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Fucoid algae as model organisms for investigating early embryogenesis

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Abstract: In the past few years, there have been exciting advances in our understanding of the mechanisms that control morphogenesis in fucoid embryos. In this article we review recent findings from our laboratories concerning 1) polarity establishment and expression in the zygote and 2) development of the zygote into a multicellular embryo.

Résumé : Durant la dernière décennie, des avancées importantes ont été réalisées dans la compréhension des mécanismes qui contrôlent la morphogenèse des embryons de Fucacées. Dans cette revue, nous présentons les résultats récents obtenus dans nos laboratoires respectifs concernant 1) l'établissement de la polarité et son expression dans le zygote et 2) le développement du zygote en un embryon pluricellulaire.

Keywords : *Fucus, Pelvetia,* embryogenesis, polarity.

Introduction

In addition to their importance as sources of natural polymers and foods, many marine algae also provide excellent opportunities for investigating the mechanisms that control development. For example, zygotes and early embryos of the heterokont algae of the genera *Fucus* and *Pelvetia* have long served as models for investigating fertilization, establishment of cell polarity, and embryogenesis. Several features of these organisms make them very amenable to study. Sperm, eggs and zygotes are released in large numbers from sexually mature fronds into the surrounding seawater at low tide (Quatrano, 1980). Fertilization is oogamous with small (5 µm), biflagellated sperm cells fusing with large (60 - 100 µm) sessile eggs (Fig. 1A). The fertilized eggs are dense and settle rapidly onto the substratum (rocks in the intertidal zone) where they attach tenaciously by a secreted adhesive (Vreeland et al., 1993). Rapid adhesion is critical for survival because zygotes that fail to attach are washed out to sea in the next tidal cycle. As was first recognized over 100 years ago by Rosenvinge (Rosenvinge, 1888), the attached zygotes sense vectorial information in their environment and establish a rhizoid-thallus growth axis in accordance with the perceived vectors (for review see Jaffe, 1968). Some 10 - 12 hours after fertilization (AF), growth begins at the rhizoid pole of the axis, giving the zygote a pear-shaped appearance (Fig. 1B). This process is termed germination. Presumably, the reason for monitoring the environment for spatial information is to ensure that the rhizoid, which develops into the holdfast of the plant, germinates toward the rocky substratum. The rhizoid elongates by tip growth and

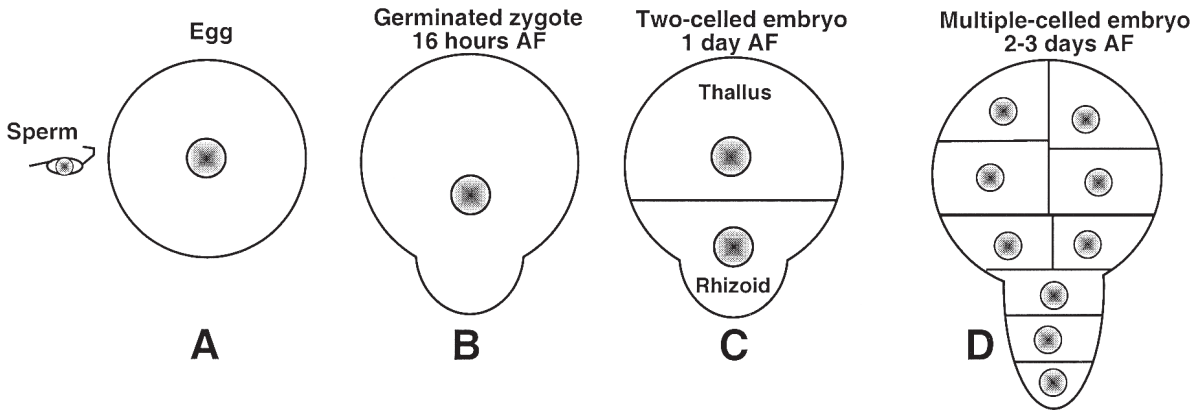

Figure 1. Embryogenesis in fucoid algae. **A.** The large sessile egg is 60 to 100 µm in diameter, depending on species, and is symmetrical. The sperm is small and biflagellated. **B.** Polarity is established and expressed in the first cell cycle, and growth is localized to the rhizoid pole at germination. **C.** The first zygotic division is an asymmetric cleavage producing thallus and rhizoid cells. **D.** The division pattern is reproducible and results in globular thallus and linear rhizoid portions of the early embryo. Shaded spheres are nuclei.

