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ELABORATION OF BODY PLAN AND PHASE CHANGE DURING DEVELOPMENT OF *ACETABULARIA*: How Is the Complex Architecture of a Giant Unicell Built?

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ABSTRACT

While uninucleate and unicellular, *Acetabularia acetabulum* establishes and maintains functionally and morphologically distinct body regions and executes phase changes like those in vascular plants. Centimeters tall at maturity, this species has allowed unusual experimental approaches. Amputations revealed fates of nucleate and enucleate portions from both wild type and mutants. Historically, graft chimeras between nucleate and enucleate portions suggested that morphological instructions were supplied by the nucleus but resided in the cytoplasm and could be expressed interspecifically. Recently, graft chimeras enabled rescue of mutants arrested in vegetative phase. Since the 1930s, when *Acetabularia* provided the first evidence for the existence of mRNAs, a dogma has arisen that it uses long-lived mRNAs to effect morphogenesis. While the evidence favors translational control, the postulated mRNAs have not been identified, and the mechanism of morphogenesis remains unknown. Amenable to biochemistry, physiology, and both classical and molecular genetics, *Acetabularia* may contribute yet new insights into plant development and morphogenesis.

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WHY USE *ACETABULARIA?*

Species of marine green algae in the order Dasycladales present opportunities to study extreme cases of organismal body plan (patterning) and development without cellularization. Despite dependence on a single nucleus for most of their life cycle, these evolutionarily successful species reach heights of 1–10 cm at reproductive maturity and undergo elaborate morphogenesis concomitant with development (7). The most studied member of this order (cf suggested elevation to class Dasycladophyceae, 109) is the type species, *Acetabularia acetabulum* (= *mediterranea*). When vegetative (Figure 1), *A. acetabulum* superficially resembles the "horsetail" *Equisetum*. When reproductive, *A. acetabulum* resembles a miniature goblet, hence the nickname, The Mermaid's Wineglass. Many aspects of growth of and development in this unicell are reminiscent of multicellular vascular plants. For example, during vegetative growth, whorls of hairs and interwhorls alternate and are stacked one on the other, just like the phyllodes of vascular plants. In addition, in preparation for reproductive phase, *A. acetabulum* goes through juvenile and adult vegetative phases (87). In any unicell, consideration of cell-cell interactions within the organism is moot, so being unicellular eliminates a layer of developmental complexity. Compared with other unicellular models for development such as oocytes and early embryos of *Fucus*, flies, and frogs, *Acetabularia* has added appeal because all of vegetative development and the switch to reproductive phase is orchestrated by a single cytoplasmic compartment in conjunction with a single nucleus.

Technical advantages made this a developmental model of choice from circa 1930–1970, as one publication history indicates (69). Simple amputations led to an accurate description of the origin and role of mRNAs in eukaryotes (35, 36) thirty years before the elucidation of the chemical nature of mRNAs in prokaryotes (45). The discovery that this alga tolerated grafting (reviewed in 40, 41) led to experiments that mixed and matched disparate nucleate and enucleate body parts within and between species and genera (13, 37, 112). Nuclear transplantations were performed to infer the contributions of the nucleus and cytoplasm to development, morphogenesis, and circadian rhythmicity (e.g. 8, 37, 55, 96, 98),

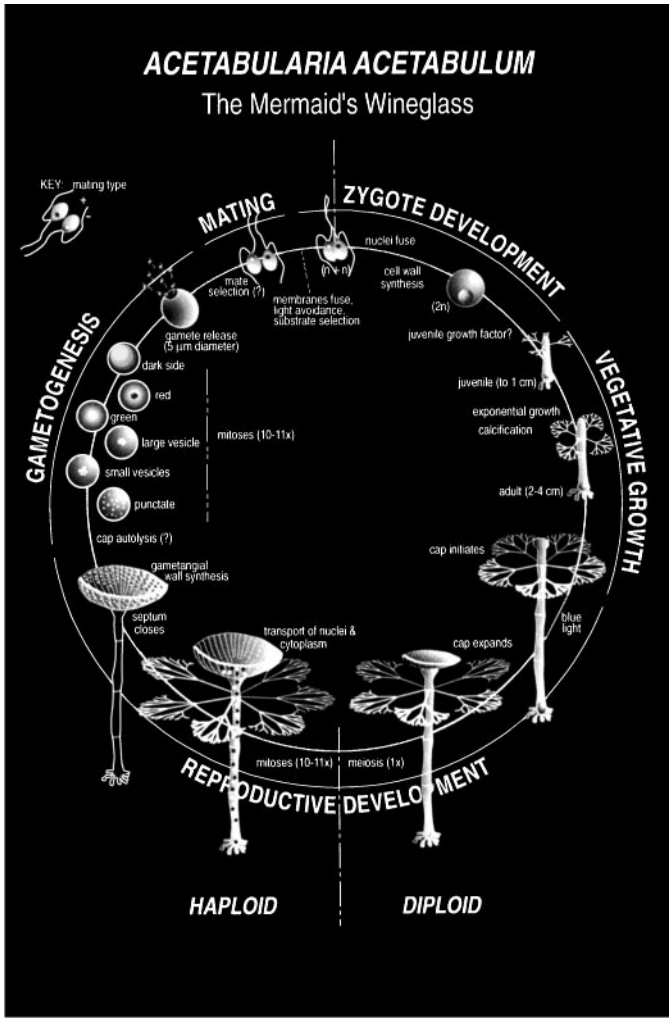

Figure 1 A view of the life cycle of *A. acetabulum*. Portions of the life cycle that are speculative or supported only by observation are flagged with *question marks*. More extensive discussion of the life cycle can be found elsewhere (69). The arrangement of the flagella during mating has not been clearly documented, but it is 11 and 5 o'clock in free-swimming gametes (see references in 109). A color rendition of this image resides at http://weber.washington.edu/~mandoli.

and to transform this plant (85). Some biochemical assays demanded as few as 1–4 plants (3, 4, 98), and the isolation of crude cytoplasm for those assays was easy: The stalk was snipped off, and the cytoplasm was stripped out just as one squeezed toothpaste from a tube. Previous researchers were cognizant that the basal portion left behind retained the ability to make progeny and potentially could serve as a renewable source of cytoplasm because it readily regrew after each amputation of the previous apical portion (see references in 5, 12, 50, 93). However, this feature of the organism was not fully exploited. Microinjection of nuclei with foreign DNA (19, 20) led to stable transformation in 70% of the plants (85). Finally, there was a hint of the potential for genetic analysis (59, 60): *A. acetabulum* has a reasonable genome size [20 chromosomes (24), 0.92 pg per haploid genome (105), small intervening sequences (106)], each plant produces millions of progeny per generation (see discussion and references in 69), and it can be selfed and outcrossed (33, 34, 59, 60). Despite its classical contributions, technical versatility, and obvious appeal, use of this taxon has decreased since the 1970s (69).

