gradient and rhizoid elongation. Addition of 5 mM $LaCl_3$ dissipated the cytosolic pH gradient and stopped growth without significantly altering the average value of pH_c (Table 1). When all treatments were considered together, the magnitude of the cytosolic pH gradient correlated well with the rate of tip growth (Fig. 4).

Although we cannot be certain whether the cytosolic pH gradient is a cause or consequence of growth, the fact that protons serve as second messengers in all eukaryotic cells raises the possibility that the pH gradient regulates regional differentiation. Cytoplasmic proton activities are typically 40 to 60 nM in resting cells, similar to, or perhaps slightly lower than, the activity of cytosolic Ca^{2+}. Because of the low resting activity, a subtle change in H^+ metabolism or transport can alter pH_c and thereby trigger a cellular response. A number of morphogenetic processes, including egg activation, conidiation, germ tube formation, and cell differentiation are regulated in this fashion (*5*, *15*), and in most instances a moderate change in pH_c of a few tenths of a unit is sufficient for induction. These temporal pH_c triggers are similar in magnitude to the spatial pH_c gradient in *Pelvetia*, making it likely that regional rhizoid physiology is influenced by the gradient.

A number of cytoplasmic structures and processes are potential targets for differential regulation by the pH gradient; these include enzyme activity, membrane trafficking, exocytosis, and cytoskeletal stability (*16*). F-actin is localized in the cortical cytoplasm at the growing tip (*17*), the most acidic zone, whereas microtubules emanate from the perinuclear region (*18*), where pH_c is most alkaline. Microtubules radiate toward the apex, but are rare in the apical cortex. This general pattern of cytoarchitecture is common in tip-growing cells (*19*) and may be determined, at least in part, by the cytosolic pH gradient. Acidic conditions stabilize both plant and animal actin filaments (*20*), whereas microtubule assembly and stability are increased at alkaline pH (*21*).

Growing rhizoids also generate cytosolic and membrane-associated Ca^{2+} gradients that superimpose on the pH gradient (*2*, *14*). La^{3+} reduces cytosolic Ca^{2+} concentrations in the rhizoid (*14*) and eliminates the pH gradient, indicating that the pH gradient is dependent on Ca^{2+} homeostasis. Although the relation between the two gradients is unclear, it seems likely that they operate coordinately in regulating tip growth. The cytoskeleton is a plausible candidate for coordinate regulation because F-actin and microtubules are affected by both pH_c and cytosolic Ca^{2+} (*20*, *21*).

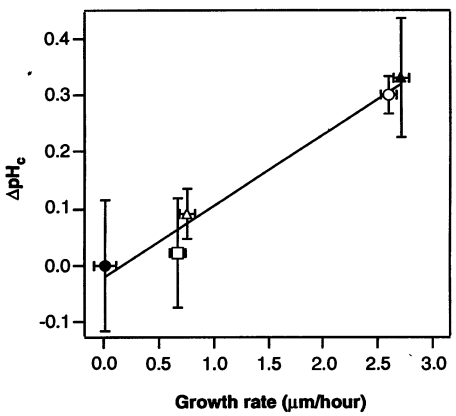

Fig. 4. Relation between the magnitude of the pH_c gradient (ΔpH_c) and rhizoid growth rate. We calculated ΔpH_c by subtracting the mean apical pH_c from the mean basal pH_c shown in Table 1. Correlation coefficient (r^2) = 0.97. ○, ASW pH 8.2; ▲, ASW pH 7.0; ●, ASW containing 5 mM $LaCl_3$; △, ASW containing 500 μM PA; and □, ASW containing 10 mM PA.

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Cell Fate Determination by the Cell Wall in Early *Fucus* Development

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In multicellular plants, development starts with an asymmetric division of the zygote into two differentiated cells. The nature and distribution of fate-determining factors operating during embryogenesis remain largely obscure. Laser microsurgery was used here to dissect two-celled embryos of the alga *Fucus spiralis*. Removal of protoplasts from the cell wall induced dedifferentiation. However, isolated cells within the walls followed their restricted fate. Moreover, contact of one cell type with the isolated cell wall of the other cell type caused its fate to be switched. The cell wall thus appears to maintain the differentiated state and to direct cell fate in plant development.