Figure 1. Embryogenèse des Fucacées. **A.** L'oosphère est une grosse cellule symétrique de 60 à 100 µm de diamètre, suivant l'espèce. Le spermatozoide est une petite cellule biflagellée. **B.** Une polarité s'établit et se manifeste au cours du premier cycle cellulaire, une croissance polarisée se met en place au pôle rhizoïdien où a lieu la germination. **C.** La première division zygotique est un clivage asymétrique produisant une cellule thalle et une cellule rhizoïdienne. **D.** Le patron des divisions est constant et conduit à la formation de la partie globuleuse du thalle et à l'allongement du rhizoïde de l'embryon. Les sphères ombrées sont des noyaux.

approximately 24 hours AF the zygote completes the first cell division. This division is an asymmetric cleavage oriented transverse to the growth axis and produces rhizoid and thallus cells of distinct morphology (Fig. 1C). Continued growth and division over the next days result in a multicellular embryo in which the thallus is composed of two cell layers (Henderson et al., 1998) and the rhizoid is composed of a file of cells (Fig. 1D).

In this article we review recent findings from our laboratories concerning 1) polarity establishment and expression in the zygote (Kropf lab) and 2) development of the zygote into a multicellular embryo (Bouget lab).

Polarity Establishment and Expression (Kropf lab)

Much of the work in our laboratory in the past decade has focused on establishment and expression of polarity in zygotes of the brown alga *Pelvetia compressa* (proposed to be renamed *Silvetia compressa* by Serrao et al., 1999). Specifically, we would like to know how the rhizoid-thallus axis is generated and how it positions the site of growth and orients the first cell division. Toward this goal, we have been investigating the roles of the cytoskeleton, ionic gradients and secretion in these processes. A summary of our current understanding is described below and is depicted graphically in Figure 2.

Growth Axis Establishment

By all accounts, the egg appears to be a uniform, spherical cell without polarity. About two hours AF, the zygote secretes an adhesive uniformly over its surface, immediately attaches to the substratum, and begins to survey the surroundings for vectorial information (Hable & Kropf, 1998). As might be expected, the zygotes are very sensitive to directional light. When incubated continuously in directional light, zygotes form a rhizoid pole on the shaded hemisphere of the cell within one hour of attachment. The process is termed photopolarization and an entire population is photopolarized by four hours AF (Fig. 2A). The rhizoid pole of the nascent axis is marked by a cortical patch of F-actin that assembles there within minutes of photopolarization (Alessa & Kropf, 1999). What causes the patch to assemble at the shaded pole is unknown, but local assembly is likely regulated by actin binding proteins, phosphorylation cascades and/or local changes in ion activity. Recently it was reported that cytosolic Ca^{2+} increases at the shaded pole during photopolarization (Pu & Robinson, 1998) and this Ca^{2+} may help induce F-actin assembly at that site. Once assembled, the F-actin patch apparently serves as a target site for secretion of additional adhesive. By five hours AF, approximately one hour after photopolarization and assembly of the F-actin patch, secretion of adhesive becomes localized to the rhizoid pole (Fig. 2B; Hable & Kropf, 1998).

Over the next several hours the zygote remains sensitive to external cues and the axis can be reoriented by changing