Three major roadblocks contributed to reduction in use of the system (69): the life cycle was too long, it was labor intensive to obtain and keep clean monocultures for routine use, and large-scale genetic analyses were not feasible. Significant headway has been made on all three fronts. The life cycle, which runs 1–2 years in the wild and 6 months in most laboratories, was reduced to 94 days for heterogeneous wild type (69) by improving the physiological conditions in which the species was grown. The first significant reductions in the duration of the life cycle were obtained when cultures were rendered axenic (42, 74), when plants were grown in an artificial seawater tailor-made for the type species (44), and when the effect of population density on the rate of development was understood (22, 69, 113). Later, a method that yielded single-cell suspensions of zygotes (73) made it possible to adjust the population densities of cultures (69). This method was important because zygotes tend to attach to each other, creating sheets or balls of plants, a physical arrangement that seems detrimental to growth. In contrast, populations of plants that are not attached to each other are fairly synchronous in development and are healthier and faster growing than siblings that are clumped (see further discussion in 69). An initial foray into genetics was made feasible by developing a mating matrix that allowed virtually all progeny to be recovered (73) and by beginning to study phenotypes defective in development and morphology (68, 70). In sum, improved understanding of the physiology of the plant has enabled these three roadblocks to be removed or substantively reduced.

Now, large-scale genetic analyses are practical in this species (69). Inbreeding and selection have decreased the genetic load carried by wild-type laboratory strains, promising uniform genetic background for mutant analyses, and have

resulted in homogeneous wild-type lineages of plants with uniform wild-type morphology that self well (BE Hunt & DF Mandoli, unpublished data). We have defined conditions for long-term storage of haploid germplasm [15 months with 89% recovery (43)] and have begun to adapt microparticle bombardment to *A. acetabulum* (C Geil & DF Mandoli, unpublished data). Although further improvements in culture conditions, strains, and genetic manipulations can and will be made, the minor problems with culture that remain do not preclude full use of the system (69), and genetic analyses of interesting developmental mutations are under way (e.g. 72).

APICES ESTABLISH THE BODY PATTERN DURING PHASE CHANGE

Images and description of the life cycle of this organism abound in the literature (23, 54, 61, 69, 93, 100), and reviews covering diverse topics are numerous (2, 5, 12, 15, 16, 32, 39–41, 50, 54, 68, 69, 93, 97, 108). Details of the life cycle of *A. acetabulum* as we now understand it (Figure 1) are offered because, in my opinion, a deep understanding of development throughout the life cycle can only increase the utility of any model system.

The body plan of *A. acetabulum* is elaborated by three types of growth regions that are regulated in number and location on the body during development and that have functions, behaviors, and morphologies that distinguish them from each other (Figure 2). I suggest that the term *apex* is applicable to each of these localized regions of growth. The *stalk apex* (1 per plant) grows upward and generates the stalk and the whorls of hairs during vegetative growth and generates whorls of the cap during reproductive growth (Figure 2). The *hair apices* that are arranged in rings (10–19 whorls of hairs per stalk with >4 more arising from the cap) initially grow upward then settle perpendicular to the stalk axis and, except the first few made during juvenile growth, bifurcate as they grow. The *rhizoid apices* ($\sim$8–10 per plant) grow downward and generate the digits of the rhizoid. The stalk and hair apices make straight cell walls, and rhizoid apices make curvilinear ones. To the best of my knowledge, the mechanism(s) of growth at these apices are not defined for any species in the Dasycladales, and this is a fundamental gap in our knowledge. Identifying the borders of and potential subdomains within the apices of *A. acetabulum* with molecular and genetic markers may also be useful, especially in light of the insights gained by understanding functional domains and organogenesis (see 21) within the shoot apical meristem of vascular plants (see 51). The biology of the apices of *A. acetabulum* suggests that these growing regions are biochemically distinct and spatially restricted, but how such regional specificity is established and maintained given the lack of cellularization is entirely unknown.

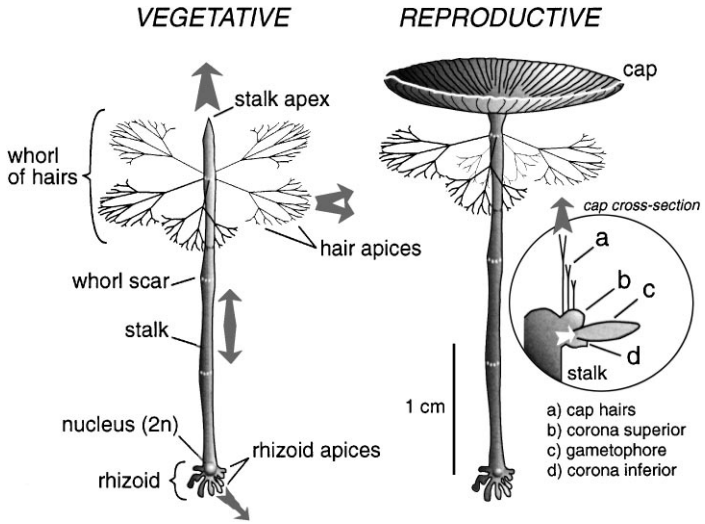

Figure 2 Anatomy of vegetative and reproductive plants are shown with each region of growth on *A. acetabulum* highlighted with an *arrow* indicating the major direction(s) of growth (modified from 86). In the cap cross section, the *white arrow* indicates the position of continued growth of the stalk apex, and the *gray arrow* indicates growth of the hair apices of the cap. For the sake of clarity, only one whorl of hairs is depicted on the vegetative plant that would normally have 10–19 whorls of hairs (see Figure 4). During reproductive phase, the whorls of hairs fall off as shown.