Early development of *Fucus spiralis* follows a similar pattern to zygotic embryogenesis in higher plants (*1*), the first asymmetric cell division giving rise to a rhizoid cell that develops into the basal root–like tissue (holdfast) and an apical thallus cell that produces the vegetative and reproductive shoot. Polarization processes taking place before the first cell division have been extensively studied (*2*). The use of enzymatically produced wall-free protoplasts has shown that the cell wall is required for fixation of the polar axis (*3*). It is assumed that bridges involving a vitronectin-like protein connect the cell wall to the plasma membrane (*4*). However, mechanisms in-

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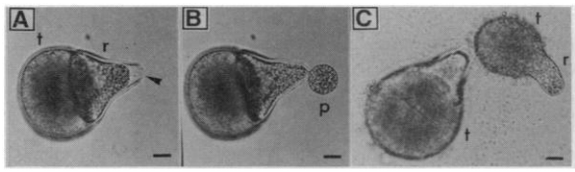

Fig. 1. Production and regeneration of a protoplast from a rhizoid cell of *F. spiralis*. (**A**) Osmotic shrinkage of the cytosol of a 24-hour zygote and laser ablation (arrowhead) of cell wall at the rhizoid tip (t, thallus cell; r, rhizoid cell). (**B**) Extrusion of a rhizoid protoplast (p). (**C**) The same protoplast after a further 24 hours, showing spontaneous polarization into rhizoid and thallus cells. Bars represent 15 μm. The gamete liberation from mature *F. spiralis* plants, fertilization, ultraviolet laser microsurgery, and protoplast extrusion were as described in (*16*). Embryos were cultured in sea water filtered with a 0.2-μm filter, containing streptomycin (20 μg cm^{-3}), under continuous white fluorescent light (50 μE m^{-2} s^{-1}) at 18°C.

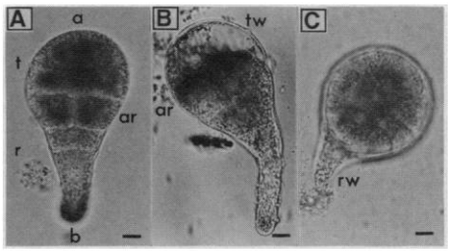

Fig. 2. Early division patterns in normal embryos and isolated, intact rhizoid and thallus cells. (**A**) Normal embryo after 60 hours (a, apical pole; b, basal pole; ar, apical rhizoid cells). (**B**) Division of an isolated rhizoid cell 60 hours after laser ablation of the thallus cell contents at the two-cell stage (tw, wall of former thallus cell). (**C**) Division of isolated, intact thallus cell, 72 hours after laser ablation of the rhizoid cell contents (rw, wall of former rhizoid cell). Bars represent 15 μm.

volved in the differentiation of apical and basal cells remain unknown. In *Arabidopsis thaliana*, numerous developmental mutants have been isolated, leading to an understanding of the genetic control of early embryogenesis (*1*). Unfortunately, access to the higher plant embryo remains difficult. The *Fucus* zygote develops autonomously in sea water and is readily amenable to laser microsurgical manipulations. This allows for the direct study of inductive processes and fate determination at the cellular level.

Localized ablation of the rhizoid cell wall allowed the extrusion of a spherical apolar protoplast (Fig. 1, A and B). Protoplasts regenerated a cell wall within an hour and then polarized and divided as normal zygotes within 24 hours (n = 79) (Fig. 1C), eventually forming complete miniature embryos (*5*). This totipotency of the rhizoid protoplast indicates that the differentiated state is not based exclusively on intracellular factors.

In contrast, rhizoid cells isolated in their original cell walls, after laser ablation of the thallus cell contents, followed their normal fate, elongating by tip growth and showing the division pattern characteristic of the rhizoid cell in the intact embryo (n = 24) (Figs. 2, A and B, and 4A). As with rhizoid cells in normal embryos, isolated, tip-growing rhizoid cells contained few pigments and chloroplasts. Similarly, isolated intact thallus cells always followed the initial thallus division pattern of the intact embryo (n = 109) (Figs. 2, A and C, and 4A). Thus, as well as being required for polar axis fixation (*3*), the cell wall is necessary for maintenance of the differentiated state of the embryonic cells.

