J Plant Growth Regul (2000) 19:253–264
DOI: 10.1007/s003440000035

J P G R
Journal of Plant Growth Regulation

Acetabularia: A Unicellular Model for Understanding Subcellular Localization and Morphogenesis during Development

Jacques Dumais,[1] Kyle Serikawa,[2] and Dina F Mandoli[3]*

[1]*Department of Biological Sciences, Stanford University, Stanford, California 94305-5020, USA;* [2]*Department of Biochemistry, University of Washington, Seattle, Washington 98105-5325, USA;* [3]*Department of Botany & Center for Developmental Biology, University of Washington, Seattle, Washington 98105-5325, USA*

Abstract

Acetabularia acetabulum is the organism that provided the first compelling experimental evidence both for the role of an organelle whose function was unknown, the nucleus, and for the existence of "morphogenetic substances," the behavior of which presaged the discovery of mRNA in other organisms. This giant unicell holds special appeal as a model system, because the contribution of its diploid nucleus to cellular processes can be assessed using simple amputation and grafting experiments and because it lends itself to a wide range of methods in cell, molecular, and developmental biology. It remains an excellent model system for understanding how body regions are functionally and structurally distinguished from each other without cellular compartmentation. Advances in genetics (that is, mutant selection and analysis, large-scale transformation) will greatly increase the power of this system to address fundamental questions in development and morphogenesis. We discuss strengths and weaknesses of the system and outline the body of knowledge that would make the system more powerful and broadly appealing.

Key words: Chlorophyta; Ulvophyceae; Dasycladales; Development; Morphogenesis; Bauplan; Cytodifferentiation; Unicellular; Uninucleate; Nuclear behavior

On first viewing, the complex morphology of the "Mermaid's Wineglass" (Figs. 1, 2) usually evokes gasps of awe for its beauty closely followed by expressions of disbelief: how can this be a single cell? The 3- to 4-cm height of the organism challenges the common view that all cells are microscopic. The wonder increases when the first-time observer learns that for most of its life this alga contains just a single nucleus. The complex architecture, with its whorls of branched cell extensions, challenges the common view that the shape of a free-living cell must be simple.

Acetabularia acetabulum holds a classic place in developmental biology (Hämmerling 1953, 1963), but fell from favor in the 1980s in part because it was difficult to culture rapidly (see discussion in Mandoli 1998b). However, recent improvements in culture conditions have reduced care during development and resulted in a shorter life cycle (Hunt and Mandoli 1992, 1996; Cooper and Mandoli 1999). Judg-

Online publication 1 March 2001
**Corresponding author; e-mail:* mandoli@u.washington.edu
Authors JD and KS contributed equally to this article.

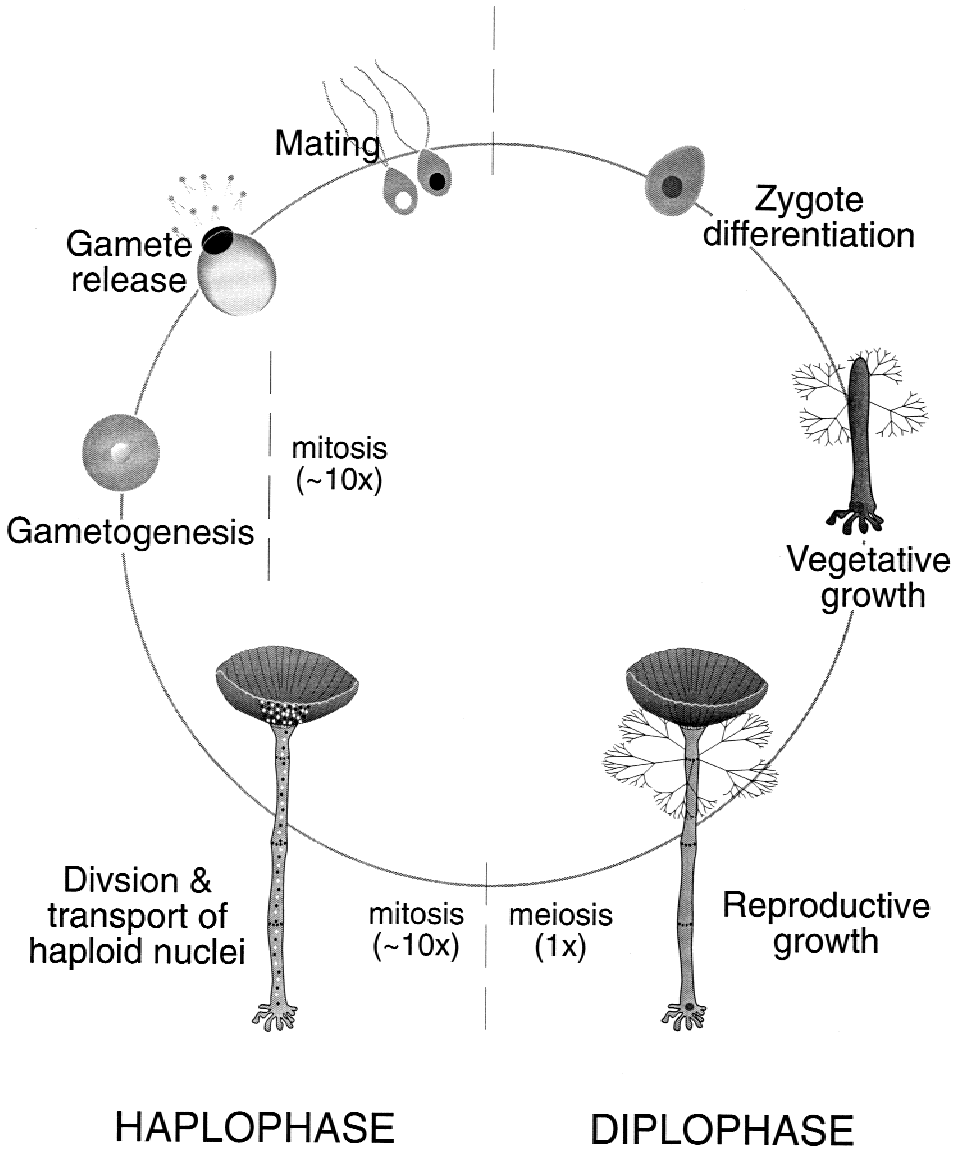

Figure 1. Life cycle of *Acetabularia acetabulum*. Details and descriptions of the life cycle can be found in Mandoli (1998b)

ing by the new authors publishing in the field and the number of inquiries for cultures, methods, and collaborations received per year (DF Mandoli, personal observations), this system may be gaining in popularity once again. With large-scale genetics (for example, selections and mutagenesis) on the horizon for the first time, this system is becoming more attractive for the study of a variety of interesting biological questions. Foremost among these is how does a unicell make and maintain such a giant, morphologically complex shape with only one nucleus?

The life cycle of *A. acetabulum* is complex (Fig. 1). The developmental biology of the nucleus and morphogenesis of the organism are both interesting (see following sections) and, because it heals well after wounding, interactions between the nucleate and enucleate portions of this unicell can be studied either in the intact organism or in amputated portions of it.