As it generates the majority of the body plan of the organism, the stalk apex of *A. acetabulum* progresses through discrete and predictable changes in shape during vegetative and reproductive development (Figure 3). The shoot apical meristem of a multicellular vascular plant partitions itself into spatially distinct stem and leaves, whereas the stalk apex of *A. acetabulum* alternately produces regions of stalk and whorls of sterile hairs during vegetative growth. The patterns of interwhorl and whorl production in *A. acetabulum* bear an uncanny resemblance to the patterns of internodes and nodes of multicellular plants. Like internodes, the interwhorls of *A. acetabulum* continue to grow in length (87) but do not alter the body plan per se. Like leaf arrangements and structures in vascular plants, the hair apices generate branched, needle-like hairs that are arranged in a ring. Unlike the leaves of vascular plants, hairs of *A. acetabulum* lack dormant growing regions adjacent to the stalk that will grow if the primary hair apex is lost, i.e. they lack regions that function like axillary buds. Akin to flower production in vascular plants, during reproductive growth the stalk apex makes a morphologically distinct, trilobed-whorl which is called

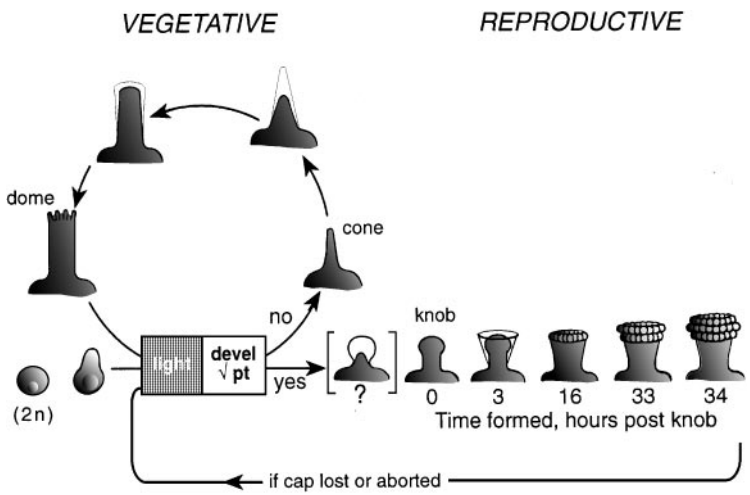

Figure 3 Shape changes in the stalk apex during vegetative and reproductive phases (adapted from 62). Vegetative shapes are based on data of Schmid (95). Reproductive onset depends on completion of vegetative phases, i.e. the plant must pass some "developmental checkpoint" and is marked by "*knob*." Knob ($t = 0$) is the only light-regulated shape change in the switch from vegetative to reproductive growth (62). The time below each *apex shape* indicates the hour at which 50% of a population of plants had a stalk apex of that shape.

the "cap" (Figures 1 and 2). The shape changes characteristic of the hair and rhizoidal apices have not been documented. However, especially given the paucity of data on the apices of *A. acetabulum*, it is premature to speculate whether any of the resemblances to vascular plants noted above are any more than superficial.

On a larger temporal and spatial scale, *A. acetabulum* progresses through juvenile and adult phases in development (69, 80, 87) as do vascular plants (63). These phases are morphologically distinct (Figure 4), temporally sequential and predictable, spatially stacked as they are in *Zea*, and physiologically distinct (Table 1). The basic features of juvenile and adult phases are similar in genetically heterogeneous wild type (87) and in two inbred wild-type strains (Table 2; 80). In general, the borders between the phases or "phase transitions" have been poorly characterized, and the molecular basis of phase change is unknown in *A. acetabulum* and has only begun to be elucidated in vascular plants (18).

Juvenile phase (Figure 4) comprises the first 1 cm of growth, represents 25–28% of the life cycle, and includes about 5–6 whorl-interwhorl units (87).

Table 1 Summary of phase characteristics of *Acetabularia acetabulum*[a]

	Juvenile phase	Adult phase	Reproductive phase
Physiology[b]			
Population density required	100 per mL	0.4 per mL	0.4 per mL
Growth rates[c]			
Rhizoids, mm per day[d]	0.04	0.02	?
Stalk, mm per day	0.17	0.61	0.19
Whorls of hairs, # per day	0.16	0.35	4–5 sets of cap hairs
Morphogenetic potential[c] of			
Apical portion			
Whorls of hairs, mean	0.2	2	<1 set of cap hairs
Cap	0	1	Not applicable
Basal portion			
Whorls of hairs, mean	13	10	10
Cap	1	1	1

[a]Values pertain to axenic growth and development in iterations of an artificial seawater (44).
[b]See References 69, 113.
[c]Hunt & Mandoli, unpublished data; 80.
[d]See Reference 86.

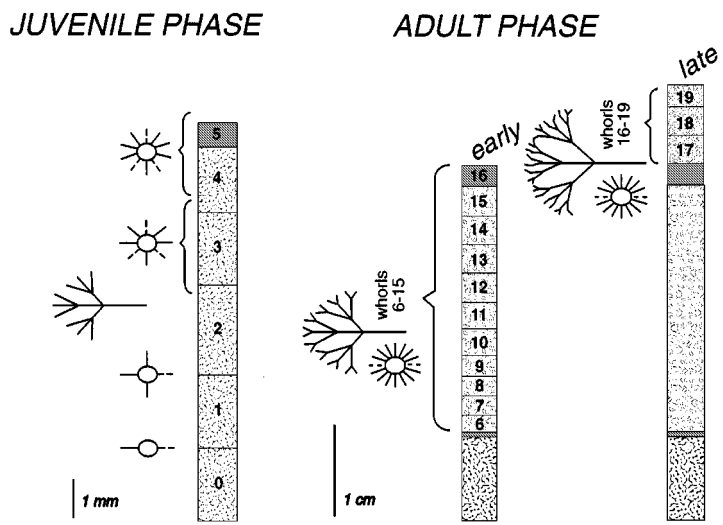

Figure 4 Summary of the spatial and morphological features that distinguish the vegetative phases from each other (derived from original data in 87). Note the separate scales relevant to juvenile and adult phases. Interwhorl lengths are drawn to scale and cross-referenced between the cartoons by common *shading textures* with likely regions of phase transitions indicated with a distinct texture. Positions of the whorls of hairs on each stalk are demarcated by *light horizontal lines*—these *lines* do not represent crosswalls. To the left of each stalk the number of hairs in a whorl and the branching patterns of those hairs is indicated for groups of whorls. *Dashed lines* indicate the standard errors for the hair arrangements in a group of whorls.

