Development 125, 1999-2008 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV0149

Position dependent control of cell fate in the *Fucus* embryo: role of intercellular communication

François-Yves Bouget[1], Frédéric Berger[2] and Colin Brownlee*

Marine Biological Association, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK
[1]Present address: Centre d'Etudes Océanologiques et de Biologie Marine, CNRS-UPR 9042, F-29680 Roscoff, France
[2]Present address: Ecole Normale Supérieure de Lyon, Reconnaissance Cellulaire et Amélioration des Plantes, 69364 Lyon-cedex 07, France
*Author for correspondence (cbr@wpo.nerc.ac.uk)

Accepted 10 March; published on WWW 6 May 1998

SUMMARY

The early embryo of the brown alga *Fucus* comprises two cell types, i.e. rhizoid and thallus which are morphogically and cytologically distinguishable. Previous work has pointed to the cell wall as a source of position-dependent information required for polarisation and fate determination in the zygote and 2-celled embryo. In this study we have analysed the mechanism(s) of cell fate control and pattern formation at later embryonic stages using a combination of laser microsurgery and microinjection. The results indicate that the cell wall is required for maintenance of pre-existing polarity in isolated intact cells. However, all cell types ultimately have the capacity to re-differentiate or regenerate rhizoid cells in response to ablation of neighbouring cells. This regeneration is regulated in a position-dependent manner and is strongly influenced by intercellular communication, probably involving transport or diffusion of inhibitory signals which appear to be essential for regulation of cell fate decisions. This type of cell-to-cell communication does not involve symplastic transport or direct cell-cell contact inhibition. Apoplastic diffusible gradients appear to be involved in pattern formation in the multicellular embryo.

Key words: *Fucus* embryogenesis, Laser microsurgery, Pattern formation, Cell fate determination, Positional information, Intercellular communication

INTRODUCTION

In animals, cell fate decisions during embryogenesis can arise from cellular interactions with neighbouring cells (induction) and from the asymmetric segregation of cellular factors which specify cell lineages (Gurdon, 1992). A major distinguishing feature of plant development is its indeterminate nature which, coupled to a considerable morphogenetic plasticity in response to external stimuli, allows the plant, by modifying its shape and physiology, to adapt to its environment. Thus the mechanisms underlying developmental pattern must also accommodate the plasticity of plant development. In plants, only the developmental axis and zones of continuously differentiating tissues (meristems) are laid down during early embryogenesis (e.g. Goldberg, 1994) and new organs and tissue are continuously formed throughout the life of the plant. Therefore by the end of embryogenesis overall pattern is established but morphogenesis is limited. In root and shoot apical meristems, positional information rather than cell lineage plays an essential role in fate decisions (Van den Berg et al., 1995; Steeves and Sussex, 1989). Several lines of evidence suggest that cell fate determination and pattern formation in plant embryos are also under the control of positional signalling. Transected carrot embryos are capable of restoring missing parts in a position-dependent manner (Schiavone and Racusen, 1991). However, this regeneration involves de-differentiation and re-differentiation of cells at the cut surface unlike, for example, limb regeneration in amphibians in which cell migration plays an important role (Brockes, 1994). The nature of positional information in plant embryos remains obscure. At least three mechanisms have been invoked to account for positional signalling. Intercellular diffusion of morphogens may occur via plasmodesmata (Lucas, 1995) and hormonal gradients have been shown to be involved in patterning plant tissue and organs (Liu et al., 1993). Finally positional information appears to be imprinted in the cell wall of the 2-celled *Fucus* embryo (Berger et al., 1994).

The embryo of the brown alga *Fucus* presents a very simple system to study early zygotic development in plants. The axis of embryonic polarity is established in the zygote in response to external cues (e.g. Goodner and Quatrano, 1993). The early embryo is composed of only two cell types, i.e. thallus cells and rhizoid. Moreover, the embryo is free of maternal tissues and easy to manipulate. We have undertaken a systematic microsurgical approach to understand better the mechanisms of cell fate control and patterning in the developing embryo. Here we show that the cell wall is essential for maintenance of polarity in isolated cells. A gradient of positional information regulates the nature of cell regeneration in response to ablations. Later stages of embryogenesis are characterised by

increased plasticity in the response to cell ablation or microsurgical dissection. After the 2-cell stage, intercellular communication involving extracellular diffusible factors appears to play an important role in fate decisions and in patterning the embryo.

MATERIALS AND METHODS

Algal culture

Mature receptacles of *Fucus spiralis* were washed, blotted dry and stored at 4°C in the dark. Gametes were released into sterile filtered sea water (FSW) at 17°C over a period of 1 hour by standard procedures (Quatrano, 1980). Fertilised zygotes were settled on coverslips in culture chambers and were polarised in unilateral white light and cultured for up to 10 weeks at 17°C in FSW.

Preparation of protoplasts

Culture chambers were placed on the stage of an inverted microscope (Nikon, Tokyo, Japan). Embryos were plasmolysed in FSW containing 1 M sorbitol. Plasmolysis produced a 10-20 μm space between the plasma membrane and the cell wall. The cell wall was removed locally using laser microsurgery (Berger et al., 1994; Taylor et al., 1996). Osmoticum was then gradually replaced by FSW containing 0.6 M sorbitol. During subsequent deplasmolysis, the rhizoid or thallus protoplast expanded through the ablated opening in the cell wall. Nucleated protoplasts which regenerated cell wall within 1-2 hours following extrusion (Berger and Brownlee, 1995) were subsequently cultured in FSW.

Laser microsurgery

Specific cells in embryos were ablated with a pulsed nitrogen UV laser (Berger et al, 1994). Cells were either completely ablated (removing most of the cytoplasm) or simply irradiated (i.e. killed by laser irradiation leaving cellular debris and cell walls intact (e.g. Berger et al., 1994). Laser-treated embryos were subsequently cultured for up to 6 weeks in FSW. For photomicroscopy of embryos older than 12 days, embryos were mounted in Hydramount (Burr, Searle Scientific Services, High Wycombe, Bucks, UK) before observation. This allowed visualization of individual cells in the multicellular embryo. Typically, approximately 100 regenerating embryos (n=50-200) were counted for each type of dissection.

Microinjection of FITC dextran

Rhizoid cells of 3-celled or 8- to 12-celled embryos were microinjected with FITC dextran (10 kDa) using pressure pulses applied to an intracellular electrode as described previously (Roberts et al., 1994; Taylor et al., 1996). Embryos were pre-incubated in FSW containing 0.7 M sorbitol to reduce internal turgor pressure prior to injection. Following injection, embryos were transferred to FSW and cultured as described above. Dye fluorescence was recorded with a cooled CCD camera (Digital Pixel, Brighton, UK) on a Nikon Diaphot fluorescence microscope.

RESULTS

Cell division pattern during normal development in *Fucus* embryos

The first asymmetric division of the polarised zygote (Fig. 1A,B) produces two cells with striking morphological differences. The thallus cell is spherical and highly pigmented while the rhizoid cell is elongated and has fewer chloroplasts (Fig. 1C). Embryonic pattern becomes well established during subsequent divisions. The second division normally occurs in the rhizoid, producing 3 cells: thallus (T), apical rhizoid (AR), and basal rhizoid (BR) (Fig. 1D). The embryo further divides to produce apical thallus (AT), basal thallus (BT), apical rhizoid (AR) and basal rhizoid (BR1 and BR2) cells (Fig. 1E). After 48 hours, 8 cells (2 each of AT, BT, AR and BR) are visible when viewed from above although a further longitudinal division of thallus cells occurs in a plane perpendicular to the first longitudinal division to produce 8 thallus cells. Since the precise timing of this division is not certain we will refer to the 48 hours embryo as 8- to 12-celled. The thallus cell gives rise to an elongated multicellular