Strengths of *Acetabularia acetabulum* as a Model System

It Has a Relatively Small Genome and (Probably) Normal GC Content

Although this genome is larger than that of many other green algae, it is small compared with vascular plants that average 3.0 pg and 8.5 pg 1C values for annuals and nonannuals, respectively (Bennett and Smith 1991). Microspectrophotometry suggests n = 0.92 pg of DNA for *A. acetabulum* (propidium iodide staining, Spring and others 1978). It is about the size

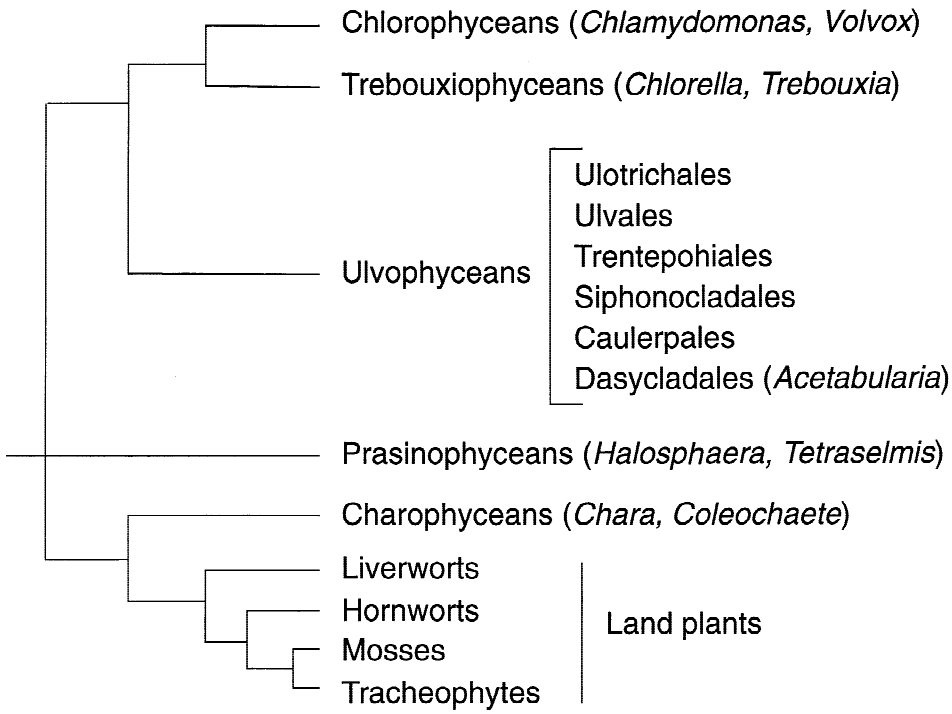

Figure 2. Phylogeny of *A. acetabulum* and its close relatives. Based on structural, biochemical, and molecular data (Graham and Wilcox 2000, for example Table 17–1), the green algae (chlorophytes) are grouped into three evolutionary lineages. Of these, only the two major lineages contain multicellular organisms. One major lineage gave rise to land plants (right branch). Charophycean algae, including *Chara,* are basal in this lineage. The second major lineage (left branch), the ulvophyceans, trebouxiophyceans, and the chlorophyceans, probably arose from unicellular flagellates in the prasinophycean lineage (middle branch). The third (minor) lineage are the prasinophyceans, which are polyphyletic. The ulvophyceans are monophyletic and, based on several lines of evidence (reviewed in Graham and Wilcox 2000), are the earliest of the three groups in this major lineage to diverge. Ulvophyceans and prasinophyceans are primarily marine, whereas the trebouxiophyceans and the chlorophyceans are usually fresh water or land species. This phylogeny is redrawn from Fig. 17–7 in Graham and Wilcox (2000) with additions closely following their text.

of the tomato genome, and 4.6 times larger than the *A. thaliana* genome (Feulgen staining, Bennett and Smith 1976, 1991). In the only full-length gene cloned to date (carbonic anhydrase-1, Serikawa and others 2001), the number and sizes of the introns were not unusual, but any general statement about genomic structure (that is, mean size of exons and introns, amount of nongenic DNA) is obviously premature.

Probably none of the three genomes in this alga is GC-rich. Chemical analysis revealed that the haploid genome was 29% adenine (Baltus and others 1968), giving a calculated 42% GC content. Lacking direct measurements of the other nucleotides, this one value is not well supported. Bouyant density measurements suggested that the haploid was 38% GC (Green and others 1970). Looking at individual genes rather than the genome as a whole, *A. acetabulum* sequences in Genbank also provide an estimate of only 44% GC (calculations based on 16 coding domains using the Codon Usage Database at http://www.kazusa.or.jp/codon/). The chloroplast genome is 45–50% GC (Green and others 1970; Leible and others 1989), and the mitochondrial genome is 55% GC (Green and others 1970). GC-rich sequences can complicate cloning and heterologous expression, but the available data do not suggest severe problems are looming.

The Large Nucleus Has Interesting Cell Biology and Biochemistry

In the 1930s, simple amputation experiments that separated the alga into nucleate and anucleate portions suggested that the role of the nucleus was to store the blueprint of the species (Hämmerling 1932, 1934). This was the first compelling evidence for the role of the nucleus in any organism.

The diploid nucleus lends itself to studies of chromosome behavior (for example, Koop and others 1978, 1979), because it is so large and has a fixed position in a relatively transparent unicell. The diploid nucleus in this and related species ranges from 50–120 µm in diameter (Berger and others 1994; Shihira-Ishikawa 1984). This is large compared with other organisms studied for chromosome dynamics. For example, although the *A. acetabulum* nucleus shrinks to 40 µm just before meiosis (Koop and others 1979), it is still one third larger than the maize nucleus in meiotic prophase, which is 30 µm in diameter (Z. Cande, personal communication, July 2000). Remarkably, the spindle during meiosis

reaches 150 μm in diameter (Koop and others 1979). During diplophase, the nucleus is anchored in the base of the plant and surrounded by a dense perinuclear matrix (Berger and Schweiger 1975; Berger and others 1975; Franke and others 1974; Menzel and others 1996). During vegetative development, lampbrush chromosomes have been observed (Berger and others 1994; Spring and others 1975, 1978), and the size of the nucleus increases dramatically during exponential vegetative growth (Franke and others 1974; Spring and others 1978). Indirect immunolabeling combined with confocal microscopy distinguishes different antigens in chromosomal regions during development (Berger and others 1994). A karyotype made with 4′,6-diamidino-2-phenylindole (DAPI) stained nuclei suggests that the diploid has 40 chromosomes (De and Berger 1990), but older methods suggest there are half this number (see references in De and Berger 1990). Chromosomal gels and direct examination of *both* meiotic divisions of the diploid nucleus could help resolve the number of chromosomes.

Meiosis occurs during cap expansion (Fig. 1) (De and Berger 1990; Runft and Mandoli 1996; Shihira-Ishikawa 1984) and then rounds of mitosis generate thousands of haploid nuclei (for example Nishimura and Mandoli 1992a). These nuclei are loaded onto the cytoskeleton and transported up into the cap (Menzel 1986; Menzel 1994). The bulk of the cytoplasm moves into the cap with these nuclei (Fig. 1). The fate of the large central vacuole during nuclear and cytoplasmic transport is unknown. If nuclei are left in the stalk, some cytoplasm stays with them, and viable gametes or gametangia can form there (Hämmerling 1963; Puiseux-Dao 1962), suggesting that the haploid nuclei are associated with cytoplasm. If there are physical and/or chemical associations of each haploid nucleus to a specific region of cytoplasm, a kind of cellularization without wall deposition, perhaps the cytoplasm, is passively dragged upward during transport of these nuclei.