Juvenile stalks are threadlike and make whorls of just a few hairs that are clear (87). Juvenile rhizoids grow rapidly (Table 1; 86). In addition, juveniles may be uniquely able to attach readily to substrata (93), may retract their cytoplasm when overcrowded (93), and, unlike adults, may tend not to calcify (75). Physiologically, juvenile phase initiates well only in crowded conditions; zygotes differentiate best at a population density one million times the optimum for reproductive onset (22, 113), a population density at which adults die (113). One interpretation of the need for crowding of zygotes is that zygotes or juveniles make a growth factor that must accumulate in the medium to trigger differentiation to the siphonous growth habit, but this remains conjectural. In addition, juveniles may be more cold tolerant than adults. In the wild, observations suggest that "young algae overwinter" by retracting the cytoplasm into the rhizoid (93), and laboratory-grown juveniles, but not adults, can be stored in the cold often without adverse effects (7, 69). Apical portions amputated from juveniles survive but cannot initiate a cap without the nucleus (Table 2; 80), supporting application of the term juvenile for this portion of development.

Adult phase (Figure 4) comprises the remaining 2–3 cm of growth, represents 20–21% of the life cycle, and includes the next 10–14 whorl-interwhorls, i.e. the sixth to nineteenth ones made (87). Adult stalks grow rapidly (Table 1), average ~0.3 mm in width (71, 72), and make hairs that contain chloroplasts (87). Adult phase was split into early and late portions based on the morphology of the whorls of hairs: Hairs are more ramified and live twice as long in the late portion of adult phase (87). In general, the apical region of an early adult looks bald, and that of a late adult looks tufted because there are more whorls of hairs close to the stalk apex late in adult phase. Adults grow well only at low population densities, conditions in which zygotes fail to differentiate and juveniles grow poorly (22, 113). The stalk apex becomes competent to make a

Table 2 How phases in *Acetabularia acetabulum* might relate to morphogenetic potential

Primary bioassay	Interpretation of bioassay	Juvenile	Adult		Reproductive
			Early	Late	
When the intact plant makes a cap	Cytosolic inhibitor prevents premature reproductive onset	0%	0%	0%	100%
Apical portion (enucleate) makes a cap	Stable, cap-specific mRNAs are localized in the apical portion	0%[a]	15%[b]	60%[b]	Not applicable

[a]See Reference 80.
[b]See Reference 94.

cap without the nucleus during adult phase (Table 2). Adult phase terminates with initiation of the cap by the stalk apex.

Possible Functional Significance of Phases in A. acetabulum

Juvenile growth may serve to embed the sole nucleus rapidly in a protected locale, and, to a lesser degree, juvenile responses to body loss and adverse physiological conditions suggest that this phase might aid species survival. After the nascent zygote finds and adheres to a solid substratum, in the wild, the digits of the juvenile rhizoid grow into crevices in shells and pebbles (92, 93), and in the laboratory they spread out and flatten so as to grip the bottom of the Petri dishes. Because it houses the nucleus, rapid growth of the rhizoid (86) may quickly sequester the sole nucleus in a protected location away from grazers such as the ascoglossan, *Elysia timida* (75). While the extent to which these attributes are confined to juvenile phase and are missing in adult phase is not yet clear, assessing their functional significance using genetic analyses is now possible and looks promising.

Adult phase may function to increase the volume and surface area of the plant in preparation for the prolific reproduction of this unicell. Adult growth accounted for the major increase in plant height with a concomitant increase in the number of whorls of hairs (80, 87), implying that both the calculated surface area (31) and the volume of the plant increased more dramatically during adult phase. It is plausible that an increase in surface area may be important for nutrient uptake and for photosynthesis: Adult whorls of hairs contain chloroplasts (see Figure 2 in 31), each has a calculated surface area equal to that of the entire stalk (31), and each incorporates radioactive DNA and protein precursors (e.g. 11). The number of chloroplasts increases during development (67), with the main increase probably occurring during what we now call adult phase, an inference based on plant size. The response of the adult basal portion to loss of the apical portion is to repeat adult growth (Figure 5), which may reflect an important function: Given the fecundity of the organism and the presumed need for each gamete to have mitochondria and a chloroplast to be viable, successful reproduction may demand a full complement of the cytoplasm made in adult phase.

Responses to Removing and Adding Body Regions

In a unicell, recovery from loss of body parts cannot be achieved by replacing cells or recruiting new cells to fulfill the role of the lost body regions as a multicellular species would. The primary response to wounding by *A. acetabulum* is to limit loss of body contents (cytoplasm, periplasmic space, vacuole, etc) using the cytoskeleton and wound plug formation to heal the breach rapidly and efficiently (29, 77, 79). The secondary response of the body regions of

A. acetabulum to body loss depends on whether the nucleus is present, when in development that body loss was sustained, and how much of the body was lost (Figure 5). Hence, although *A. acetabulum* lacks structural redundancy, i.e. it lacks cell populations that can de-differentiate to heal a wound, it has functional redundancy because the basal portion can repeat development.