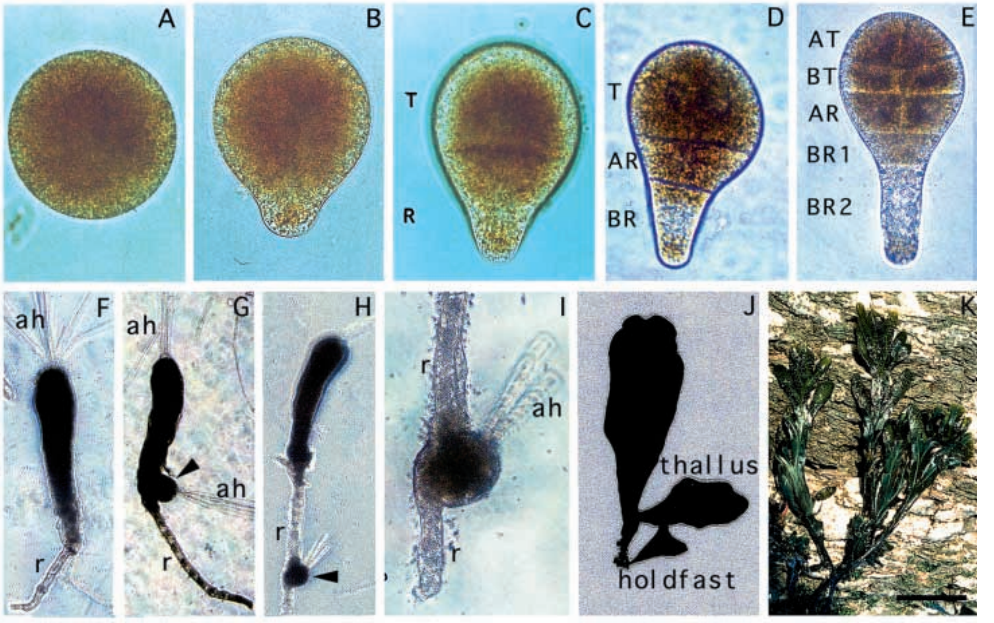

Fig. 1. Normal development in *Fucus*. (A) The fertilised egg and zygote are apolar. (B) Polarisation occurs in response to unidirectional light resulting in rhizoid outgrowth. (C) The first asymmetric division is perpendicular to the polar axis, producing a thallus cell (T) and a rhizoid cell (R) with different fates. (D) The next division of R generates one apical rhizoid (AR) and one basal rhizoid cell (BR). (E) At 48 hours four different cell types are recognisable, apical thallus (AT), basal thallus (BT), apical rhizoid (AR) and basal rhizoid (BR1 and BR2). (F) After 3 to 4 weeks, the thallus produces an apical meristematic region preceded by the emergence of apical hairs (ah). (G) At 7 to 8 weeks 'adventive' thallus regions (arrowhead), identified by the presence of apical hairs (ah) can develop from the rhizoid (r). (H) In some embryos the 'adventive' thallus region develops close to the rhizoid base (arrowhead). I is a higher magnification of H. At about 2 months the young plantlet exhibits a holdfast and ramified thallus branches (J) typical of the adult alga (K). Bar, 30 μm (A-C and I), 40 μm (D-E), 100 μm (F-H), 400 μm (J) and 20 cm (K).

structure which after 3 to 4 weeks usually produces filamentous hair cells at its apex (Fig. 1F). An apical meristem is subsequently laid down at the base of the hair cells (Nienburg, 1931). The primary rhizoid cell generates branches of secondary rhizoid cells which allow the embryo to attach firmly to the substratum. Thus, two different developmental programs can be distinguished in the early embryo. However these programs are not irreversibly fixed in later developmental stages, even during normal development. At about 8 weeks, changes frequently occur (in up to 90% of embryos in culture) locally in the orientation of cell divisions in the rhizoid associated with increased pigmentation of these cells. Thallus-like structures develop from these cells as confirmed by the presence of filamentous apical hairs (Fig. 1G). Occasionally these structures can be observed very close to the base of the rhizoid (Fig. 1H,I), indicating clearly their rhizoid origin. Hence the first rhizoid cell generates not only the holdfast but also a large part of the adult plant (Fig. 1J,K), whereas the initial thallus cell normally gives rise only to thallus tissue.

Absence of cell lineages in the *Fucus* embryo

Laser microsurgery was used to ablate specific cells in the embryo (Berger et al., 1994). After ablation of all rhizoid cells in 8- to 12-celled embryos (Fig. 2A), thallus cells above the ablation switch their fate and re-differentiate into rhizoids in less than 3 days (Fig. 2B). Following the ablation of all thallus cells and AR cells (Fig. 2C), filamentous BR cells remaining below the ablation become spherical and pigmented, eventually forming a thallus-like structure after approximately 2 weeks (Fig. 2D). The fact that isolated cell types have the ability ultimately to regenerate embryos demonstrates the absence of long-lived cytoplasmic determinants.

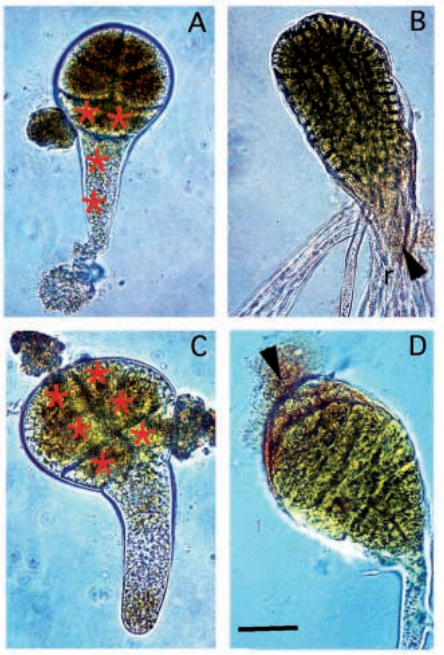

Fig. 2. Absence of cell lineages in the early *Fucus* embryo. Specific cell types were ablated (red asterisks in A and C) using a u.v. pulsed nitrogen laser and regeneration was monitored for up to 6 weeks. (A) Following ablation of all rhizoid cells (AR+BR), rhizoid regeneration occurred from thallus cells as early as 3 days after the dissection and after 6 weeks (B) the overall pattern of the embryo appears normal. (C) Following ablations of all thallus cells and AR, rhizoid cells below the ablation divide parallel to the polar axis, become more pigmented and can produce thallus structures at 4 weeks (D). Arrowheads show cellular debris remaining after ablations. Bar, 30 μm.

Features of regeneration in 8 week old embryos

Eight-week old multicellular embryos were surgically transected using a microscapel (Fig. 3A, B). After 2 weeks, rhizoid regeneration was observed from both cut surfaces in more than 80% of transected embryos (n=50) (Fig. 3C-F). This regeneration occurred independently of the level of the transection along the apical-basal axis of polarity, suggesting that at this stage of development, rhiozoid regeneration can be induced following any type of dissection.

Evidence for positional information in the early embryo

Following sequential laser ablations of all types of basal cells in 8- to 12-celled embryos, e.g. AR+BR (Fig. 3A,B), BR (Fig. 4) or BR+AR+BT (not shown), cells immediately apical to the dissection, including thallus cells, have the ability to regenerate rhizoids from their basal pole. We took advantage of this to try to understand how rhizoid regeneration is regulated in early embryos. All cells of a specific type (i.e. AT, BT, AR or BR) were ablated (Fig. 4A) and regeneration was monitored for up to 6 weeks following ablation (Fig. 4B,C). All cell types were ultimately capable of producing rhizoids in response to ablations (Fig. 4C). However, the frequency and the timing of rhizoid regeneration were highly dependent on the position of

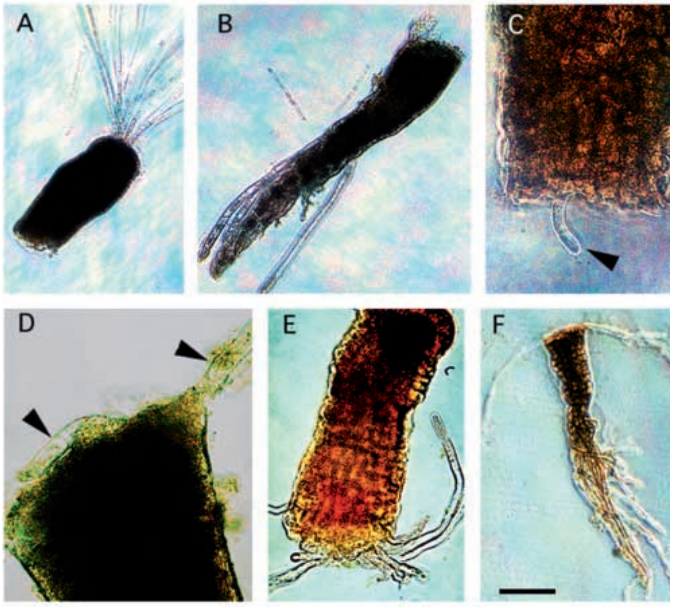

Fig. 3. Apolar regeneration of rhizoids in transected embryos. Six week old embryos were surgically transected to give separate apical (A) and basal (B) regions. Rhizoid production (arrowheads) was first observed about 2 weeks after transection at both apical and basal edges of the surgical margin (C and D). At 21 days multiple rhizoids were observed at the basal (E) and apical (F) poles of the transected segments. Bar, 50 μm (A,B,F), 50 μm (C,D), 25 μm (E).

the ablation. Three days following BR ablation all embryos exhibited new rhizoids arising from adjacent AR cells (Fig. 4B,C). In contrast, no rhizoids were observed for up to 12 days following AT or BT ablation and fewer than 25% of these embryos regenerated rhizoids even after 6 weeks, ablated cells being largely replaced by new thallus cells (Fig. 4B).