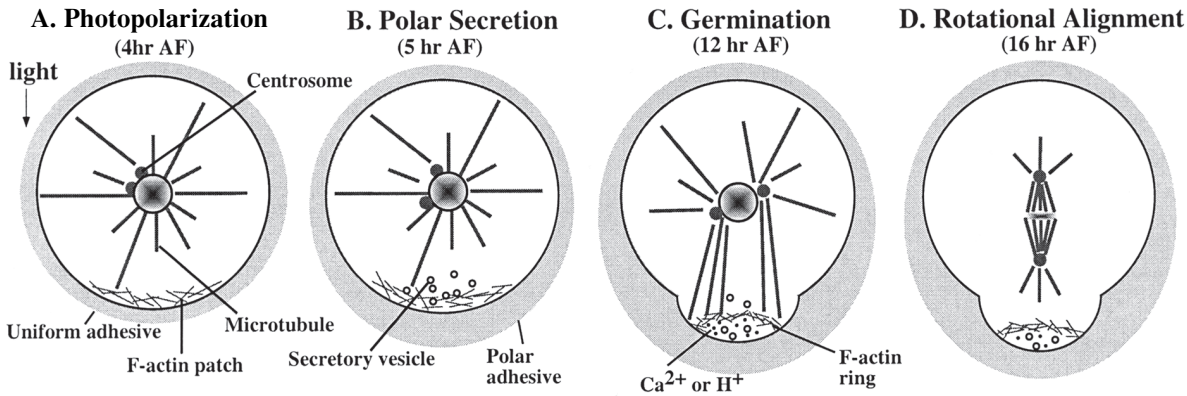

Figure 2. Polarity establishment and division plane alignment. **A.** Photopolarization is characterized by an F-actin patch at the (shaded) rhizoid pole and centrosomes that have not yet separated. **B.** Polar secretion occurs rapidly following photopolarization resulting in thicker adhesive at the rhizoid pole. Centrosomes are still only beginning to separate. **C.** By germination the centrosomes are separated but are randomly aligned with the growth axis. Microtubules extend from the centrosomes to the F-actin ring in the rhizoid cortex. Distinct gradients of Ca^{2+} and H^{+} are present by germination. **D.** Microtubules anchored in the rhizoid cortex likely effect rotational alignment of the nucleus using microtubule-dependent motors (kinesins or dyneins). The spindle forms axially and first division bisects the spindle forming a transverse cell plate (not shown).

Figure 2. Etablissement de la polarité et alignement des plans de division. **A.** La photopolarisation se caractérise par un amas d'actine F au pole rhizodien (ombre) et par des centrosomes qui ne sont pas séparés. **B.** Des secrétions polarisées suivent la photopolarisation et assurent une adhésion forte au pôle rhizoïde. **C.** A la germination, les centrosomes se séparent, mais sont alignés au hasard par rapport à l'axe de croissance. Les microtubules s'étendent des centrosomes vers l'anneau d'actine F dans le cortex rhizoidien. Des gradients distincts de Ca^{2+} et de H^{+} sont présents à la germination. **D.** Les microtubules ancrés dans le cortex rhizoïdien sont probablement les effecteurs de la rotation du noyau par des moteurs dépendants (kinésines ou dynéines). Le fuseau se forme axialement et la première division partitionne le fuseau formant une plaque de division tranversale. (non présenté).

the direction of the orienting vector. When the direction of orienting light is changed, the initial F-actin patch disassembles and a new patch assembles at the new shaded pole. Polar secretion then shifts to the new shaded pole. This process can be repeated several times until just prior to germination, at which time the axis becomes fixed in space.

At germination, the rhizoid emerges from the spherical zygotes at the site of the cortical F-actin, which has expanded from a patch into a ring (Fig. 2C). Growth initiation involves local weakening of the cell wall (Hable & Kropf, 1998) and an increase in the rate of secretory vesicle fusion at the rhizoid pole (Quatrano, 1972). The rhizoid elongates by tip growth and is associated with cytosolic gradients of both H^{+} and Ca^{2+} (Berger & Brownlee, 1993; Gibbon & Kropf, 1994).

Division Plane Alignment

Although substantial deviation from transverse alignment of the first asymmetric division is tolerated, divisions that approach longitudinal orientation severely inhibit embryo growth (Bisgrove & Kropf, 1998). To prevent longitudinal division, the zygotes have evolved a mechanism to ensure that cell wall forms transverse to the growth axis in nearly every zygote. This mechanism involves alignment of the spindle. The spindle is positioned parallel to the growth axis and cytokinesis then bisects the spindle; the cell plate therefore forms transversely. Because spindle orientation determines division alignment, we have been investigating the positioning of centrosomes which serve as spindle pole bodies during mitosis. As in all brown algae, centrosomes are inherited paternally (Nagasato et al., 1998) and migrate with the sperm pronucleus to the egg pronucleus. Following karyogamy (two hours AF), the two centrosomes reside close together on the zygotic nucleus (Fig. 2A). The centrosomes slowly separate from one another over the nuclear envelope in a process that is dependent on microtubules (Bisgrove & Kropf, 1998). Centrosomal separation is completed in all zygotes prior to mitosis, which begins at 16 hours AF, but the orientation of the axis defined by the two centrosomes is random with respect to the growth axis (Fig. 2C). The nucleus then rotates such that the centrosomes come into longitudinal alignment, in perfect register with the growth axis, and the aligned centrosomes almost immediately nucleate a spindle (Fig. 2D).