Responses to body loss have been interpreted to reflect both the morphogenetic capacity of each of the amputated body regions and the influence that that body region has on the morphogenetic potential of the remainder of the organism. As both portions of the organism survive, it is trivial to build simple and elegant internal controls into such amputation experiments. Interactions between the chloroplast and nuclear genomes have been inferred by comparing the numbers of organelles (e.g. 67, 99) or enzymatic activity (e.g. 25) in intact and amputated body portions (see other references in 12). Although nuclear implantations (e.g. 8, 37, 55, 96, 98) allow the roles of the nucleus and the cytoplasm to be further explored, what Hämmerling (40) called "nucleocytoplasmic"

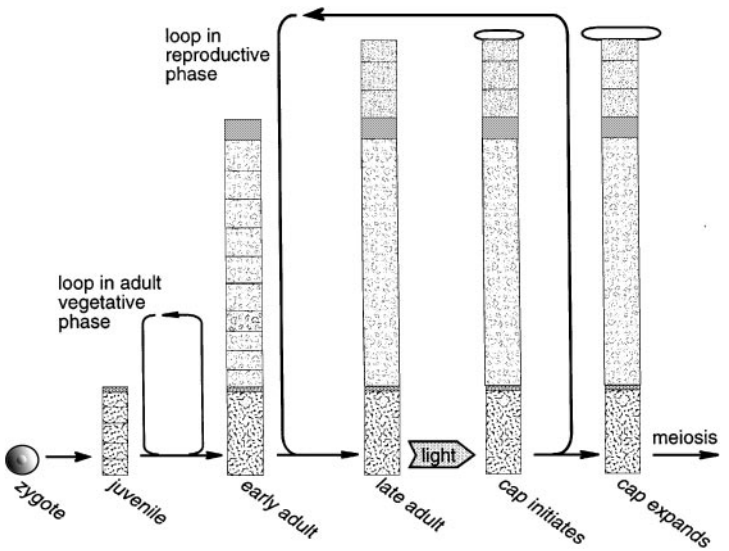

Figure 5 Amputation of the stalk apex prior to meiosis induces the rhizoid to repeat portions of development, i.e. to loop back through development (derived from 80, 94). The portion of development repeated depends on the volume of the apical portion removed and on when in development the amputation occurred. The vegetative loop shown was induced by removing all the apical portion, whereas the reproductive loop shown was triggered by removing just the cap and the whorl of hairs immediately below it. Textures of the phases and interwhorl lengths are as in Figure 4.

interactions, the responses of body portions that result from amputation are not synonymous with the responses of the cytoplasm to the loss of the nucleus and vice versa because there is always cytoplasm left with the rhizoid.

The responses to amputation of body portions during different phases in development suggest that the strategy of the intact plant is to render the stalk apex morphologically competent to reproduce before the nucleus commits to meiosis (33, 59, 61, 94) and that the nucleus then regulates the timing of reproductive onset. Here, *basal portion* means the entire rhizoid that contains the nucleus plus a variable amount of stalk and whorls depending on the experimental design, and *apical portion* means the stalk and whorls but does not include the nucleus or rhizoid. Note that these terms do not imply a time in development, e.g. an apical portion from a plant in reproductive phase would include a cap. Apical portions removed from juveniles grew, but few made a whorl of hairs (Figure 5), and none made a cap [0 out of 100 plants in Table 2 (80)]. In contrast, many apical portions removed from adults morphologically skipped the rest of vegetative development, making just 1–2 whorls of hairs before making a cap (36, 80). Taken together, these data suggest that the juvenile stalk apex has a limited ability to undergo morphogenesis or development without the nucleus and that the adult stalk apex is poised to reproduce but in some way is inhibited by the nucleus. In contrast, basal portions removed from juveniles finished vegetative growth without repeating vegetative development and without a delay in cap initiation [i.e. juvenile phase has no loop in Figure 5 (80)], as if loss of this part of the juvenile body was inconsequential. In contrast, basal portions removed from plants in adult or reproductive phase recapitulated the growth characteristic of adult phase both morphologically and temporally (*small loop* in Figure 5). These behaviors are reminiscent of the responses of portions excised from vascular plants (see references in 76, 76a–c). The response of the adult apical portion to loss of the basal portion implies that if nuclear function were blocked in an intact adult plant, then the organism would form a cap prematurely (Table 2); this is exactly what happened when transcriptional inhibitors were applied (114).

The responses of *A. acetabulum* to adding or replacing body parts, achieved by amputation followed by grafting, show that it responds to changes in body make-up and hint at how the enucleate and nucleate portions of the organism interact during development (Table 3). Interspecific grafts that combined the apical portion of one species with the basal portion from another could make viable progeny (reviewed in 12), whereas intergeneric grafts frequently died and failed to make progeny as if the apex and rhizoid were incompatible (13). In preliminary graft chimeras using the apical portion of one developmentally arrested (*da*) phenotype to the basal portion of another, interactions ranged from kill of one partner by the other to full acceptance and use of the donated

Table 3 Opportunities with graft chimeras of *Acetabularia* species

Nature of grafts	Purposes	References
Basal portion with other basal portion(s)[a]	Rescue putative mutants	71
	Infer interactions of nuclei in trans	86
	Infer nuclear dosage effects	14, 86
Apical portion with a basal portion	Define contribution of apical and basal portions to morphogenesis both intra- and interspecifically	10, 37, 41, 112
	Compensate putative mutants	71
	Characterize mutant biology (dependence on the polarity of the graft partner, etc)	70
	Compensation analysis (i.e. interactions of rhizoid of one mutant with apical portion of another or of wild type)	DF Mandoli & BE Hunt, unpublished data
	Intergenomic interactions (i.e. between organelle populations)	

[a]Number of rhizoids per graft chimera can be varied from 2 to 9 (14).

apical portion, suggesting that the graft partners both sensed and responded to changes in body composition (BE Hunt & DF Mandoli, unpublished data). These graft chimeras frequently form functional ectopic stalk apices, a response to replacement of body parts that has not been well studied and the significance of which is not known.