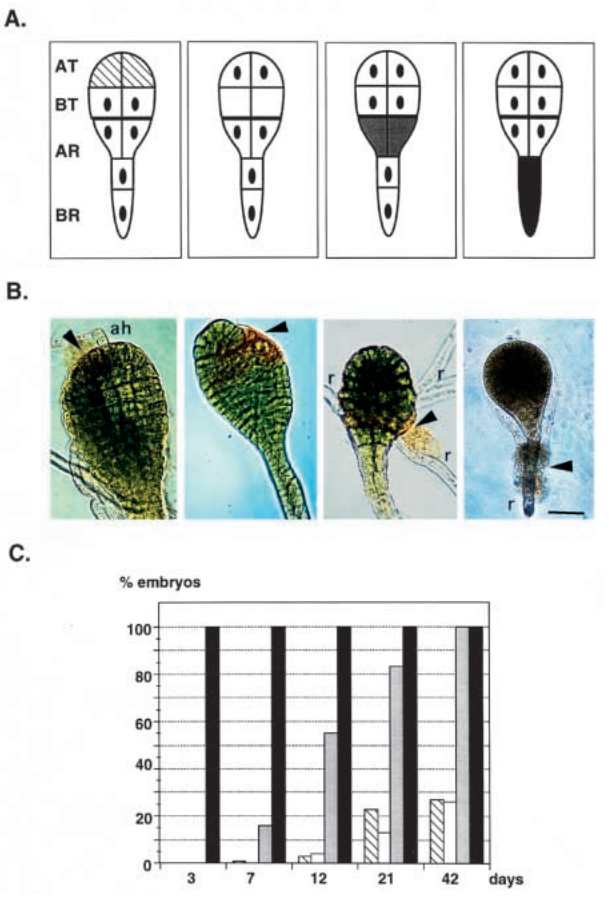

Fig. 4. Position-dependent rhizoid regeneration in the 8- to 12-celled embryo. (A) Specific cell types (AT, BT, AR or BR, denoted by different shading) were ablated and regeneration was monitored for up to 6 weeks. Responses to ablations are shown in B below the corresponding ablations in A. (B) Rhizoid regeneration (r) was occasionally observed 21 days following AT ablation (1st panel in A and B) or rarely 12 days following BT ablation (2nd panel), though in most cases missing thallus cells were replaced by neighbouring cells of the same type, producing embryos indistinguishable from normal embryos after 6 weeks. Apical hairs (ah) were occasionally detected following AT ablation (A,B) demonstrating position-dependent replacement of AT. When AR cells were ablated (3rd panel), most embryos produced rhizoids (r) from descendants of BT after 12 days and rhizoid regeneration always occurred after 6 weeks. Three days following ablation of BR (4th panel), new rhizoids (r) always grew from AR (B). The percentage of embryos regenerating rhizoids following each type of ablation is shown in C as a function of time after the ablation (n=100). Ablated cell types are represented by the same shading in A and C. The ability to regenerate rhizoid cells is greatest at the rhizoid pole of the embryo. Furthermore the regeneration frequency is higher and the time shorter following ablation of BR in comparison to ablation of AR suggesting the existence of an apical-basal gradient of positional information throughout the embryo. Bar, 30 μm.

Following AR ablation, rhizoid regeneration was detected from adjacent BT cells after 12 days in 70% of embryos. These results indicate the existence of an apical-basal gradient of positional information controlling rhizoid development in the embryo.

Role of the cell wall in maintaining polarity and controlling cell fate

Protoplasts extruded from either thallus or rhizoid cells of 2-celled or 8- to 12-celled embryos are able to re-polarise in response to the prevailing light direction, produce a new rhizoid within 24 hours and develop into apparently normal embryos (Fig. 5A,B; see also Berger et al., 1994). In contrast, following ablations of all neighbouring cells in 8- to 12-celled (Fig. 5C) or 3-celled embryos (Fig. 5E), all types of cells conserve the ability to re-differentiate forming new rhizoids at their former basal pole and thallus tissue at their apical pole even when the polarising unidirectional light is reoriented (Fig. 5D,F). Rhizoid regeneration from BT-derived cells could be observed albeit with low frequency (25% only after 42 days) following ablation of AT cells (Fig. 5G,H, see also Fig. 4C). In this case it is unlikely that rhizoid induction is under the control of factors in the cell wall.

Role of cell-to-cell communication in cell fate regulation

Rhizoid regeneration following ablation of all cells of a specific type in 8- to 12-celled embryos was monitored with respect to the presence of remaining rhizoid cells below the ablation (Fig. 6). Thus, cells adjacent to BT were sequentially ablated, (i.e. ablation of AR, AR+BR1, AR+BR1+BR2 or AT; Fig. 6A). To determine precisely the proportion of embryos in which rhizoids regenerated from BT, control ablations were also performed. For example, the control for AR ablation consisted of leaving only BR1 and BR2 alive and following rhizoid regeneration (not shown). In this case, rhizoid regeneration against the polar axis from isolated BR2 or BR1+BR2 after 21 days occurred in fewer than 5% of dissections. More than 50% of embryos regenerated rhizoids from BT within 3 days following ablation of AR+BR1+BR2, 5 days following AR+BR1 ablation and 12 days following AR ablation (Fig. 6B,C). Rhizoid regeneration from BT following ablation of AT was not observed until 12 days and reached its maximum (30% of embryos) only after 21 days (Fig. 6C). Therefore the timing and frequency of rhizoid induction from BT is correlated to the number of rhizoid cells left below the dissection. We propose that an inhibitor of rhizoid regeneration may be produced by rhizoid cells and can diffuse or is transported to the thallus where it normally prevents rhizoid formation (Fig. 6D).

Parallel experiments were carried out with 3-celled embryos (Fig. 7). When AR alone was ablated (Fig. 7A), leaving BR intact, rhizoid regeneration from T was not detected until 5 days and a majority of the embryos produced new rhizoids only after 12 days (Fig. 7B,C: left graph). All embryos regenerated rhizoids from T within 3 days after the ablation of AR+BR. Ablation of T in 3-celled embryos allowed rhizoid regeneration from AR against the normal embryonic polarity, to be monitored (Fig. 7A, right graph). Rhizoids were produced earlier and more frequently from the thallus pole of AR when BR was also ablated (Fig. 7B,C, right graph). Of course in this case new rhizoids were also produced within 3 days at the