Although the mechanism of rotational alignment is not fully understood, it involves microtubules that emanate from the centrosomes and extend into the rhizoid where they terminate in the cell cortex (Bisgrove et al., 1997). These microtubules are thought to anchor in the cortex and, via

microtubule-dependent motors, to pull the centrosomes toward the rhizoid tip (Fig. 2C). One centrosome wins this tug of war and moves toward the tip while the other centrosome moves toward the thallus pole. The anchoring of microtubules in the tip requires the cortical F-actin ring, which is itself part of a transmembrane adhesion linking the cortex to the cell wall (Henry et al., 1996). Thus, rotation alignment of the nucleus appears to rely upon structural linkages all the way from the centrosomes to the cell wall at the rhizoid tip.

From zygote to multicellular embryo (Bouget lab)

Prerequisites for normal embryonic development

Polarity establishment in the zygote is an absolute requirement for later steps of development including cell fate determination, cell differentiation and pattern formation. When unpolarized zygotes are treated early with agents that inhibit axis formation, such as inhibitors of protein phosphorylation, pattern is deeply and irreversibly disturbed (Fig. 3D) (Corellou et al., 2000). In contrast, transient treatments that mildly affect the orientation of the first division plane [such as microtubules inhibitors (Fig. 3A, 3C; Pollock, 1968), or Brefeldin A (Shaw & Quatrano, 1997)] do not prevent the overall patterning of the embryo. Therefore the global body organization appears to rely on the establishment of the initial axis of polarity in the zygote rather than on the precise orientation of the initial division planes. This is in good agreement with results coming from genetic analysis of higher plants mutants such as *FASS* in *Arabidopsis* (Torres-Ruiz & Jurgens, 1994) or *TANGLED 1* in maize (Smith et al., 1996) where misaligned division planes do not affect pattern in the long term. In contrast polarity mutants such as *GNOM* in *Arabidopsis* exhibit an abnormal apical-basal pattern (Mayer et al., 1993).

Nature of developmental mechanisms

Classically, cell fate of embryonic cells can be determined by two different types of developmental processes, i. e. autonomous versus non autonomous (Gurdon, 1992). In