When a wild-type apical portion from a reproductive plant was grafted to the basal portion of a vegetative one from the same species, the graft chimeras generated haploid nuclei sooner than the nucleus in the basal portion would have been expected to had it been left intact (39) and much sooner than if the apical portion had simply been removed. Electron microscopy indicated that once associated with an older enucleate graft partner, a young nucleus would take on the appearance of an older one; it was smaller in volume and had reduced periplasmic space (6, 8), as if it were from an older plant about to enter meiosis. These results suggest that the reproductive apical portion hastened the vegetative nucleus to assume a reproductive morphology and induced karyokinesis, but this result has not been repeated or corroborated by other means, e.g. with molecular markers. Taken together, data from graft chimeras suggest that both the apical and basal portions mutually influence each other to orchestrate age progression and reproductive onset and have the potential to provide insight about how the identities of body regions are established and maintained.

The ability to compare genes *in trans* and *in cis* exists at several levels in *A. acetabulum*. For example, graft chimeras that combine from 2–9 rhizoids,

producing bi- or multinucleate heterokaryons, enable rescue of mutants or nuclear dosage experiments (Table 3). Given that genetic analysis is just beginning, it is hard to predict what comparison of complementation analysis and the cell biological cognate, *compensation analysis* (interactions of a cytoplasmic genotype with a different nuclear genotype; Table 3), will reveal. The utility and power of such genetic and cell biological comparisons will become clear only once the organelle and nuclear genomes of *A. acetabulum* are better characterized.

A MECHANISM OF MORPHOGENESIS?

Words change meaning as a field or idea evolves, so when the language used to describe events is questioned, it signals increased understanding. Conversely, when word choices are not challenged, they can inadvertently hamper understanding as much as missing or poor controls, preconceived notions, or hidden (therefore untested) assumptions (see discussion of "homologous" versus "analogous" in 49). In the case of *Acetabularia*, use of the term "mRNA" in relation to morphogenesis of the cap is a word choice that needs revisiting. In the 1930s, when this unicell first made its mark, the complexity of the RNA world—that is, the diversity of types, functions, and catalytic abilities of RNA was unknown. Accordingly, the criteria for applying the term mRNA as it is used today have dramatically changed. Curtailing use of the phrase "translational control" in view of the current criteria that term now implies and the limited data available on *Acetabularia* species seems similarly apropos. Hence, in the section that follows, every attempt has been made to stick closely to what was actually done in the experiments that originally engendered the somewhat entrenched belief that morphogenesis of the hair and cap whorls in this unicell is under translational control and that cap-specific mRNAs exist.

The Case for Translational Control of Morphogenesis During Development

The concept that morphogenesis is directed by unique information concentrated in the stalk apex arose from four kinds of data: fates of body regions post amputation, distributions of biochemical activities, interspecific grafting, and inhibitor studies.

The first important result was that the nucleus was the source of morphological information: An implanted nucleus alone could confer on a middle portion the ability to make a rhizoid or a stalk apex (this and other experiments on this point are reviewed in 40). Three laboratories used grafts to show that the nucleus contained morphological information that was species-specific (9, 13, 37, 111). A vegetative apical portion and a vegetative basal portion of

two species that make caps of different shapes were joined. Usually, cap morphology was detailed (e.g. 111), and then the cap was amputated each time one formed. In succession, the cap shape followed that of the donor of the apical portion, then was intermediate in shape, and finally was that of the donor of the basal portion (37). Note that while these data suggest that some aspects of cap shape are conserved enough to function across species lines, they do not indicate the chemical nature of the information. While the role of the nucleus as a source of species-specific morphological information may seem obvious now, in the 1930s it was revolutionary.

The basic finding that the enucleate apical portion can undergo morphogenesis is robust. Several independent studies corroborated that apical portions removed from older plants (based on their size and age, these were probably adults) could make one or two whorls of hairs and then a cap after amputation (9, 35, 36, 80), but apical portions removed from juveniles could make one whorl of hairs at best (80). The enucleate middle portion had a lower probability of making a cap at the apical pole than an apical portion did, and middle portions occasionally made a rhizoid-like structure at their basal poles (37). Primarily based on these amputations and the morphology and development of the intact plant, Hämmerling (reviewed in 37, 38, 66) proposed that there were two gradients of morphogenetic information in this species such that instructions for differentiating the rhizoid and stalk apices were concentrated at the basal and apical poles respectively.

There is independent biochemical evidence for apical-basal gradients of protein synthesis (30, 66, 89, 90), of ribosomal and mRNA (30, 40, 46, 47, 56–58, 65, 84, 97), of thiol groups (110), and even of specific enzymatic activities (e.g. 116) in this alga. These gradients differ widely in degree or strength of the gradient, in the crudeness of the assay used, and in direction, i.e. some constituents of the plant are concentrated at one pole and some at the other. It is possible that comparing gradients of total proteins, ribosomes, and redox potential in diverse wild-type and mutant backgrounds might provide insight into the strongly directional growth of the organism. However, the relevance of any gradient to pole differentiation and morphogenesis can only begin to be addressed using specific molecular markers and can be tested only by manipulation of the most important molecules in defined genetic backgrounds.

Two additional kinds of experiments suggest that at least one morphogenetic event, cap initiation, entails translation of relatively few proteins. For example, stalk apices treated with ultraviolet radiation are half as likely to make a cap (17, 91)—results that are consistent with RNA playing an important role in cap morphogenesis. However, such fairly nonspecific treatments do not rule out alternate explanations, e.g. preventing the growth needed to fashion the shape can account equally well for these results and similar types of experiments that

prevent morphogenesis (reviewed in 12). When analyzed on 2-D gels, in vitro translation of mRNAs from plants poised to make caps revealed just 29 proteins made de novo at cap initiation (101–104). Taken together, these data argue that cap initiation does not entail general up-regulation of translation. However, to the best of my knowledge, these experiments on cap morphogenesis have not been independently corroborated, and the question of the specificity of translation for morphogenesis of the whorls of hairs has not been addressed even to the limited extent it has been for cap morphogenesis.