Nucleic acid metabolism in this unicell is impressive. There are 3800 rDNA genes and 32 nucleoli in the diploid nucleus (Spring and others 1978). In contrast, other green algae have approximately 400 copies of rDNA (see references in Spring and others 1978). Presumably, so many copies of rDNA are necessary to build a giant vegetative organism from just one diploid nucleus (Fig. 1). With 0.92 pg of DNA per haploid nucleus (Spring and others 1978) and production of 2–6 million gametes per unicell (see table and references in Mandoli 1998b), each individual makes 1.8–5.6 μg of DNA per generation! Not surprisingly, inhibition of folate-dependent one-carbon metabolism, the biochemical pathway that generates purines and thymidylate in all organisms, curtails development and limits the number of progeny (Richmond and Mandoli, unpublished), suggesting that efficient nucleic acid metabolism is important in this primitive eukaryote.

The Diploid Thallus Is Large

Amputation is a powerful yet simple way to look at the developmental and physiologic potential of nucleate and enucleate portions of the plant. In addition, determination of RNA and protein half-life with and without the nucleus are easy amputation experiments and not fraught with the ambiguities inherent in using inhibitors. Amputation can even be used to determine subcellular enzyme activity on living fragments of the thallus (for example, carbonic anhydrase: Serikawa and others 2001; Zetsche and others 1970).

The large size of *Acetabularia* facilitated one of the first examinations of gradients in morphologic and developmental potential within a unicell (for instance, Hämmerling 1936). Examination of the mRNA distribution within the thallus for specific genes suggests that genes fall into four classes: those ubiquitously expressed throughout the organism, those more abundant in the base, those more abundant in the stalk apex, and those whose distribution changes during development (Serikawa and Mandoli 1999; Serikawa and others 2001; Vogel 1998). These studies were by no means exhaustive, however, and other patterns may exist.

The Diploid Thallus Develops an Elaborate Shape

At first glance, the most striking aspect of this unicell is its elaborate architecture (Fig. 3). The thallus consists of a central axis, the stalk, and two kinds of lateral structures: hairs and gametophores (Fig. 3). Both lateral structures are arranged in whorls. Fourteen to 19 whorls of branched hairs are initiated at more or less regular intervals during vegetative growth (Nishimura and Mandoli 1992b). One whorl of gametophores, called a "cap," initiates at reproductive onset (see Fig. 3).

Morphogenesis in *A. acetabulum* occurs principally at the stalk apex. Careful observations subdivide whorl initiation into discrete events (Fig. 4) (Dumais and Harrison 2000). Stalk elongation (Fig. 4A) (Von Dassow and others 2000) may be the "default" growth pattern, because under restrictive conditions (Goodwin and others 1983; Schmid and others 1987), these unicells grow at the apex without ever forming whorls of hairs. The growing stalk apex shares features with tip-growing cells (Harrison and

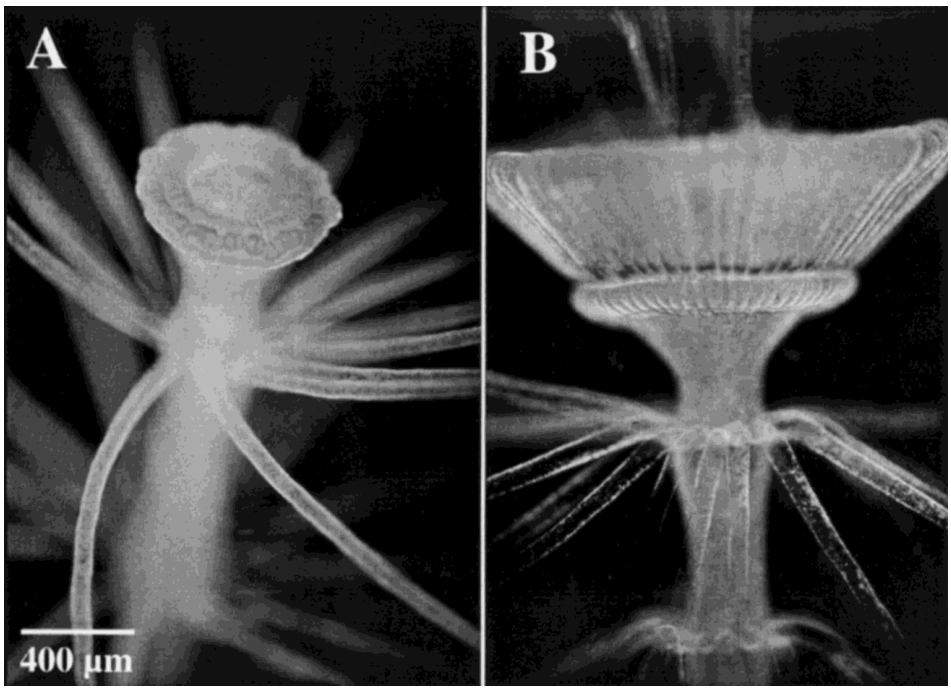

Figure 3. *A. acetabulum* is a striking example of complex cellular morphogenesis. **(A)** Top view of a developing "cap" (reproductive whorl). The whorl of gametophores has initiated and formed a staggered series of bulges around the periphery of the cap. A whorl of hairs is visible below the cap. The hairs branch just outside the picture. **(B)** Underside of an expanding cap. The cap has reached approximately one-fifth of its final diameter. The fold at the base of the cap is the corona inferior. Two whorls of hairs are also visible below the cap. Some hairs that have emerged from the corona superior (not visible) are visible above the cap.

others 1988; Reiss and Herth 1979; Schmid and others 1987). Just before initiation of a whorl of hairs, the tip flattens (Fig. 4B). Visualization of membrane-bound and free cytosolic calcium redistributions during this event (Dumais and Harrison 2000; Harrison and others 1988) suggests that the region of maximal wall expansion shifts from the apex to the subapical region during apex flattening. Next, small lytic pits on the inner side of the wall (Fig. 4C) mark where the lateral appendages will emerge. Uncharacterized proteins (Werz 1959), the endoplasmic reticulum (Werz 1959) and actin filaments (Menzel 1996), are associated with regions of lytic activity, suggesting that the pits result from coordinate secretion of lytic enzymes. The lysis pits develop into small chambers in the wall corresponding to outward bulges on the surface of the tip (Fig. 4D). Each bulge elongates and branches three to four times to form a branched hair. A septum concurrently forms at the base of the appendage (Fig. 4E), but a large central pore preserves the continuity between the lateral appendage and the central cavity. Meanwhile, the main stalk resumes apical growth until initiation of the next whorl (Fig. 4F).

One of the most intriguing morphogenetic features of *Acetabularia* is the establishment of the nicely spaced whorl pattern (Fig. 4C). Beyond the few observations mentioned earlier, little is known of the molecular basis of this process. However, observations of a different kind have provided additional information. First, the spacing between hairs in a whorl is affected by temperature (Harrison and others 1981) and exogenous Ca^{2+} concentration (Harrison and Hillier 1985; Harrison and others 1997) in a manner suggestive of kinematic regulation. Second, the whorl pattern is initiated simultaneously, as revealed by the uniform degree of lysis in a whorl. Together, these observations suggest that the whorl pattern is established in a single event by a kinetic process that encompasses the whole apex (Dumais and Harrison 2000). Whorl formation in *Acetabularia* presents a beautiful challenge for those interested in cellular morphogenesis. Putative mechanisms for the control of whorl morphogenesis (Goodwin and Trainor 1985; Harrison and others 1981; (Martynov 1975) offer a wealth of hypotheses that await experimental verification.

Classical Genetics, Including Selfing and Out-crossing, Are Feasible.

