6.53±0.26	6.92±0.32
Number digestive cells/bud	4614±83	6508±153	9284±1416	12,278±3015

Moreover, the budding rate for my hydra, *Chlorohydraviridisima*, was optimal in the vicinity of 23° (actually closer to 21°) centigrade, trailing off both above and below this optimum. As one might imagine, the number of buds initiated per day and the duration of bud development varied as functions of temperature, but the number of tentacles on each bud did not change significantly. Thus, hydras maintained on different feeding schedules and incubated at different temperatures, produced buds at different rates and with different numbers of cells, but with the same number of tentacles. The determination of tentacle numbers was, therefore, under different controls than the rate of budding and size of buds.

Since then, it was suggested that hydra's budding region occurs at the point where downward moving cells from the gastric region encounter upward moving cells from the peduncle. When a minimum number of these cells accumulate, they constitute a bud module and initiate a bud's development while determining the number of a bud's tentacles that will ultimately be present on the bud. The module proceeds to break with the "parent" hydra's symmetry redirecting growth outward. Ultimately, the rate at which buds develop and detach is a function of feeding rate and temperature as parental cells continue to feed buds that also grow intrinsically. Thus, the size of a bud module determines the number of tentacles produced by the bud while the number and size of buds produced per unit time is governing by the rate of cell division on the "parent" and bud.

Under both regimes, the number of tentacles per buds ranged from [5-7]. Larger animals fed more often tended to produce buds with slightly more tentacles (i.e., the regression of tentacles per feeding days differed significantly [21-23]. But, the number of tentacles per bud did not differ statistically as either a function of temperature or feeding schedule. The number of buds initiated per day, the number buds developing on a parent at any time, and the duration of bud development did differ significantly as a function of

four days a week while cultured at 21° C.

temperature; and the number of digestive cells per bud differed significantly as a function of feeding schedule.

How many parental cells comprise a bud module?

One may answer this question with data in (**Table 1**) and assumptions about the average rate of cell division in hydras fed on different schedules. Extrapolating back, the 12,278±3015 (rounded to 12,000) digestive cells present in freshly detached hydras cultured at 21° C and fed 4 days a week would have been produced in 2.04±0.12 (two days) by an initial mass of 2000 to 3000 digestive cells dividing twice a day. Likewise, the 6500 digestive cells present in freshly detached buds of parents fed 2 days a week would have been produced by 2000-3000 digestive cells dividing once every day, and the 4000-5000 digestive cells present in buds from parents fed one day a week would have been produced by about 2000-3000 digestive cells dividing once in two days.

Thus, the bud module would contain 2000-3000 digestive cells at the initiation of bud development in parents fed one to four days a week (and maintained at 21°C). If the number of digestive cells is less than half the number of epidermal cells, and the entire epithelium is half the size of the interstitial cell population [24], then an initial bud module would consist of about 15,000 cells.

Intriguingly, an estimate of 200-600 digestive cells found in tentacle rudiments during regeneration [22] is consistent with the estimate of 2000-3000 digestive cells present in bud modules were the size of the initial module to determine the tentacle number on buds.

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