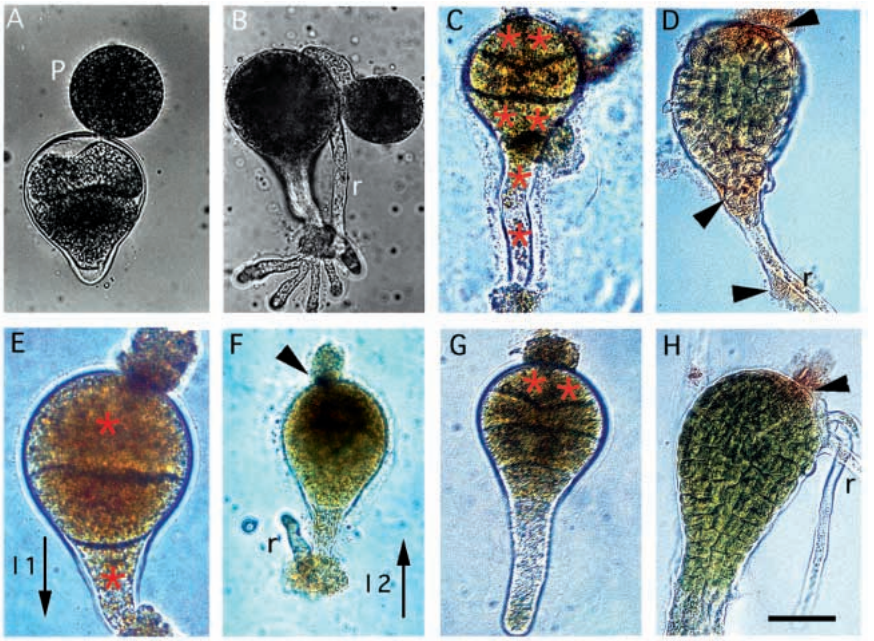

Fig. 5. A thallus protoplast (P) extruded from a 2-celled embryo following laser ablation of the cell wall (A) re-polarizes de novo and produces a new rhizoid (B). (C) BT cells in 8- to 12-celled embryos isolated by ablation of all neighbouring cells (red asterisks) are capable of regenerating rhizoids (r) at the former rhizoid pole and thallus at the apical pole 4 weeks following the ablation (D). Arrowheads show cellular debris remaining following ablation. In 3-celled embryos, isolated AR cells (E) regenerate rhizoids according to their polarity prior to isolation, irrespective of the light direction (F). Arrows labelled l1 and l2 show light direction before and after laser ablation. (G) Ablation of AT can ultimately result in low frequency rhizoid regeneration (H) from BT-derived cells in the absence of contact with remaining rhizoid cells. Bar, 30 μm.

rhizoid pole of AR as early as 3 days following ablation. These results also indicate that a diffusible factor produced by rhizoid cells may inhibit thallus-to-rhizoid re-differentiation (Fig. 7D, left).

Taken together these results suggest that re-differentiation of thallus cells into rhizoid cells in response to ablation of adjacent cells is strongly influenced by the presence of pre-existing rhizoid cells. Thus, remaining rhizoid cells are able to inhibit the re-differentiation of new rhizoids from thallus cells. However, it is notable that rhizoid cells have greater potential to regenerate rhizoids from their basal pole, even in the presence of remaining rhizoid cells. Thus, following ablation of BR1 in 8- to 12-celled embryos, rhizoids were produced as quickly from AR (100% after 3 days) in the presence as in the absence of BR2 (not shown).

Symplastic versus apoplastic communication and control of rhizoid regeneration

To test whether intercellular communication was involved in the control of rhizoid regeneration by existing rhizoid cells, FITC-dextran was injected into individual rhizoid cells of 3-celled or 8- to 12-celled embryos. FITC-dextran injected into the cytoplasm of rhizoid cells could be detected in all other cells of the embryo, including thallus cells within 24 hour (Fig. 8A-C). When sub-basal cells (e.g. BR1 in 8- to 12-celled embryos) were injected, fluorescence was detected first in more basal cells (Fig. 8A) and subsequently in more apical cells (Fig. 8B). Thus molecules at least as large as 10 kDa are able to move via symplastic connections between cells in both directions along the axis of polarity. Normal embryogenesis proceeded after injection of any cell type, resulting in multicellular embryos with all cells fluorescently labelled (Fig. 8C).

Microinjections of FITC dextran were also carried out in conjunction with laser ablations. One day after microsurgery rhizoid cells (i.e. BR) immediately basal to ablated AR cells were microinjected. Ablation of AR (Fig. 8D,G) irreversibly prevented the movement of FITC dextran to the cells above the ablation (Fig. 8E,H). Fluorescence was still detected in all cells below the ablation 1 week after the microinjection but was never observed in the cells above the ablation or their derivatives (Fig. 8E,H). The absence of fluorescence in regenerated rhizoids confirms their origin from cells apical to the ablation gap (Fig. 8E,F). The inhibitory effect on rhizoid regeneration by pre-existing rhizoid cells in microinjected embryos, despite the disruption of symplastic connections (Fig. 8E-H, see also Figs 6 and 7), suggests that symplastic communication was not involved in this control of rhizoid regeneration.

DISCUSSION

Plasticity and developmental stage

The results presented here show that a frequent response to cell ablation in 3-celled to 8 week old embryos is the production of cells that are morphologically indistinguishable from rhizoid cells. In early embryos (up to 48 hours), rhizoid production in response to cell ablation is tightly regulated in a position-dependent manner whereas in older multicellular embryos (3 to 8 weeks), rhizoid regeneration occurs independently of the level of the dissection and embryo polarity. In adult vegetative fronds of *Fucus*, the outgrowth of rhizoids from cortical cells has also been observed (Fries, 1984). Thus, developmental plasticity appears to be more restricted in early embryonic stages compared to later stages.

Absence of cell lineage involvement in pattern formation in *Fucus* embryos

Clonal analysis in maize (Dawe and Freeling, 1991) as well as fate mapping experiments in shoot and root meristems of *Arabidopsis* (Van den Berg et al., 1995; Szymkowiak and Sussex, 1996) have indicated that fate determination and differentiation are dependent on positional cues. Genetic analysis of *Arabidopsis* mutants such as *fass* (Torrez-Ruiz and Jurgens, 1994), reveals that pattern formation in embryos does

not require properly oriented cell divisions, suggesting indirectly that pattern and cell fate determination is position rather than lineage sensitive in plant embryos. In *Fucus* embryos, normal embryonic pattern also appears to be independent of the pattern of cell division since transient treatments with brefeldin A (Shaw and Quatrano, 1996) or β-oestradiol (Pollock, 1969), which lead to abnormal patterns of cell division, can produce apparently normal polar embryos.

Analysis of *Arabidopsis* mutants such as *monopteros*, which lacks the basal or central embryonic regions and subsequently the root and hypocotyl, suggests that the embryo forms from autonomous regions arising from rigid lineages in the very early embryo (Berleth and Jurgens, 1993). Here we present evidence that pattern formation in early plant embryos proceeds by positional control rather than cell lineage. Both rhizoid and thallus cells and protoplasts have the ability to regenerate the other cell type following surgical ablation. Another striking example illustrating the lack of lineages is the regeneration of apical hair and meristem cells from cells descended from BT which occurs when cells which normally give rise to these cell types (i.e. AT), are ablated in the 8- to 12-celled *Fucus* embryo. Finally during normal development in late embryos and young adult plants, thallus tissue can form from rhizoid cells, suggesting that rhizoid cells are not irreversibly committed to a particular fate.

Cell wall contribution to positional information

Despite the evidence that positional information is an important factor in plant development (Jurgens, 1995), its nature, particularly during embryogenesis is poorly understood. Previous work with 2-celled *Fucus* embryos has demonstrated that rhizoid development from isolated thallus cells following laser ablation can be rapidly induced by contact of the remaining cell with the residual cell wall of the ablated cell but not if this contact was prevented (Berger et al., 1994; Kropf et al., 1993). Here we show that at later developmental stages, rhizoid production from a thallus cell can ultimately occur in the absence of intimate contact between a cell and the remaining wall of the dissected cell. Thus no differences were observed in the times and frequencies of regeneration between embryos in which cells were simply killed by laser ablation and those in which cells were completely ablated (not shown). Moreover, thallus cells which never come in contact with rhizoid cells, e.g. following dissection of AT in 8- to 12-celled embryos, are ultimately capable of re-differentiating into rhizoids in up to 25% of embryos. However, this re-differentiation from later stage embryos takes much longer in