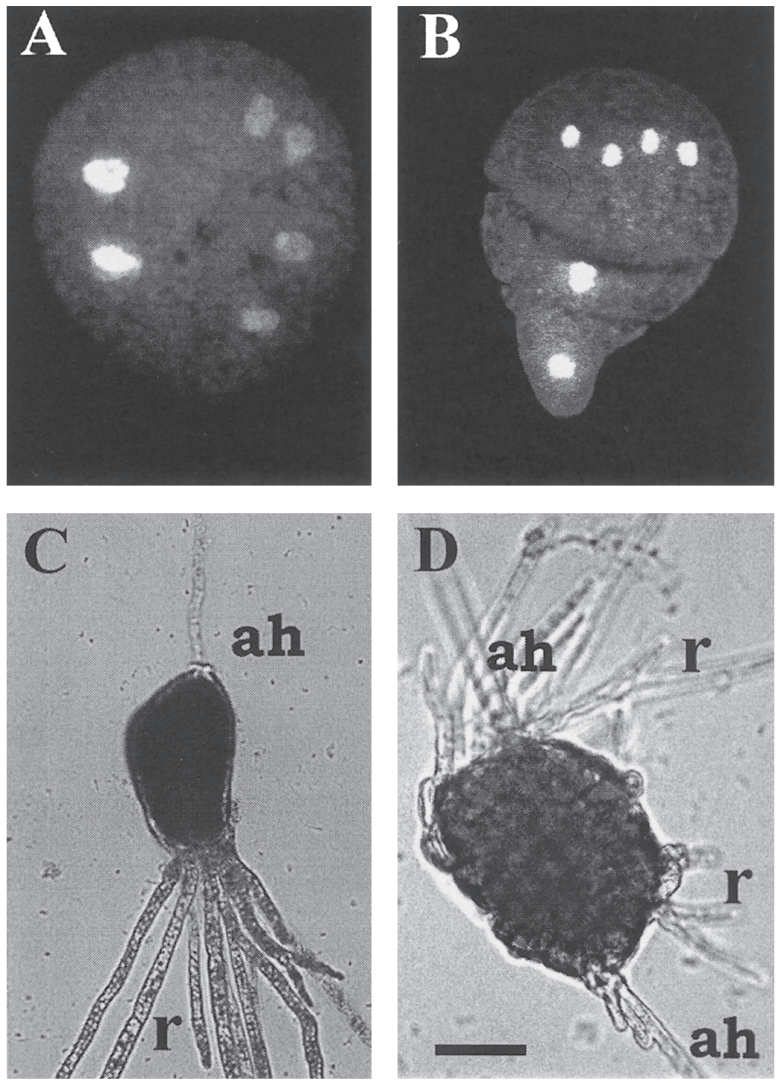

Figure 3. Prerequisites for normal embryogenesis. **A.** Mithramycin A staining of nuclei in 72 h old embryo, recovering from a 24h inhibition of cell division by Nocadazole (0.1 µM), compared to **B.** normal embryo. **C.** After 2 weeks in culture, pattern of embryo recovering from nocodazole is apparently normal with correctly localized apical hair (*ah*) and rhizoids (*r*). **D.** In contrast, embryo recovering from a 24h inhibition in the inhibitor of protein kinase Herbimycin A (0.3 µg ml^{-1}) exhibit a deeply perturbed pattern, with delocalized apical hair (*ah*) and rhizoids (*r*), after 2 weeks in culture. Scale bar: 25 µm in A, 30 µm in B, 100 µm in C and 70 µm in D.

Figure 3. Prérequis pour une embryogenèse normale. **A.** Coloration à la Mithramycin A du noyau d'un embryon de 72 h après une inhibition de 24 h de la division par du Nocadazole (0.1 µM) comparé à **B.** un embryon normal. **C.** Après deux semaines de culture, le patron de division des embryons traités 24 h au nocodazole est apparemment normal avec des poils apicaux (*ah*) normaux et des rhizoïdes (*r*). **D.** Par contre, les embryons traités 24 h avec l'inhibiteur de proteine tyrosine kinases Herbimycin A (0.3 µg ml^{-1}) montrent des patrons de division très perturbés avec des poils apicaux délocalisés (*ah*) et des rhizoïdes (*r*) après 2 semaines de culture. Echelle : 25 µm en A, 30 µm en B, 100 µm en C et 70 µm en D.

animal embryos such as *Caenorhabditis elegans*, either asymmetric segregation of cytoplasmic determinants specify cell lineages (autonomous type) or neighbouring cells can induce a cell to adopt a new fate (non autonomous type). Both mechanisms are reciprocally non-exclusive. Two main developmental programs are distinguished in Fucales embryos, i.e. rhizoid and thallus. These cell types are easily recognizable: rhizoid cells are filamentous and display few chloroplasts, whereas thallus cells are spherical and highly pigmented. Recent development of laser microsurgery techniques have allowed the investigation of the mechanisms of cell fate determination in *Fucus* young embryos (Berger et al., 1994). Following ablations of all rhizoid cells, thallus cells above the dissection can switch their fate and produce rhizoid cells. Similarly, rhizoid cells can compensate the ablation of thallus cells (Bouget et al., 1998). Furthermore, protoplasts extruded from either rhizoid or thallus cells are capable of developing like normal zygotes suggesting again the absence of long-lived cytoplasmic determinants (Berger & Brownlee, 1995; Bouget et al., 1998). Taken together, these data suggest that embryo development is non-autonomous in *Fucus* embryos.