This species is monoecious. *A. acetabulum* makes gametes of two mating types that are packaged into different gametangia (Fig. 1), that is, gametes from the same gametangium cannot mate (Green 1973; Koop 1975). Outcrosses and selfs involve pairing two gametangia from different or the same parent, respectively. Given the millions of progeny per individual per generation, segregation and propagation of defects are easy. Although the thallus is large,

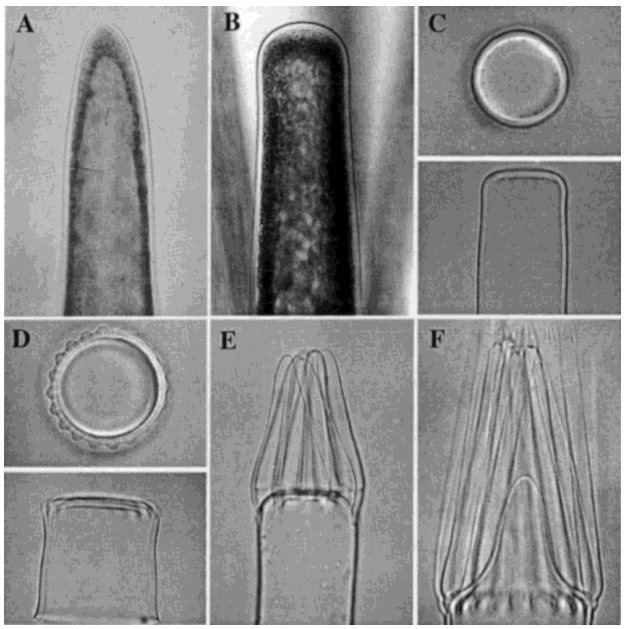

Figure 4. Morphogenesis of the vegetative whorl of *A. acetabulum.* **(A)** Growth at the stalk apex. The large central vacuole is surrounded by a thin layer of cytoplasm and a relatively thick cell wall. **(B)** Flattening of the tip at the onset of whorl formation. **(C)–(F)** The cytoplasm was removed to reveal the shape of the cell wall (from Dumais and Harrison 2000). **(C)** Top and side views of a cell at the lysis stage. A regular array of lysis pits is seen at the periphery of the tip (upper panel). **(D)** Top and side views of a young whorl. **(E)** Septum formation. The septa forming are above the junction between the hair segments and the main stalk. The hair segments have flattened tips indicating the initiation of branching. **(F)** Resumption of growth at the stalk apex. The young hair segments that surround the apex have branched once.

one can grow millions of these organisms easily in a small space.

Koop (1977a, 1977b) isolated a spontaneous mutant from the wild that arranged the gametangia in the cap in an aberrant pattern. This proved to be a recessive, single gene trait that segregated in Mendelian fashion (Koop 1977a, Koop 1977b). During creation of inbred near isogenic lineages, we have isolated several spontaneous mutations that are arrested in vegetative development (Mandoli 1996; Mandoli and Hunt 1996) or altered in morphogenesis (Mandoli 1996, 1998a). The developmentally arrested defects were grouped into four classes on the basis of which portion of the bisected mutant, nucleate or enucleate, could make a cap in the absence of the other (Mandoli 1998a). Unfortunately, the two of these genotypes that are the best studied do not segregate as single gene traits (Mandoli and Hunt, unpublished).

The Dasycladales Have an Extensive Fossil Record

Acetabularia belongs in the ulvophyceans (Fig. 2). The earliest ulvophycean alga arose 700–800 M years ago in the Precambrian period. The Dasycladales, the order to which *A. acetabulum* belongs, has a nearly continuous fossil record because many members of this order calcify in the wild (Berger and Kaever 1992; see also the Web ring at http://faculty.washington.edu/mandoli). The most ancient remains that resemble modern species in the Dasycladales are 600 M years old. Interestingly, this ancestral Dasyclad, *Yakutina,* is thought to be the oldest calcified green alga (Graham and Wilcox 2000). There are about 175 fossil dasycladacean genera and 11 extant ones (Berger and Kaever 1992; for cultures see http://www.bio.utexas.edu/research/utex). The modern genus *Acetabularia* first appeared about 38 M years ago and includes about eight species.

The fossil record has been studied from a paleontological standpoint (Deloffre 1988, Pia 1920) and also provides valuable insights into the morphogenesis of extant species. For example, analysis of the fossil record and of morphogenesis in extant Dasycladales reveals structural similarities between the vegetative and reproductive whorls (Church 1895; Dumais and Harrison 2000; Pia 1920; Solms-Laubach 1895; Valet 1968). Whorls made by the Dasycladales share the same basic architecture (Bauplan) but take multifarious shapes in different species. The vegetative and reproductive whorls of *A. acetabulum* may constitute the most extreme case (Fig. 3). Their common Bauplan (Fig. 5) suggests that the reproductive whorl derived from the hair whorl, so it would be interesting to compare their morphogenesis so as to understand how such striking morphological differences are generated. As models for cellular morphogenesis, the favorable features of *Acetabularia* and other extant Dasycladales are greatly augmented by their extensive fossil record.

Structure and development in the Dasycladales reflects that of vascular plants, and such comparisons may help us determine which features are independent of cellularization (Mandoli 1996, 1998a). Structural similarities to vascular plants have been recognized since Nägeli (1847). For example, the architecture of *Acetabularia* (rhizoid-stalk-hair-cap) resembles that of vascular plants (root-stem-leaf-flower). But the most surprising similarities between this alga and vascular plants are developmental. One of these, regular progression through juvenile,

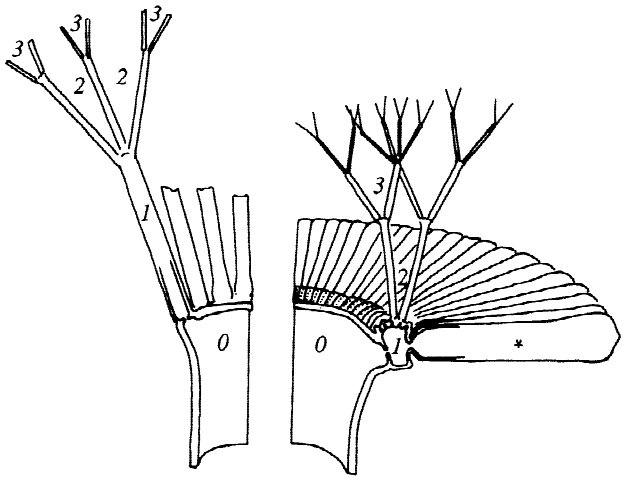

Figure 5. Homology between the whorl of hairs and the cap of *A. acetabulum.* Homologous structures were given the same number. The cap gametophores (asterisk) are considered an evolutionary innovation and therefore do not correspond to any structure in the whorl of hairs. This scheme, proposed by Solms-Laubach (1895), is the most compatible with what is currently known about morphogenesis and evolution of whorls in the Dasycladales. Other models exist (see Dumais and Harrison 2000).

adult, and reproductive phases during development, is reviewed elsewhere (Mandoli 1998a).

Are there fundamental developmental rules common to algae and vascular plants that transcend cellularization? Perhaps comparison of the fossil records of vascular plants and the Dasycladales will reveal shared aspects of the evolution of development as well. We thus concur with Chadefaud (1952) in that there is much to be learned from the algae, both with regard to their own morphogenesis and the light they shed on the morphogenesis of vascular plants.