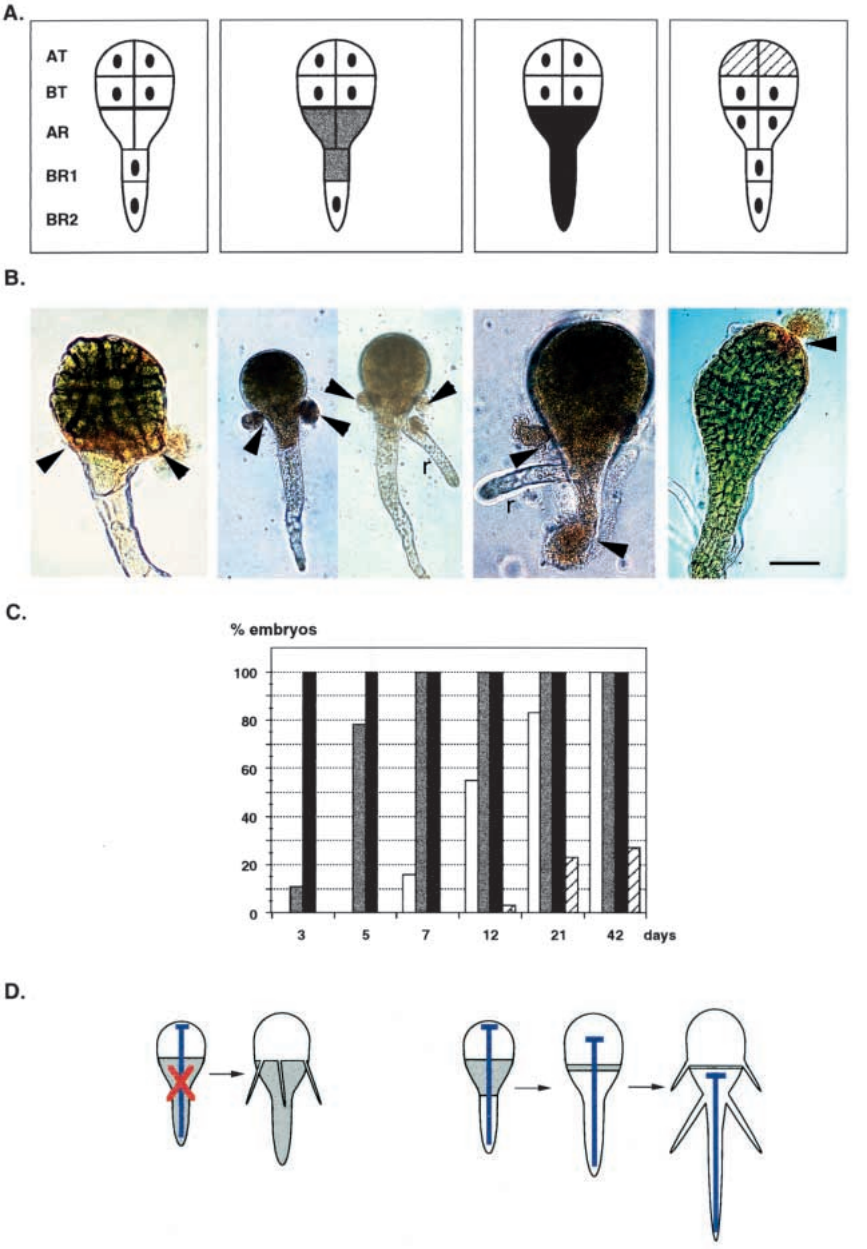

Fig. 6. The effect of neighbouring cells on rhizoid regeneration was monitored following ablation of all cells of a specific type (shadings in A and C correspond as in Fig. 4) in 8- to 12-celled embryos. Responses to ablations are shown in B below the corresponding ablations in A. Arrowheads indicate debris from ablated cells. (C) The percentage of embryos regenerating rhizoids following each type of ablation is shown as a function of time after the ablation (n=100). When AR was ablated (A), remaining BR1 and BR2 could prevent rhizoid regeneration from BT for at least 5 days (B). More frequent regeneration of rhizoids from BT was observed following ablation of AR+BR1 (both non-regenerating and regenerating embryos are shown in B below the corresponding ablation in A). Ablation of all rhizoid cells elicited rhizoid regeneration from BT in all embryos after 3 days. Rhizoid regeneration from the apical pole of BT after ablation of AT (hatched bars) was infrequent and only observed after 12 days. In D the results are interpreted in terms of a diffusible inhibitor (blue) of thallus cell re-differentiation produced by normal rhizoid cells (left). This inhibitor can cross an ablation gap but is effective over a limited range, allowing for more thallus-to-rhizoid re-differentiation as embryo size increases (right). Bar, 30 μm.

comparison with re-differentiation in ablated 2-celled embryos and only after a number of cell divisions have taken place. It can therefore be argued that different mechanisms are operating in the control of re-differentiation in embryos beyond the 2-cell stage. Furthermore isolated single cells in 3-celled and older embryos have the ability, as do isolated protoplasts, to de-differentiate and re-differentiate, though while isolated protoplasts fix a new polar axis before regenerating (Kropf et al., 1988; Berger et al., 1994), isolated single cells retain their original apical-basal axis. However differences in patterns of re-differentiation must be considered when comparing protoplasts from 2-celled embryos with isolated cells from older embryos. Whereas the isolated protoplast re-differentiates directly, in isolated cells of older embryos re-differentiation is only apparent from daughter cells following a number of cell divisions. Together, these results suggest that after the 2-cell stage, pattern formation is not exclusively under the control of positional information in the cell wall. Therefore the extracellular matrix could provide the necessary information required for polarisation (Kropf et al., 1988) and fate specification (Berger et al., 1994) in both the zygote and the 2-celled embryo and for the retention of polarity in isolated intact cells from older embryos, whereas additional mechanisms are also likely to be involved in directing pattern in later embryonic stages.

Nature of cell fate control

Laser microsurgery experiments with 3-celled and older embryos indicate that all cells of the *Fucus* embryo have the potential ultimately to produce rhizoids in response to ablations. It is clear that cells derived from those adjacent to ablated cells take much longer to regenerate rhizoids against the embryonic polarity. Cells derived from rhizoid cells have greater capacities to regenerate rhizoids than those derived from thallus cells. Moreover, our results show that rhizoid regeneration from thallus tissue (but not from rhizoid tissue) is strongly influenced by the presence of residual intact rhizoid cells.

Cell contact inhibition could possibly account for the inhibition of rhizoid regeneration from apical thallus tissue in ablation experiments. However this is unlikely for the following reasons. Firstly, residual cell wall prevents complete restoration of cell continuity between neighbouring cells following cell ablation, as indicated by the loss of symplastic connections. Secondly, the timing and frequency of rhizoid