In brown algae, a common response to wounding is the production of filamentous cells that are morphologically identical to rhizoids (Fries, 1984; Benet et al., 1997). After the two celled stage, all cells of *Fucus* embryos are indeed capable of producing rhizoids in response to laser ablations (Bouget et al., 1998). However rhizoid production by the cells adjacent to the dissection is tightly regulated in a position dependent manner. Interestingly the production of rhizoid cells by thallus cells nearby the dissection appears to be inhibited by more basal rhizoid cells. A combined approach of micro-injection of high molecular weight fluorescently labelled marker and laser ablation has shown that the inhibitory signals is apoplastic (Bouget et al., 1998).

There is increasing evidence that hormones such as auxin are involved in plant patterning (Uggla et al., 1996) and many phytohormones. Auxin has been found in algae and it appears to play a role in determining apical dominance in adult plants of *Fucus* (Moss, 1964). A tempting hypothesis is that the long range apoplastic inhibitory signal in *Fucus* embryos is hormonal in nature. However among all hormones and inhibitors tested, including those reported to disrupt apical dominance in adult algae (Moss, 1964), none of them had any significant effect on embryogenesis (Table 1). Therefore the role of hormones in the control of morphogenesis and cell differentiation in *Fucus* embryos remains an open question.

Table 1. Effect of phytohormones and inhibitors on *Fucus* early development.

Tableau 1. Effet de phytohormones et d'inhibiteurs sur le développement précoce de *Fucus*.

	Effective concentrations on higher plants (µg ml^{-1})	**Concentration tested on *Fucus* zygotes (µg ml^{-1})**	**Effect on *Fucus* zygotes**
	AUXINS		
IAA	0.01-3	0.01-50	No
2,4 D	0.01-5	0.01-50	No
IBA	0.1-10	0.01-50	No
NAA	0.1-10	0.01-50	No
PAA	0.1-50	0.01-50	No
	ANTIAUXIN		
NPA	0.02-20	0.001-50	No
	CYTOKININS		
Kinetin	0.01-5	0.01-50	No
Zeatin	0.01-5	0.01-50	No
	GIBBERELLIN		
GA3	0.01-5	0.01-50	No

The role of the cell wall

Over the last twenty years, much attention has been devoted to the cell wall of *Fucus* early embryos (for review see Brownlee & Berger, 1995). The cell wall is required for anchoring the initial of axis of polarity and required to maintain polarity in older embryos (Kropf et al., 1988; Bouget et al., 1998). Following ablation of a rhizoid cell in two celled embryos, the remaining wall can provide a positional signal to the thallus cells and trigger the dedifferentiation and redifferentiation into a rhizoid cell (Berger et al., 1994). This type of signalling is compatible with non autonomous development but does not account for the long range inhibitory effect observed in older embryos (see above). Furthermore, thallus cells below dissections are capable of producing rhizoids in the absence of contact with rhizoid walls (Bouget et al., 1998). Therefore, position-dependent signal by the cell wall is unlikely to be a major mechanism involved in cell fate determination after the two-cell stage.

Conclusion

Unlike the embryo of most other plants, the free embryo of Fucales is suitable for most experimental approaches, including electrophysiology (Taylor et al., 1997), cell biology (Alessa & Kropf, 1998), biochemistry (Kropf et al., 1989, Corellou et al., 2000) and molecular biology (Bouget et al., 1996). For this reason, *Fucus* and *Pelvetia* have been

used for over a century to study basic and universal developmental processes such as fertilization (Roberts et al., 1994), signal transduction (Robinson & Miller, 1997) and polarization (for review see Goodner & Quatrano, 1993). More recently *Fucus* has emerged as model system (like the sea urchin in animal) in experimental embryology (Berger et al., 1995, Bouget et al., 1998). In the future it may be a very good experimental system to test hypotheses drawn from genetic analysis of plant models such as *Arabidopsis*. Thus, it will be very important to continue to develop and refine techniques for micro-injection, molecular biology (construction of libraries, gene isolation, *in situ* hybridization, transgene expression), biochemistry, cell biology (immunolocalization, laser microsurgery), and electrophysiology (patch-clamp) in fucoid zygotes.

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