A. acetabulum Is Amenable to a Wide Range of Experimental Approaches, Including Transformation

Physiological studies are attractive because one can perform feeding studies in axenic cultures (Hunt and Mandoli 1992) in completely defined, artificial medium (Hunt and Mandoli 1996). Uptake and depletion studies are facilitated by growth in a liquid medium.

Electrophysiology (Mummert and Gradmann 1991a, 1991b) is attractive because of the size of the thallus, the ease with which one can make wall-less spheres of cytoplasm bounded only by a membrane, and the ability of the amputated individual to heal. For example, fluxes of ions can be measured in relation to the physiology and differentiation of body regions (Serikawa and others 2000). The potential for examining calcium gradients (Reiss and Herth 1979; Harrison and others 1988) during polar growth at the stalk apex and the redistribution of membrane-bound and free cytosolic Ca^{2+} that accompanies morphogenesis of the whorl of hairs (Dumais and Harrison 2000; Harrison and others 1988) has barely been tapped.

Evidence for stable transformation on the small scale is robust: several heterologous genes have been expressed (Brown and others 1986; Cairns and others 1978a, 1978b, 1978c; Neuhaus and others 1984; Neuhausand others 1986), one over three generations (Neuhaus and others 1986). The method entails removing the nucleus from one plant, microinjecting it with the DNA of choice, and implanting that nucleus back into a second, enucleate individual. CAMV35S and T-antigen promoters work, and plants are stably transformed to neomycin resistance (Cairns and others 1978a; Cairns and others 1978c; Neuhaus and others 1984, 1986). The caveats are that the method is labor intensive, the amount of DNA injected is hard to control (we calculate that they injected 330 ng of adenovirus type 2 DNA, or about 10^{10} genomes per nucleus: Cairns and others 1978a), and it results in many inserts per genome (Neuhaus and others 1986). To the best of our knowledge, only Schweiger's laboratory has published success with this method, although perhaps no one else has tried, and there is no question that the method is relatively "high tech."

Localized Determinants: The Key to Morphogenesis and Development in a Unicell?

Of the arenas in which *A. acetabulum* could excel as a model system, perhaps none is more exciting than understanding how it partitions its subcellular space into differentiated body regions. In situ hybridization with poly(U) probes indicates that mRNA in *A. acetabulum* is present in a gradient with abundance highest in the base near the nucleus (Garcia and Dazy 1986). Messenger RNAs of some genes are localized (Serikawa and Mandoli 1999; Serikawa and others 2001; Vogel 1998), and enzyme (uridine diphosphate glucose pyrophosphorylase (UDPG-P) and others) activities, can be asymmetrically distributed along the stalk (Zetsche and others 1970). The existence of phenotypes that are morphologically altered (Mandoli 1996) suggests that one can genetically perturb the creation and maintenance of this presumed organization. Clearly, these data indicate that subcellular localization of mRNAs and proteins

occurs and that mutations that perturb morphology can be found. What is needed is to identify specific determinants that make or regulate morphogenesis in wild-type *A. acetabulum* and that cause changes in morphology when they are mislocalized or mutated.

As with other large algae such as *Fucus* (Bouget and others 1996; Kropf and others 1999; Shaw and Quatrano 1996), in situ hybridization, injection of ion-sensitive dyes, and immunolocalization allow excellent resolution of the subcellular localization of various developmentally important molecules, such as actin and calcium (Harrison and others 1988; Menzel 1994). Amputated subcellular portions can also be assayed for enzyme activity and mRNA localization (Keck and Clauss 1958; Serikawa and Mandoli 1999; Serikawa and others 2001). *A. acetabulum* surpasses *Fucus* in the potential for genetics and for the continued unicellularity throughout most of development. Again, although all of these data are consistent with subcellular localization being important, the mechanisms and determinants involved in this process have yet to be explored.

Post-transcriptional Control: Distinguishing Two Possible Mechanisms?

The ability to survive and develop when enucleated suggests that posttranscriptional regulation controls much of gene expression in *A. acetabulum.* With a single nucleus, other control mechanisms, such as differential regulation of nuclear genes in tissues with different fates, are impossible. With a giant unicell, more conventional strategies for responding to changes in the environment (stimuli perception and signal transduction to the nucleus leading to changes in gene expression) may be too slow to be practical. Its reliance on posttranscriptional control may be rivaled only by *Drosophila* or *Xenopus* oocytes, or neurons.

Seen through the lens of current biological knowledge, Hämmerling's amputation experiments suggest at least two posttranscriptional mechanisms that may regulate *A. acetabulum* development: subcellular localization of mRNAs (discussed earlier) and selective translation of mRNAs. Protein abundance and activities of several enzymes such as UDPG-P and pyruvate kinase increase when transcriptional, but not translational, inhibitors are added to algal populations (Berger and others 1987; Nickl and others 1988). Enzyme activities of proteins can also increase in enucleated algae, suggesting controlled translation of a long-lived mRNA pool (Keck and Clauss 1958; Nickl and others 1988; Spencer and Harris 1964). Li-Weber and Schweiger (1985) demonstrated that some mRNAs are actively recruited to polysomes in both nucleated and enucleated cells at specific times in development.

The ability to grow large cultures of these algae should facilitate biochemical approaches to isolating regulatory components of the posttranscriptional apparatus. Microinjection will be useful for introducing reporter constructs and for identifying *cis* and *trans* acting factors that control posttranscriptional regulation.

Weaknesses of *Acetabularia acetabulum* as a Model System

It has Unusual Codon Use: An Unchangeable Issue.

A. acetabulum reads only one of the three normal stop codons as a stop and the others as glutamine. Sequences of 10 cDNAs and of the protein for ribulose bisphosphate carboxylase confirmed that the stop codons had been translated as glutamine (Schneider and others 1989). This has been borne out by comparing DNA sequences of other genes from plants to their homolog in *A. acetabulum* (see http://www.kazusa.or.jp/codon/). It is not clear whether this codon use has functional significance or is merely a relic of evolution (Judson and Haydon 1999; Wilting and Böck 1996). Although codon bias can preclude expression of *A. acetabulum* genes in other organisms, the problem can be circumvented. An *Escherichia coli* strain expressing a suppressor tRNA has been engineered to read these stop codons as glutamine (Cohen and others 1990). This bacterial strain was designed for expressing ciliate genes and theoretically should work for Dasycladales as well, but in practice most ciliate genes are modified before heterologous expression (Meng-Chao Yao, personal communication to DFM, 1998). Conversely, *A. acetabulum* expresses heterologous genes well; the maize storage protein zein, adenovirus T-antigen, and tobacco mosaic virus (TMV) sequences are all expressed (Brown and others 1986; Cairns and others 1978a; 1978b; 1978c; Neuhaus and others 1984; Neuhaus and others 1986). Certainly, the unusual codon use helps to confirm that one has a bona fide gene from *A. acetabulum.*

Life Cycle Duration Could Be Shorter

Physiological improvements shortened the life cycle by 75% over that in the ocean (Mandoli 1998b). At 97 days from zygote to zygote, it is workable, but being greedy, we would like to see it shortened further. One modification to our culture methods that might further decrease the life cycle is prevention of

culture recontamination. Although cultures are started from axenic zygotes, contamination (by *Pseudomonas* sp, *Staphlococcus* sp., and a yeast) occurs during vegetative growth (Mandoli unpublished), probably because we open the culture boxes frequently when we remove individuals. Microorganisms have a shorter generation time and may also out-compete the slower-growing alga for vitamins. Adding antibiotics to the cultures might reduce the duration of the life cycle, especially if vitamins essential to one-carbon metabolism (for example, B_{12}) are limiting to development.