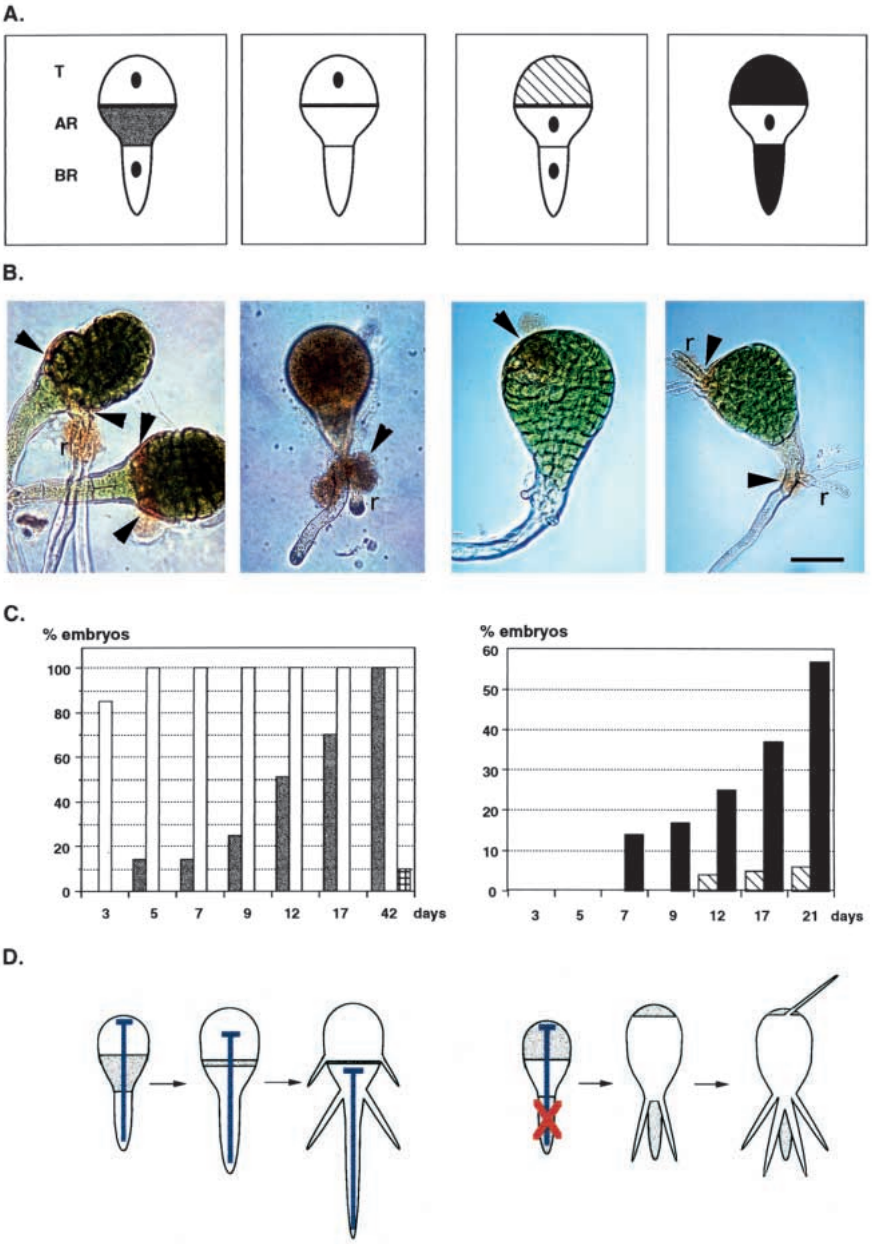

Fig. 7. In 3-celled embryos ablation of AR+BR (A, 2nd panel) results in the emergence of new rhizoids (r; B) from T, 3 days following the dissection (C, left chart). Ablated cells types are represented by the same shading in A and C and typical responses to ablation in A are shown directly below in B. In C for each type of ablation the percentage of embryos regenerating rhizoids is represented as a function of the time after the dissection (*n*=50-200). When BR was left intact (1st panel in A) 50% of embryos regenerated rhizoids (r) from T after 12 days but a significant proportion did not. The checkered bar in C shows a low frequency of rhizoid regeneration from isolated BR against the polar axis even after 42 days (dissection not shown). Regeneration from AR was also monitored following T ablation (3rd panel) compared to T+BR ablation (4th panel). When T alone was ablated, most embryos did not regenerate rhizoids from AR (C, right chart). However, following ablation of T + BR, while rhizoids (r) regenerated predominantly from the rhizoid pole of AR (B), significant regeneration also occurred from the thallus pole after 12 days (C, right chart, black bars). (D) The results can be explained in terms of the production of a diffusible inhibitor (blue) which prevents rhizoid regeneration from both the basal (left) and apical (right) poles of the thallus cells. Bar, 30 μm.

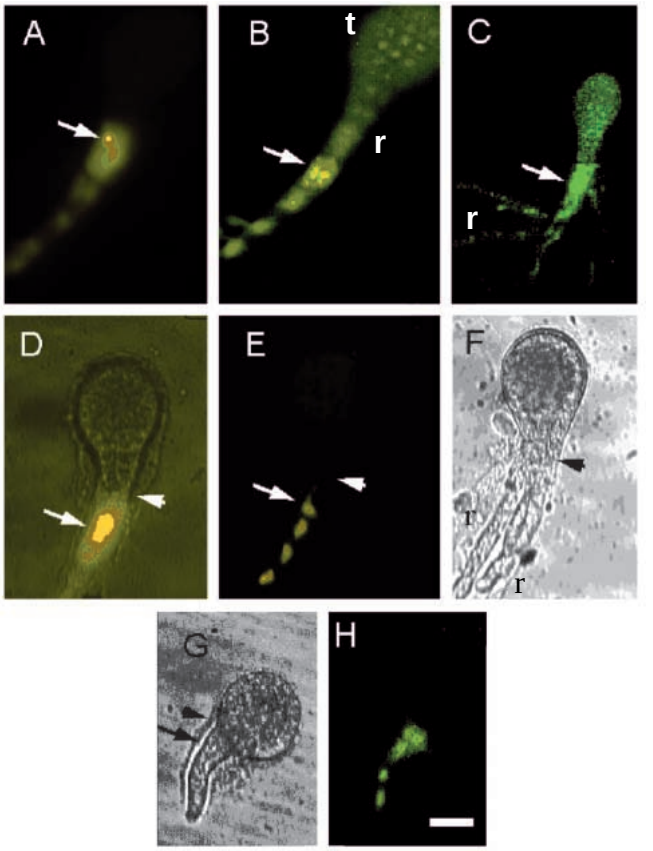

Fig. 8. Symplastic communication during embryogenesis and disruption following cell ablation. FITC dextran (10,000 kDa) microinjected into the rhizoid cell of an 8- to 12-celled embryo (arrow in A-C) can be detected initially in more basal rhizoid cells after 24 hours (A). After 3 days (B, same embryo) fluorescence can be detected in all rhizoid (r) and thallus (t) cells. After 10 days (C, same embryo), fluorescence is detected throughout the embryo, including new rhizoid cells (*n*=5). (D) Microinjection of FITC dextran into BR1 (arrow) following ablation of both AR cells (arrowhead) results in restriction of the dye to the injected rhizoid (E, same embryo 10 days after microinjection, *n*=14). In this embryo, additional rhizoids were initiated from BT apical to the ablation (F, arrowhead) (see also Fig. 4). (G,H) Following ablation of AR and microinjection of FITC dextran into BR in 3-celled embryos, dye remained restricted to rhizoid cells during subsequent development (shown 48 hours following ablation of AR, *n*=6). Arrow shows injected cell, arrowhead shows ablation of AR. Bar is 20 μm in A, D-H; 30 μm in B and 60 μm in C.