The suggestion that the timing of cap initiation is regulated by an inhibitor that is made by the nucleus has support from three kinds of studies: amputation, grafting, and inhibitor studies in wild type and now in one mutant, *nightstick*. Since an apical portion taken from a heterogeneous wild-type plant formed a cap faster than an intact plant did (Table 2, Figure 5), Beth (10) surmised that "the nucleus" actively prevents premature cap initiation in the intact plants that, based on their size (about 2 cm tall at amputation), were probably adults. Because this result has not been extended with nuclear implantations, it is safest to state that the rhizoid actively prevents cap initiation by the (adult) apex. Consistent with this hypothesis, the transcriptional inhibitor, actinomycin D, turned on cap initiation in intact (probably adult) wild-type plants (115), implying that cessation of transcription turned on cap morphogenesis once the stalk apex had gained competence to make a cap. Intact adults make an average of 2.2 whorls of hairs in actinomycin D (80), suggesting that the information for making hairs may also be preloaded into the stalk apex. Although the lack of temporal delays in cap onset in interspecific grafts led to the suggestion that this inhibitor may be species-specific in nature (1), it is not clear that this effect, a whole plant bioassay, can be solely attributed to a molecule that remains putative. Finally, the amputation behavior of the *da* mutant *nightstick* (*nst*; Figure 6) supports the existence of a cytosolic inhibitor of cap initiation. In brief, *nst* is a recessive trait with a terminal morphology that arrests late in adult phase, i.e. it is capless when intact or wounded. After amputation of the apical portion, the *nst* basal portion made a cap. Furthermore, the probability that a *nst* basal portion would make a cap after amputation of its own cytoplasm was directly proportional to the volume of the apical portion that had been removed (72). In sum, the hypothesis that the nucleus produces a cytosolic inhibitor of cap initiation that plays a role in the timing of reproductive onset (9) deserves further consideration.

Taking a Genetic Approach: Are the Controls Spatial, Temporal, or Both?

Clearly, the solid foundation of knowledge about morphogenesis in *Acetabularia* species provides a springboard for further advances in understanding how

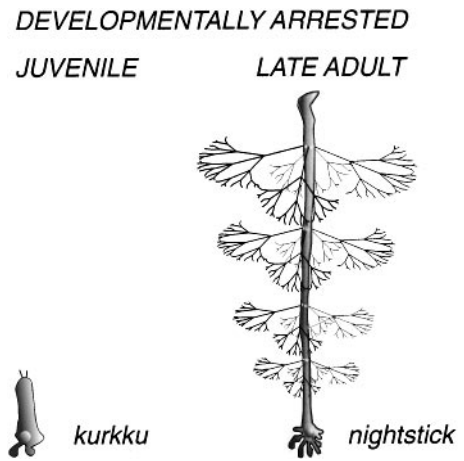

Figure 6 Phenotypes of two developmentally arrested mutants of *A. acetabulum* (68), both of which are recessive in outcrosses to wild type (71, 72). *kurkku* makes 0–1 whorl of hairs per plant consisting of 1–2 hairs each (70, 71). *nightstick* progresses normally through development, making an average of 10 ± 0.5 whorls of hairs until it arrests late in adult phase (72). Classes of *da* defects can be distinguished by their amputation behavior (68).

body plan is established and maintained in a unicellular context. However, a wealth of questions remain: How distinct are vegetative and reproductive whorls?—e.g. are hair and cap morphogenesis effected by distinct or overlapping sets of mRNAs, proteins, etc? How are juvenile and adult phases related—i.e. how abrupt is the switch and how is it timed? How is localized differentiation achieved without cellular partitioning?

The naiveté of the regulation of *Acetabularia* morphogenesis (e.g. Table 2) can be most succinctly illustrated by an example from a unicellular system about which more is known, the fly egg. As background, the gene *oskar* is central to posterior pole determination. When *oskar* activity is missing, *Drosophila* females make oocytes that lack a germ line or abdomen (64). If *oskar* is expressed in the wrong place, oocytes form posterior pole features ectopically (28). Three aspects of *oskar*'s role in germ plasm determination are of particular interest to thinking about *Acetabularia*. *oskar* RNA is found throughout the early oocyte, it becomes physically localized in several steps during oocyte development (27, 53). *oskar* mRNA that is not at the posterior pole is translationally repressed (52). oskar protein is needed to keep *oskar* RNA localized (27, 53, 75a, 93a; see also *staufen* e.g. 107). In sum, *oskar* activity becomes tightly restricted to the posterior pole of the oocyte by two mechanisms: by localization of *oskar* RNA and by spatially confined translation of *oskar*. It

seems reasonable to anticipate that pole determination and differentiation may be as complex in *Acetabularia* as it is in *Drosophila.*

One powerful aspect of a genetic approach is that prior knowledge of the target is unnecessary. This statement comes with the proviso that the phenotypes sought must be sufficiently broad to encompass all likely targets. Since it is premature to predict whether spatial or temporal cues will prove more important or experimentally tractable in identifying factors relevant to morphogenesis in *A. acetabulum*, we study two broad groups of phenotypes. Developmentally arrested (*da*) phenotypes (Figure 6) fail to make a cap but are normal in morphology until the point of arrest. In contrast, morphologically altered (*ma*) phenotypes are aberrant in body plan (Figure 7). Study of these phenotypes with genetic and cell biological manipulations is under way (71, 72). One way to explain development in this unicell would be to alter the apices (*ma* genes) as the organism ages (*da* genes) (Figure 8). That is, it is theoretically possible to regulate all of the patterning of the elaborate body plan of the Dasycladales just by locally controlling differentiation of the critical subcellular regions—the apices—and to regulate phase change by globally controlling development of the organism as a whole. Clearly, the first part of this working concept

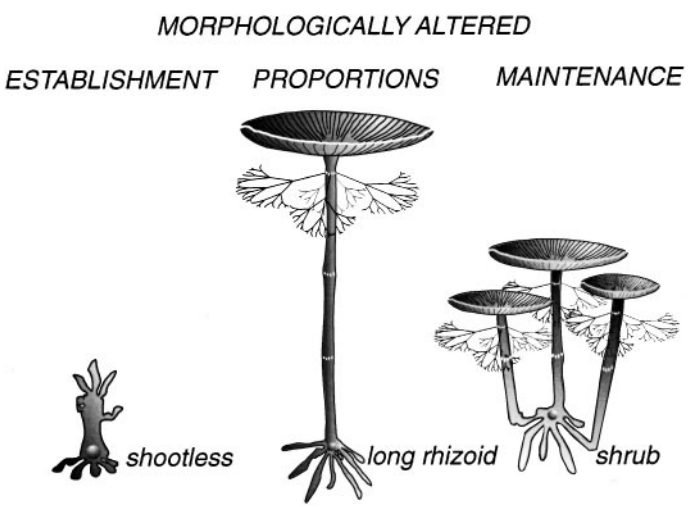

Figure 7 Morphologically altered phenotypes of *A. acetabulum* fall into three classes: those that fail to establish a pole, those that make too much of one body region, and those that fail to maintain the identity of a region after it is made (drawn based on photographs in 68). These phenotypes have been assigned a brief, descriptive name, but it is not yet known whether these represent genes.