Infancy of Genetics

The main barrier to genetics in this unicell has been a high genetic load; on self-crossing more than 95% of the 2nd generation died, suggesting that outcrosses are the norm in the wild. Over the last 7 years, we have inbred near-isogenic lines (NILs) for 10 sequential generations (Mandoli, unpublished), which should make mutagenesis practical for the first time.

Significantly, because of its size and unicellularity, this species offers interesting ways to study mutant phenotypes. Traditional complementation analysis is possible, because one can both self and outcross each individual. Complementation analysis by means of microinjection of the candidate mRNA is also possible because the thallus is so large. RNA interference (for example, Fire and others 1998) by microinjection of the cap when it is a syncytium filled with haploid nuclei may be an attractive means to study defects in early development. Finally, interactions between genotypes can be studied by grafting a wild-type enucleate apex to a mutant nucleate rhizoid to determine whether the mutation is compensated by wild-type cytoplasm alone (Mandoli and Hunt 1996). One can also compensate the mutant *in trans* by grafting a wild-type nucleate rhizoid to a mutant nucleate rhizoid (Mandoli and Hunt 1996).

Infancy of Molecular Biology

The news that there are little molecular data for this species is a double-edged sword; whereas virtually everything one finds is new and publishable, basics are not in place. Of 10 cDNAs we sequenced at random, 5 were novel (Serikawa and Mandoli 1999 see Genbank acessions)! Of the remaining five genes, one was a nicotinamide nucleotide transhydrogenase common in bacteria and animals that has never before been found in a plant (Arkblad and others 2001). We (Grotewold, Blackstone, and Mandoli) are now sequencing 1,000 putatively phase-specific expressed sequence tags (ESTs), which will increase the database by more than 50 fold, give us molecular markers, and perhaps tell us something about genes expressed in this organism.

Its Value as a Model System Would Increase If we Had . . .

Reliable, Easy Transformation

Microparticle bombardment of gametangia-bearing caps will transform the haplophase to neomycin resistance or to express MUG (the soluble form of B-glucuronidase (GUS)) (Hollis, Ivey and Mandoli, unpublished). We have been able to confirm transformation in the second generation of progeny (T2) with polymerase chain reaction amplification of the neomycin sequence but not yet by Southern blotting of the genome. The latter was not possible previously, because the NILs were not ready. Interestingly, the frequency of transformation was high — about 34–61% of the progeny were neomycin resistant—because we transformed while the haploid nuclei were still dividing inside the gametangia. These are unlikely to be independent events but probably represent cohorts of sibling gametes with the same transformation event. Having cohorts of a given mutation is a distinct advantage for a unicell, particularly if one needs to rescue the mutation in some way before studying it, for example, by grafting to wild type as we did with *kurrku* (Mandoli and Hunt 1996).

Tagged Mutagenesis

The fecundity of the organism makes transformation feasible on a large scale, and access to NILs makes tagged mutagenesis possible. Of the 2 to 6 million progeny per individual and with 34–61% transformation of progeny (Hollis, Ivey and Mandoli, unpublished), it is not unreasonable to expect that some fraction of the millions of transformants per individual will have been mutated by insertion of foreign DNA into a nonlethal gene. Large-scale selection of mutants is theoretically possible after transformation to neomycin resistance (Neuhaus and others 1986), and large-scale screening may also eventually become a reality using fluorescence-activated cell sorting of zygotes (Krueger and Mandoli, unpublished). Ideally, GFP-fusions will permit direct visualization of proteins that localize while subcellular regions are being made and maintained during development and morphogenesis.

Homologous Recombination

The ability to substitute an in vitro engineered copy of a gene for the one in the genome has been an incredible boon to studies of gene function in *Saccharomyces cerviseae.* Obviously, one needs a single copy nuclear gene to be able to see a phenotype resulting from recombination with a mutated copy of the gene or to rescue a mutant phenotype with a wild-type gene. The dearth of molecular data—especially of cloned genes—is a clear prerequisite before we can determine whether homologous recombination is feasible.

Genomic Map

Mapping the locations of genes and (ESTs) onto individual chromosomes isolated on gels will be a first, solid step toward a genomic map. Chromosome gels of the haplophase will be easier than that for the diplophase because haploid nuclei are so much easier to obtain in large numbers (Fig. 1).

Once the current round of genome sequencing is done, there will be machinery and people who could then sequence the genomes of less well-established model systems. We hope that attention will be turned to the organisms that have the greatest potential to broaden what we learn about genes in one branch of the phylogenetic tree to other, less well-studied branches (Fig.1 in Mandoli and Olmstead 2001). For forays into novel biochemical pathways, novel uses of well-known genes and evolution of gene function, genomic maps of emerging model systems like *A. acetabulum* will be exciting gold mines for future bioinformatic analyses.

NOTE: As enticement to new investigators interested in joining the fun, a bibliography of the Dasycladaceae is available in hardcopy (for example, Bonotto and Lüttke 1980) and will be available soon in electronic format (http://www.faculty.washington.edu/mandoli).

ACKNOWLEDGMENTS

The work of the authors mentioned in this article was supported by NSF IBN96-30618 (DFM), by an NSF postdoctoral fellowship in biosciences related to the environment (KAS while in Mandoli's laboratory), and by a doctoral NSERC graduate fellowship (JD while at the University of British Columbia).

REFERENCES

Arkblad EL, Betscholz C, Mandoli DF, and Rydström J. 2001. Characterization of a nicotinamide nucleotide transhydrogenase gene from the green algae *Acetabuluria acetabulum* and comparison of its structures with those of corresponding genes in mouse and *Caenorhabditis elegans.* Submitted to Biochim Biophys Acta on Jan. 16, 2001.

Baltus E, Edström JE, Janowski M, Hanocq-Quertier J, Tencer R, Brachet J. 1968. Base composition and metabolism of various RNA fractions in *Acetabularia mediterranea.* Proc Natl Acad Sci USA 59:406–413.

Bennett MD, Smith JB. 1976. Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond B Biol Sci 274:227–274.

Bennett MD, Smith JB. 1991. Nuclear amounts in angiosperms. Philos Trans R Soc Lond B Biol Sci 334:309–345.

Berger S, de Groot EJ, Neuhaus G, Schweiger M. 1987. *Acetabularia:* a giant single cell organism with valuable advantages for cell biology. Eur J Cell Biol 44:349–370.

Berger S, Herth W, Franke WW, Falk H, Spring H, Schweiger H-G. 1975. Morphology of the nucleo-cytoplasmic interactions during the development of *Acetabularia* cells. II. The generative phase. Protoplasma 84:223–256.

Berger S, Kaever MJ. 1992. Dasycladales: An illustrated monograph of a fascinating algal order. London: Elsevier Press. 247 p.

Berger S, Menzel D, Traub P. 1994. Chromosomal architecture in giant premeiotic nuclei of the green alga *Acetabularia.* Protoplasma 178:119–128.

Berger S, Schweiger HG. 1975. Cytoplasmic induction of changes in the ultrastructure of the *Acetabularia* nucleus and perinuclear cytoplasm. J Cell Sci 17:517–529.

Bonotto S, Lüttke A. 1980. List of references of *Acetabularia* and the other *Dasycladaceae* for the periods 1960–1980. Brussels: Centre d'Etude de l'Energie Nucleaire. 133 p.

Bouget FY, Gerttula S, Shaw SL, Quatrano RS. 1996. Localization of actin during the establishment of cell polarity and early cell divisions in *Fucus* embryos. Plant Cell 8:189–201.