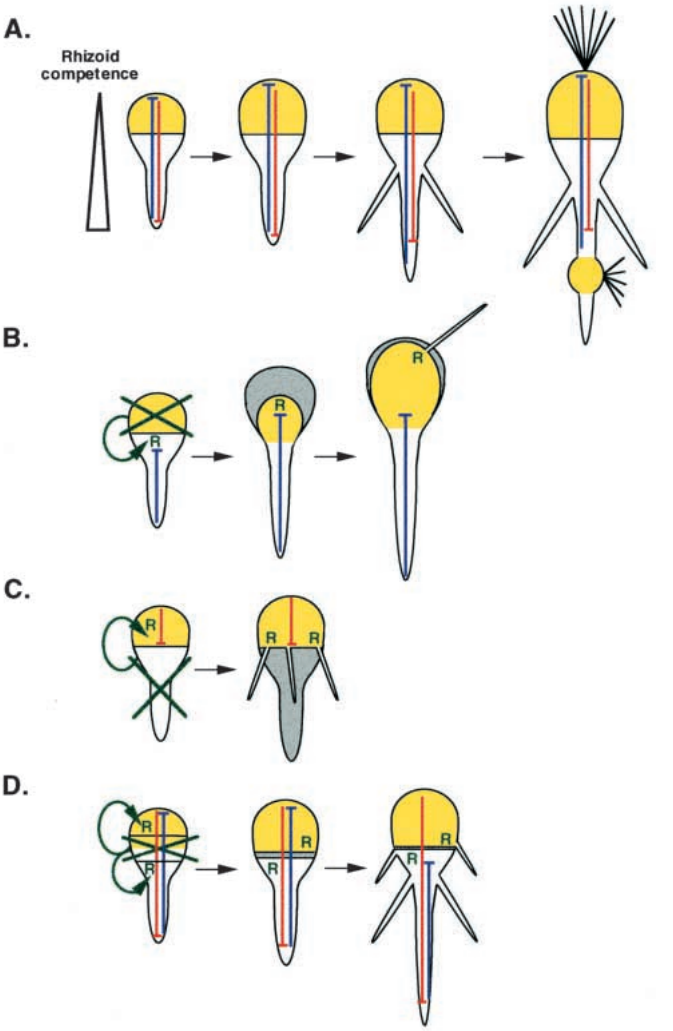

Fig. 9. Summary model of intercellular signalling during pattern formation in the *Fucus* embryo. (A) In the early embryo thallus cells produce a diffusible inhibitory signal (red bar) which acts to prevent rhizoid-to-thallus re-differentiation. Rhizoid cells produce an equivalent signal (blue bar) which inhibits thallus-to-rhizoid re-differentiation. As embryo size and rhizoid length increase new 'adventive' thallus tissue can develop out of range of the inhibitory signal produced by the thallus. Inhibition of thallus-to rhizoid re-differentiation can be maintained by the production of inhibitory signals from lateral rhizoids. These inhibitory signals thus act to prevent re-differentiation of one cell type into another cell type. (B) While the most frequent response to ablation of thallus cells is replacement with more thallus cells, thallus cell ablation (green cross) can stimulate the induction of new rhizoid cells, possibly via the wounding-associated release of a rhizoid inducer (green arrow). However, the presence of inhibitory signals from residual rhizoid cells delays the appearance of new rhizoids. (C) Ablation of rhizoids in early embryos stimulates the production of new rhizoids by thallus cells. In this case rhizoid induction is not inhibited by signals from residual rhizoids. (D) When intact rhizoid cells are left below an ablation gap they may continue to produce an inhibitory signal that would prevent rhizoid regeneration from thallus cells until later stages. This inhibitory signal can operate across an ablation gap.

regeneration from similar cell types, i.e. from AT following dissection of BT or from BT following dissection of AR, are fundamentally different despite a quick re-establishment of physical contact between neighbouring cells.

The frequency of rhizoid regeneration following different ablations is directly influenced by the number of rhizoid cells remaining basal to the ablation. Thus an inhibitory substance may be produced by rhizoid cells and move between ablated cell layers to thallus cells (Fig 9). The fact that the capacity of cells to regenerate rhizoids following dissections increased with time after ablation suggests that the influence of factors causing inhibition of rhizoid generation decreased with time. Thus one possible explanation is that rhizoid inhibitory factors become progressively diluted or that intercellular communication is decreased at later embryonic stages (Fig. 9).

In older embryos and young plants, control of thallus differentiation from rhizoid tissue is likely to be exerted by existing thallus tissue (Fig. 9). Thallus tissue and meristem normally differentiate from rhizoid tissue after 8 weeks. The process of re-differentiation of rhizoid cells into thallus cells was originally described as 'adventive embryony' (McLachlan et al., 1971). This normal stage of development in *Fucus spiralis* is likely to account for the ramification often observed in older embryos and adult plants (Fig. 1) (McLachlan et al., 1971). The model of control of thallus differentiation from rhizoid cells is compatible with the demonstration of apical dominance in *Fucus* whereby a pre-existing apical meristem inhibits the development in its vicinity of new meristematic regions (Moss, 1966).

Parallels have been established between the morphologies and division patterns of *Fucus* and *Arabidopsis* early embryos (Brownlee and Berger, 1995). The analysis of *Arabidopsis* suspensor mutants such as *raspberry*, *twin* or *sus* demonstrates the potential of the suspensor to develop into embryo-proper like structures (Yadegari et al., 1994; Vernon and Meinke, 1994; Schwartz et al., 1994). It has been proposed recently (Zhang and Sommerville, 1997) that the likely involvement of intercellular communication in patterning the *Arabidopsis* embryo suggests that differences may exist between the cell wall-related signals in *Fucus* and those involved in higher plant embryos. Here we show that in *Fucus*, as in *Arabidopsis* embryos, communication between different cell types in the embryo does indeed appear to play a role in the regulation of fate specification and pattern.

Morphogen gradients in positional signalling

Most of our knowledge about the nature of intercellular signals in the control of cell fate and differentiation come from studies with higher plants. For example genetic and molecular analysis of *pistillata* (*pi*) mutants in *Arabidopsis* have shown that *pi* acts non-cell autonomously and that it may regulate the production of a diffusible morphogenetic signal (Bouhidel and Irish, 1996). Similarly, morphogenetic gradients are likely to be involved in regulating the development of suspensor and embryo proper in *Arabidopsis* as suggested by the analysis of suspensor mutants (Schwartz et al., 1994).

Several lines of evidence suggest that cell-to-cell communication via symplastic connections can influence fate decisions in plants. In fern gametophytes the distribution and number of plasmodesmata predicts cell fate suggesting that intercellular signalling may orchestrate patterning (Tilney et al., 1990). In maize, the mRNA encoding the homeodomain protein KNOTTED 1 is selectively transported through plasmodesmata to neighbouring cells (Lucas et al., 1995). The symplastic trafficking of mRNAs or proteins such as DEF or GLO can account for non-autonomous control of meristem development in *Antirrhinum* (Perbal et al., 1996). Segregation and gradients of transcripts have also been detected in *Fucus* zygotes and embryos and may be relevant to polarisation and early embryogenesis (Bouget et al., 1995, 1996). Furthermore, plasmodesmata-like structures exist in sieve plates of midrib and wing regions of adult *Fucus* (Fielding et al., 1987) though they have not been observed in early embryos (Brawley et al., 1977). Since microinjected molecules at least as large as 10 kDa can pass between both rhizoid and thallus cells, these cells must be symplastically connected. However laser ablation irreversibly destroys symplastic communication even when physical contact between new neighbours is re-established. Thus the inhibition of re-differentiation by thallus cells above an ablation zone into rhizoid cells by the presence of rhizoid cells below the ablation zone, suggests that the operation of cell-to-cell communication influencing cell fate can occur via the apoplast.

Although there is considerable evidence for the involvement of hormonal gradients in patterning plant tissues (Uggla et al., 1996; Carland and McHale, 1996, Masucci and Schiefelbein, 1996), the contribution of hormones to positional information in embryos is less clear. In *Phaseolus vulgaris*, gibberellins produced by the suspensor enhance transcription in the embryo proper (Walthall and Brady, 1986). More significantly, the effects of polar auxin transport inhibitors and the analysis of *pin-1-1* mutants of *Arabidopsis* show that polar auxin transport is required for the establishment of bilateral symmetry during embryogenesis (Schiavone and Cooke, 1987; Liu et al., 1993). Auxin activity has long been known in *Fucus* (du Buy and Olson, 1937) and has been suggested to influence embryonic polarity (Jaffe and Neuscheler, 1969). Moreover, inhibitors of polar auxin transport suppress apical dominance in *Fucus* apices (Moss, 1964).

Overall our results support the idea that cell fate is regulated non-autonomously in *Fucus* embryos. In the zygote and 2-celled embryo positional information in the wall is required to fix the axis of polarity and establish the initial pattern (Berger et al., 1994). After the 2-cell stage, the cell wall is still required to retain the initial apical-basal polarity in intact isolated cells. However after the 2-cell stage intercellular communication is essential for regulation of cell fate decisions but appears to involve neither symplastic transport of inhibiting substances nor direct cell contact. Rather, apoplastic diffusible factors may be involved. This is associated with increased plasticity and decreased influence of the polar axis in patterning.

We would like to thank D. Nicholson, R. Bellé and J. Orion for assistance with photographic work, R. Williamson and A. Chrachri for Nomarski microscopy and A. Asensi and F. Corellou for fixation techniques and helpful comments. F.-Y. B. was a recipient of an EEC long term postdoctoral fellowship (BIO4CT965064). This work was also supported by the BBSRC and the Marine Biological Association, UK.

REFERENCES

Berger, F., Taylor, A. and Brownlee, C. (1994). Cell fate determination by the cell wall in early *Fucus* development. *Science* **263**, 1421-1423.

Berger, F. and Brownlee, C. (1995). Physiology and development of protoplasts obtained from *Fucus* embryos using laser microsurgery. *Protoplasma* **186**, 63-71.

Berleth, T. and Jürgens, G. (1993). The role of *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. *Development* **118**, 575-587.

Bouget, F.-Y., Gerttula, S. and Quatrano, R. S. (1995). Spatial redistribution of poly A^+ RNA during polarization of the *Fucus* zygote is dependent upon microfilaments. *Dev. Biol.* **171**, 258-261.