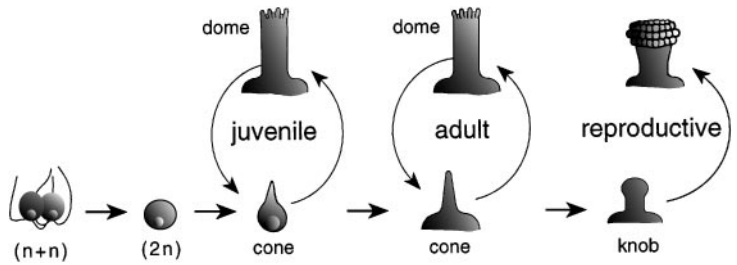

Figure 8 One idea of how morphological changes at the stalk apex and phase change might interface in *A. acetabulum*. All the whorl-interwhorl units made in juvenile or adult phases are represented by a simple alternation between a cone-shaped stalk apex or a dome-shaped stalk apex that has just initiated a whorl of hairs. It has not been proved which of these apical shapes is the default or uninduced shape, and it is not known how *ma* and *da* defects might be related.

has resonance with the emerging picture that cellular position is important to morphogenesis in multicellular vascular plants (21, 51, 81). My bias is that genes that effect the temporal sequence of development and genes that alter the spatial definition of body regions are needed if we are to begin to understand morphogenesis and phase change as well as the interface between these processes.

POTENTIAL FOR FUTURE INSIGHTS INTO DEVELOPMENT AND MORPHOGENESIS

It is striking that phase change is shared by this ancient unicellular protist, *A. acetabulum*, and by modern multicellular plants. Perhaps the study of phase change in a unicellular context will lend insight about why it evolved and into how it is accomplished. Of paramount interest would be aspects of phase change that have been evolutionarily conserved independent of whether the body plan of the organism is partitioned into one or many cells. Why is competence to reproduce, a feature of adult phase (63), acquired so early in development in *A. acetabulum* and in multicellular plants (e.g. 18)? Is the strategy to prevent premature reproductive onset rather than to delay competence an ancient one? This said, it would be folly to ignore the alternate view that the very concept of phase change is not useful in thinking about development but an artificial construct without real biological relevance (76) until we have more than an inkling of the genetic circuitry (e.g. 82) that embodies vegetative growth.

How shape and function of localized regions are established and then maintained within cells, a question of fundamental importance in development, is

one to which *A. acetabulum* is well suited. Given the central role of localized determinants to patterning of the body plan in animals, in the long term it will be interesting to compare the mechanisms for localizing determinants in species as diverse as fly oocytes (88) and *Acetabularia*. The extent to which features of the diplophase body plan are parentally derived or imprinted, e.g. maternal mRNAs in *Drosophila* or structural elements of body plan like surgically altered cilia in *Paramecium* (48), cannot be ruled out and will be important to distinguish from those which are made de novo.

The physical height of the organism may have implications for the crosstalk between the nucleus and the major site of morphogenesis, the shoot apex. Given this long-distance relationship, it is hard to envision that the cytoskeleton (78) and molecular motors would not be intimately involved in moving and targeting information for aspects of pole establishment, differentiation, or maintenance of body regions. *Acetabularia* have sizable internal currents (see references in 83), but whether such currents play any role in morphogenesis in this plant is unknown. Perhaps the physical distance between the poles of *A. acetabulum* provides a clue about why the data support translational rather than transcriptional regulation of reproductive onset. For example, if speed of execution is important to changing the shape of the apex or in coordinating apical and nuclear crosstalk at specific times in development, then the physical distance between the nucleus and the shoot apex may preclude transcriptional regulation. In sum, being a giant unicell with a single nucleus may in itself have had ramifications for the way this algal species functions and, perhaps, for how it has or has not evolved.

THE HALLMARKS OF A CLASSIC DEVELOPMENTAL SYSTEM

In these days of limited funding, technical access to cross phyla comparisons, and well-established model systems, what are the most compelling reasons to use *A. acetabulum*? For development and morphogenesis, the size of its unicellular body that still retains the architectural and developmental complexity of "higher" plants physically enables the network of internal and external cues to be partly unraveled. For molecular evolution, the Dasycladales offers a 570-M year fossil record [186 total with 11 extant genera (7)] that will help address whether the unicellular nature of *A. acetabulum* and other species is primary or derived from a multicellular ancestor, and that will be useful to analyses of the conservation of homeotic genes. For cell biology, the ability to manipulate the body of the organism (to amputate, to mix-and-match the cytoplasm of one genotype with the nucleus of another) and the chemical composition of the environment or the organism (to deliver drugs and inhibitors exogenously

in an axenic, defined growth medium or via microinjection) presents tantalizing opportunities. For routine classical genetics, the species offers self- and out-crosses, packaged mating types, amplification of nuclei, millions of progeny per plant per generation and the possibility of making stable haploids (26). For molecular genetics, it offers abundant DNA per generation, haploid and diploid transformation of high yield and stable expression. However, to me, the special appeal of *A. acetabulum* lies not just in the ability to address important questions in developmental and structural biology in the context of a physically large and architecturally complex unicell, but in being able to do so with access to a diverse and robust toolkit because this means that if one avenue of attack does not work, another probably will.

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