Brown JWS, Wandelt C, Feix G, Neuhaus G, Schweiger H-G. 1986. The upstream regions of zein genes: sequence analysis and expression in the unicellular green alga *Acetabularia.* Eur J Cell Biol 42:161–170.

Cairns E, Doefler W, Schweiger H-G. 1978a. Expression of a DNA animal virus genome in a plant cell. FEBS Lett 96:295–297.

Cairns E, Gshwender H, Primke M, Yamakawa M, Traub P, Schweiger H-G. 1978b. Translation of animal virus RNA in the cytoplasm of a plant cell. Proc Natl Acad Sci USA 75:5557–5559.

Cairns E, Sarkar S, Schweiger H-U. 1978c. Translation of tobacco mosaic virus RNA in *Acetabularia* cell cytoplasm. Cell Biol Int Rep 2:573–578.

Chadefaud M. 1952. La leçon des algues: comment elles ont évolué; comment leur évolution peut éclairer celle des plantes supérieures. Année Biol 18:9–25.

Church A. 1895. The structure of the thallus of Neomeris dumetosa, Lamour. Ann Bot 9:581–608.

Cohen J, Dupuis P, Viguès B. 1990. Expression of a ciliate gene in *Escherichia coli* using a suppressor tRNA to read the UAAA and UAG Glutamine codons. J Mol Biol 216:189–194.

Cooper J, Mandoli DF. 1999. Physiological factors that aid differentiation of zygotes and early juveniles of *Acetabularia acetabulum* (Chlorophyta). J Phycol 35:143–151.

De DN, Berger S. 1990. Karyology of *Acetabularia mediterranea.* Protoplasma 155:19–28.

Deloffre R. 1988. Nouvelle taxonomie des algues Dasycladales. Bull Centres Rech Explor Prod Elf-Aquitaine 12:165–217.

Dumais J, Harrison LG. 2000. Whorl morphogenesis in the dasy-

cladalean algae: the pattern formation viewpoint. Phil Trans R Soc Lond B 355:281–305.

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. 1998. Potent and specific genetic interference by double-stranded RNA in Caenorhabditus elegans. Nature 391:806–811.

Franke WW, Berger S, Falk H, Spring A, Scheer U, Herth W, Trendelenburg MF, Schweiger HG. 1974. Morphology of the nucleo-cytoplasmic interactions during the development of *Acetabularia* cells. I. The vegetative phase. Protoplasma 82:249–282.

Franke WW, Berger S, Scheer U, Trendelenburg MF. 1975. The nuclear envelope and the perinuclear lacunar labyrinthum. Protoplasma 83:174–175.

Garcia E, Dazy A-C. 1986. Spatial distribution of poly(A)$^+$RNA and protein synthesis in the stalk of *Acetabularia acetabulum.* Biol Cell 58:23–30.

Goodwin BC, Skelton JL, Kirk-Bell SM. 1983. Control of regeneration and morphogenesis by divalent cations in *Acetabularia acetabulum.* Planta 157:1–7.

Goodwin BC, Trainor LEH. 1985. Tip and whorl morphogensis in *Acetabluria* by calcium-regulated strain fields. J Theor Biol 117:79–106.

Graham LE, Wilcox LW. 2000. Algae. Upper Saddle River, NJ: Prentice Hall. 640 p.

Green BR. 1973. Evidence for the occurance of meiosis before cyst formation in *Acetabularia mediterranea* (Chlorophyceae, Siphonales). Phycologia 12:233–235.

Green BR, Burton H, Heilporn V, Limbosch S. 1970. The cytoplasmic DNA's of *Acetabularia mediterranea:* their structure and biological properties. In: Bonotto S, editor. Biology of *Acetabularia.* New York, London: Academic Press. p 35–59.

Hämmerling J. 1932. Entwicklung und Formbildungsvermögen von *Acetabularia mediterranea.* II. Das Formbildungsvermögen kernhaltigen und kernlosen Teilstücke. Biol Zentralblatt 52:42–61.

Hämmerling J. 1934. Über formbildende Substanzen bei *Acetabularia mediterranea,* ihre räumliche und zeitliche Verteilung und ihre Herkunft. Wilhelm Roux Arch Entwicklungsmechanik der Organismen 131:1–81.

Hämmerling J. 1936. Studien zum Polaritätsproblem I-III. Zool Jahrb Abt Allge Zool Physiol 56:439–486.

Hämmerling J. 1953. Nucleo-cytoplasmic relationships in the development of *Acetabularia.* Int Rev Cytol 2:475–498.

Hämmerling J. 1963. Nucleo-cytoplasmic interactions in *Acetabularia* and other cells. Ann Rev Plant Physiol 14:65–92.

Harrison LG, Donaldson G, Lau W, Lee M, Lin BP, Lohachitranont S, Setyawati I, Yue J. 1997. CaEGTA uncompetitively inhibits calcium activation of whorl morphogensesis in *Acetabularia* Protoplasma 196:190–196.

Harrison LG, Graham KT, Lakowski BC. 1988. Calcium localization during *Acetabularia* whorl formation—evidence supporting a 2-stage hierarchical mechanism. Development 104:255–262.

Harrison LG, Hillier NA. 1985. Quantitative control of *Acetabularia* morphogenesis by extracellular calcium: a test of kinetic theory. J Theor Biol 114:177–192.

Harrison LG, Snell J, Verdi R, Vogt DE, Zeiss GD, Green BR. 1981. Hair morphogenesis in *Acetabularia mediterranea:* temperature-dependent spacing and models of morphogen waves. Protoplasma 106:211–221.

Hunt BE, Mandoli DF. 1992. Axenic cultures of*Acetabularia* (Chlorophyta): a decontamination protocol with potential application to other algae. J Phycol 28:407–414.

Hunt BE, Mandoli DF. 1996. A new, artificial seawater that facilitates growth of large numbers of cells of *Acetabularia acetabulum* (Chlorophyta) and reduces the labor inherent in cell culture. J Phycol 32:483–495.

Judson OP, Haydon D. 1999. The genetic code: what is it good for? An analysis of the effects of selection pressures on genetic codes. J Mol Evol 49:539–550.

Keck K, Clauss H. 1958. Nuclear control of enzyme synthesis in *Acetabularia.* Bot Gaz 120:43–49.

Koop H-U. 1975. Über den Ort der Meiose bei *Acetabularia mediterranea.* Kurze Mitteilung. Protoplasma 85:109–114.

Koop H-U. 1977a. Genetic aspects of *Acetabularia mediterranea.* In: Woodcock CLF, editor. Progress in acetabularia research. London: Academic Press. p 7–18.

Koop H-U. 1977b. Selection of a morphologically changed strain of *Acetabularia mediterranea.* Protoplasma 91:224–225.

Koop H-U, Schmid R, Heunert R, Spring H. 1979. Spindle formation and division of the giant primary nucleus of *Acetabularia (Chlorophyta, Dasycladales).* Differentiation 14:135–146.

Koop H-U, Spring H, Heunert H-H, Schmid R. 1978. Continuous observation of the giant primary nucleus of *Acetabularia* in situ. Protoplasma 96:89–99.

Kropf DL, Bisgrove SR, Hable WE. 1999. Establishing the growth axis in fucoid algae. Trends Plant Sci 4:490–494.

Leible MB, Berger S, Schweiger H-G. 1989. The plastome of *Acetabularia mediterranea* and *Batophora oerstedii:* inter- and intraspecific variability and physical properties. Curr Genet 15:355–361.