Bouget, F.-Y., Gerttula, S., Shaw, S. and Quatrano, R. S. (1996). Localization of actin mRNA during the establishment of cell polarity and early cell divisions in *Fucus* embryos. *Plant Cell* **8**, 189-201.

Bouhidel, K. and Irish, V. (1996). Cellular interactions mediated by the homeotic *PISTILLATA* gene determine cell fate in the Arabidopsis flower. *Dev. Biol*. **174**, 22-31.

Brawley, S. H., Quatrano, R. S. and Wetherbee, R. (1977). Fine-structural

studies of the gametes and embryo of *Fucus vesiculosus* L. (Phaeophyta). III. Cytokinesis and the multicellular embryo. *J. Cell. Sci.* **24**, 275-294.

Brockes, J. P. (1994). New approaches to amphibian limb regeneration. *Trends Genet.* **10**, 169-173.

Brownlee, C. and Berger, F. (1995). Extracellular matrix and pattern in plant embryos: on the look-out for developmental information. *Trends Genet.* **11**, 344-348.

Carland, F. M. and McHale, N. A. (1996). *LOP1*: a gene involved in auxin transport and vascular patterning in *Arabidopsis*. *Development* **122**, 1811-1819.

Dawe, R. K. and Freeling, M. (1991). Cell lineage and its consequences in higher plants. *Plant J.* **1**, 3-8.

Fielding, P., Carter, P. L. and Smith, C. A. (1987). Sieve plates in *Fucus*: a reappraisal of size and pore distribution. *Phycologia* **26**, 501-504.

Fries, L. (1984). Induction of plantlets in axenically cultivated rhizoids of *Fucus spiralis*. *Can. J. Bot.* **62**, 1616-1620.

Goldberg, R. B., de Paiva, G. and Yadegari, R. (1994). Plant embryogenesis: zygote to seed. *Science* **266**, 605-614.

Goodner, B. W. and Quatrano, R. S. (1993). *Fucus* embryogenesis: a model to study the establishment of polarity. *Plant Cell* **5**, 1471-1481.

Gurdon, J. B. (1992). The generation of diversity and pattern in animal development. *Cell* **68**, 185-199.

Jaffe, L. F. and Neuscheler, W. (1969). On the mutual polarization of nearby pairs of fucaceous eggs. *Dev. Biol. Suppl.* **19**, 549-565.

Jurgens, G. (1995). Axis formation in plant embryogenesis: Cues and clues. *Cell* **81**, 467-470.

Kropf, D. L., Coffman, H. R., Kloareg, B., Glenn, P. and Allen, V. W. (1993). Cell wall and rhizoid polarity in *Pelvetia* embryos. *Dev. Biol.* **160,** 303-314.

Kropf, D. L., Kloareg, B. and Quatrano, R. S. (1988). Cell wall is required for fixation of the embryonic axis in *Fucus* zygotes. *Science* **239**, 187-189.

Liu, C.-M., Xu, Z.-H. and Chua, N.-H. (1993). Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. *Plant Cell* **5**, 621-630.

Lucas, W. J. (1995). Plasmodesmata: intercellular channels for macromolecular transport in plants. *Curr. Opin.. Cell Biol.* **7**, 673-680.

Lucas, W. J., Bouche-Pillon, S., Jackson, D. P., Nguyen, L., Baker, L., Ding, B. and Hake, S. (1995). Selective trafficking of KNOTTED 1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270**, 1980-1982.

Masucci, J. D. and Schiefelbein, J. W. (1996). Hormones act downstream of *TTG* and *GL2* to promote root hair outgrowth during epidermis development in the *Arabidopsis* root. *Plant Cell* **8**, 1505-1517.

McLachlan, J., Chen, L. C.-M. and Edelstein, T. (1971). The culture of four species of *Fucus* under laboratory conditions. *Can. J. Bot.* **49**, 1463-1469.

Moss, B. (1964). Apical dominance in *Fucus vesiculosus. New Phytol.* **64**, 387-391.

Moss, B. (1966). Polarity and apical dominance in *Fucus vesiculosus*. *Brit. Phycol. Bull.* **3**, 31-35.

Nienberg, W. (1931). Die Entwicklung der Keimlinge von *Fucus vesiculosus* und ihre Bedeutung fur die Phylogenie der Phaeophyceen. *Wiss. Meer. Ab. Kiel.* **1**, 52-62.

Perbal, M.-C., Haughn, G., Saedler, H., Schwarz-Sommer, Z. (1996). Non-cell-autonomous function of the *Anthirrinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell-to-cell trafficking. *Development* **122,** 3433-3441.

Pollock, E. G. (1969). Effect of 17 β-oestradiol on early cleavage patterns in the embryo of *Fucus distichus*. *Experientia* **10**, 1073-1075.

Quatrano, R. S. (1980). Gamete release, fertilization, and embryogenesis in the Fucales. In *Handbook of Phycological Methods: Developmental and Cytological Methods* (ed. E. Gantt), pp 59-68. Cambridge: Cambridge University Press.

Roberts, S. K., Gillot, I. and Brownlee, C. (1994). Cytoplasmic calcium and Fucus egg activation. *Development* **120**, 155-163.

Schiavone, M. and Cook, T. (1987). Unusual patterns of embryogenesis in the domesticated carrot: developmental effects of exogenous auxins and auxin transport inhibitors. *Cell Diff.* **21**, 53-62.

Schiavone, F. M. and Racusen, R. H. (1991). Regeneration of the root pole in surgically transected carrot embryos occurs by position dependent, proximodistal replacement of missing tissues. *Development* **113**, 1305-1313.

Schwartz, B. W., Yeung E. C. and Meinke, D. W. (1994). Disruption of morphogenesis and transformation of the suspensor in abnormal suspensor mutants of *Arabidopsis*. *Development* **120**, 3235-3235.

Shaw, S. L. and Quatrano, R. S. (1996). The role of targeted secretion in the establishment of cell polarity and the orientation of the division plane in *Fucus* zygotes. *Development* **122**, 2623-2630.

Steeves, T. A. and Sussex, I. M. (1989). *Patterns in Plant Development.* Cambridge: Cambridge University press.

Szymkoviak, E. J. and Sussex, I. M. (1996). What chimeras can tell us about plant development. *Annu. Rev. Plant Physiol. Plant Molec. Biol.* **47**, 351-376.

Taylor, A. R., Manison, N. F. H., Fernandez, C., Wood, J. W. and Brownlee, C. (1996). Spatial organization of calcium signals involved in cell volume control in the *Fucus* rhizoid. *Plant Cell* **8,** 2015-2031.

Tilney E. G., Cooke, T. J., Connelly, P. S. and Tilney, M. S. (1990). The distribution of plasmodesmata and its relationship to morphogenesis in fern gametophytes. *Development* **110**, 1209-1221.

Torrez Ruiz, R. A. and Jürgens, G. (1994). Mutations in the *FASS* gene uncouple pattern formation and morphogenesis in *Arabidopsis* development. *Development* **120**, 2967-2978.

Uggla, C., Moritz, T., Sandberg, G. and Sundberg, B. (1996). Auxin as a positional signal in pattern formation in plants. *Proc. Natl. Acad. Sci. USA* **93**, 9282-9286.

Van den Berg, C., Willemsen, V., Hage, W. and Scheres, B. (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. *Nature* **378**, 62-65.

Vernon, D. and Meinke, D. W. (1994). Embryogenic transformation of the suspensor in *twin*, a polyembryonic mutant of *Arabidopsis*. *Dev. Biol.* **165**, 566-573.

Walthall, E. D. and Brady, T. (1996). The effect of suspensor and gibberellic acid on *Phaseolus vulgaris* embryo protein synthesis. *Cell Diff.* **18**, 37-44.

Yadegari, R., de Paiva, G. R., Laux, T., Koltunow, A. M., Apuya, N., Zimmerman, J. L., Fischer, R. L., Harada, J. J. and Goldberg, R. (1994). Cell differentiation and morphogenesis are uncoupled in *Arabidopsis* raspberry embryo. *Plant Cell* **6,** 1713-1729.

Zhang, Z. Z. and Somerville, C. R. (1997). Suspensor-derived polyembryony caused by altered expression of valyl-tRNA synthetase in the *twin 2* mutant of *Arabidopsis. Proc. Natl. Acad. Sci. USA* **94,** 7349-7355.