Li-Weber M, Schweiger H-G. 1985. Evidence for and mechanism of translational control during cell differentiation in *Acetabularia.* Euro J Cell Biol 38:73–78.

Mandoli DF. 1996. Establishing and maintaining the body plan of *Acetabularia acetabulum* in the absence of cellularization. Seminars in Cell & Developmental Biology. London: Academic Press Ltd.

Mandoli DF. 1998a. Elaboration of body plan and phase change during development of *Acetabularia:* How is the complex architecture of a giant unicell built? Annu Rev Plant Phyiol Plant Mol Biol 49:173–198.

Mandoli DF. 1998b. What ever happened to *Acetabularia?* Bringing a once-classic model system into the age of molecular genetics. Intern Rev Cytol 182:1–67.

Mandoli DF, Hunt BE. 1996. *kurkku,* a phenotype of *Acetabularia acetabulum* that is arrested in vegetative growth, can be rescued with wild-type cytoplasm. Plant Cell 8:323–332.

Mandoli DF, Olmstead R. 2000. The importance of emerging model systems in plant biology. J Plant Growth Regul 19:249–252.

Martynov LA. 1975. A morphogenetic mechanism involving instability of initial form. J Theor Biol 52:471–480.

Menzel D. 1986. Visualization of cytoskeletal changes through the life cycle in *Acetabularia.* Protoplasma 134:30–42.

Menzel D. 1994. Tansley Review No. 77 Cell differentiation and the cytoskeleton in *Acetabularia.* New Phytol 128:369–393.

Menzel D. 1996. The role of the cytoskeleton in polarity and morphogenesis of algal cells. Curr Opin Cell Biol 8:38–42.

Menzel D, Jonitz H, Elsner-Menzel C. 1996. The perinuclear microtubule system in the green alga *Acetabularia:* anchor or motility device? Protoplasma 193:63–76.

Mummert H, Gradmann D. 1991a. Action potentials in *Acetabularia*—measurement and simulation of voltage-gated fluxes. J Membrane Biol 124:265–273.

Mummert H, Gradmann D. 1991b. Ion fluxes in *Acetabularia*—vesicular shuttle. J Membrane Biol 124:255–263.

Nägeli C. 1847. Die neuern Algensystem. Zürich: Schulthess.

Neuhaus G, Neuhaus-Url G, de Groot EJ, Schweiger H-G. 1986. High yield and stable transformation of the unicellular green alga *Acetabularia* by microinjection of SV40 DNA and pSV2neo. EMBO J 5:1437–1444.

Neuhaus G, Neuhaus-Url G, Gruss P, Schweiger H-G. 1984. Enhancer-controlled expression of the simian virus 40 T-antigen in the green alga *Acetabularia.* EMBO J 3:2169–2172.

Nickl B, Maass I, Schmid R. 1988. The control of metabolism by blue light in *Acetabularia mediterranea*—I. light dependency and evidence for post-transcriptional control of UDPG pyrophorylase and pyruvate kinase. Photochem Photobiol 48:745–751.

Nishimura NJ, Mandoli DF. 1992a. Population analysis of reproductive cell structures of *Acetabularia acetabulum.* Phycologia 31:351–358.

Nishimura NJ, Mandoli DF. 1992b. Vegetative growth of *Acetabularia acetabulum* (Chlorophyta): Structural evidence for juvenile and adult phases in development. J Phycol 28:669–677.

Pia J. 1920. Die Siphoneae verticillatae von Karbon bis zur Kreide. Abhandl Zool-Botan Ges Wien 11:1–263.

Puiseux-Dao S. 1962. Recherches biologiques et physiologiques sur quelques Dasycladacées, en particulier, le *Batophora oerstedii* J. Ag. et l'*Acetabularia mediterranea* (Lamouroux). Rev Gen Bot 50:1–36.

Reiss H-D, Herth W. 1979. Calcium gradients in tip growing plant cell visualized by chlorotetracycline fluorescence. Planta 146:615–621.

Runft LL, Mandoli DF. 1996. Coordination of the cellular events that precede reproductive onset in *Acetabularia acetabulum:* evidence for a "loop" in development. Development 122:1187–1194.

Schmid R, Idziak E-M, Tunnerman M. 1987. Action spectrum for the blue-light-dependent morphogenesis of hair whorls in *Acetabularia mediterranea.* Planta 171:96–103.

Schneider SU, Leible MB, Yang XP. 1989. Strong homology between the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase of two species of *Acetabularia* and the occurrence of unusual codon usage. Mol Gen Genet 218:445–452.

Serikawa KA, Mandoli DF. 1999. *Aaknox1,* a *kn1*-like homeobox gene in *Acetabularia acetabulum* undergoes developmentally regulated subcellular localization. Plant Mol Biol 41:785–793.

Serkawa KA, Potterfield DM, Mandoli DF. 2001. Asymmetric subcellular mRNA distribution correlates with carbonic anhydrase activity in *Acetabularia acetabulum.* Plant Physiol 125:900–911. http://www.plantphysiol.org/cgi/content/125/abstract/125/2/900

Serkawa KA, Potterfield DM, Smith PJ, Mandoli DF. 2000. Calcification and measurements of net proton and oxygen flux reveal subcellular domains in *Acetabularia acetabulum.* Planta 211:474–483.

Shaw SL, Quatrano RL. 1996. Polar localization of a dihydropyridine receptor on living *Fucus* zygotes. J Cell Sci 109:335–342.

Shihira-Ishikawa I. 1984. Chromosome behavior in the primary nucleus of *Acetabularia calyculus* as revealed by epifluorescent microscopy. Protoplasma 122:27–34.

Solms-Laubach H Graf zu. 1895. Monograph of the Acetabularieae Trans Lind Soc London 2 (Botany)5:1–39.

Spencer T, Harris H. 1964. Regulation of enzyme synthesis in an enucleate cell. Bioch J 91:282–286.

Spring H, Grierson D, Hemleben V, Stohr M, Krohne G, Stadler J, Franke WW. 1978. DNA contents and numbers of nucleoli and pre-rRNA genes in nuclei of gametes and vegetative cells of *Acetabularia mediterranea.* Exp Cell Res 114:203–215.

Spring H, Scheer U, Franke WW, Trendelenburg MF. 1975. Lampbrush-type chromosomes in the primary nucleus of the green alga *Acetabularia mediterranea.* Chromosoma (Berlin) 50:25–43.

Valet G. 1968. Contribution à l'étude des Dasycladales. 1. Morphogenèse. Nova Hedwigia 16:22–84.

Vogel H. 1998. Untersuchungen zu spezifischen mRNA-Gradienten in der einzelligen, siphonalen Grünalge *Acetabularia acetabulum* (L.) Silva. Giessen, Germany: Universität Geissen.

Von Dassow M, Odell GM, Mandoli DF. 2001. Relationships between growth, morphology and wall stress in the stalk of *Acetabularia acetabulum.* Planta. In press.

Werz G. 1959. Über polare Plasmaunterschiede bei *Acetabularia.* Planta 53:502–521.

Wilting R, Böck A. 1996. Die Flexibilität des genetischen Kodes. Biologie in unserer Zeit 26:369–379.

Zetsche K, Grieninger GE, Anders J. 1970. Regulation of enzyme activity during morphogenesis of nucleate and anucleate cells of *Acetabularia.* In: Brachet J, Bonotto S, Brachet J, Bonotto S, editors. Biology of *Acetabularia.* New York: Academic Press. p 87–110.