Intact thallus cells, isolated from two-celled embryos, continued to divide normally at least until the eight-cell stage. Thereafter, however, the fate of individual cells at the former rhizoid end was strikingly altered. One or more of these cells invariably redifferentiated into rhizoid cells (n = 60) (Figs. 3, A and B, and 4C). Thallus-rhizoid redifferentiation only occurred at the former rhizoid end and was independent of the direction of incident light (*5*). Up to five thallus-derived rhizoids have been observed in one embryo; each rhizoid originated from a separate cell. Usually, however, only one or two cells redifferentiated. Redifferentiation was only observed where thallus cells extended into the former rhizoid wall space and made prolonged (more than 24 hours) contact with the former rhizoid cell wall. If close wall-to-wall contact was prevented, either by removing the former rhizoid wall (Fig. 4B) or by simply killing the rhizoid cell and leaving cytoplasmic debris in the rhizoid cell compartment (*5, 6*), isolated thallus cells always developed into rhizoid-free embryos (n = 36). Thallus-derived rhizoids did not form simply as a result of stresses imposed on the cells inside the tube formed by the former rhizoid wall, as in cases in which rhizoid development (but not cell division) was delayed by external osmotic treatment (*7*); rhizoids were occasionally observed to originate through the wall (*5*).

When the rhizoid wall was removed from one longitudinal half of the ablated rhizoid cell (Figs. 3, C and D, and 4D), thallus-derived rhizoids were produced only on the side with residual cell wall (n = 13). Thallus cells on the other half, without residual rhizoid wall, never changed fate. We conclude that factors associated with the inner rhizoid wall are involved in the induction of differentiation of thallus cells into rhizoid cells. This

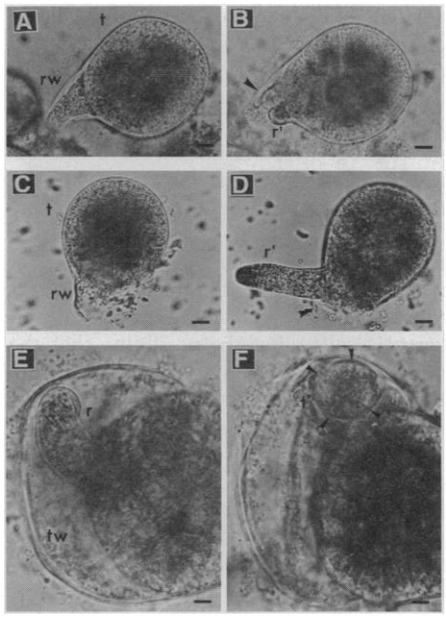

Fig. 3. Inductive effects of the cell wall. (**A** to **D**) Induction of rhizoids from thallus cells (t). (A) Ablation of rhizoid cell of a two-cell embryo, leaving the rhizoid wall (rw) intact. (B) The same embryo after a further 24 hours, showing the induction of two rhizoid cells (r') at the site of the former rhizoid wall (arrow). (C and D) Rhizoid induction from isolated thallus cell after the ablation of half of the former rhizoid wall (rw). Rhizoid induction occurs only on the side of the embryo with residual rhizoid wall (arrowhead) after 48 hours. (**E** and **F**) Rhizoid production by isolated rhizoid cells within the wall space of the former thallus cell. (E) Elapsed time of 48 hours after ablation of the thallus cell, showing initial contact of the rhizoid (r) with the former thallus wall (tw). (F) Induction of thallus-like development (t') of a rhizoid cell after 24 hours contact with the inner wall of the former thallus cell. Bars represent 15 μm in (A) to (D) and 7 μm in (E) and (F).

induction requires prolonged contact between thallus cells and the rhizoid wall.

During the development of an isolated rhizoid, a new rhizoid cell occasionally germinated within the cavity formed by the wall of the former thallus cell (n = 6) (Figs. 3, E and F, and 4, E and F). Such rhizoids had clear cytoplasm and grew by tip extension. Where such a rhizoid grew toward a hole previously cut in the former thallus wall, it continued to grow through the hole and remained unpigmented (Fig. 4F). In contrast, contact between rhizoids and the intact inner wall of the former thallus cell resulted in arrested growth followed by, after more than 24 hours, division along the longitudinal axis at the tip of the rhizoid (Figs. 3, E and F, and 4E) (n = 4) and increased pigmentation. It is unlikely that the thallus wall constituted a barrier to rhizoid growth, because growing rhizoids normally respond to mechanical barriers by altering their growth direction while maintaining their growth rate (*5*). We conclude that contact

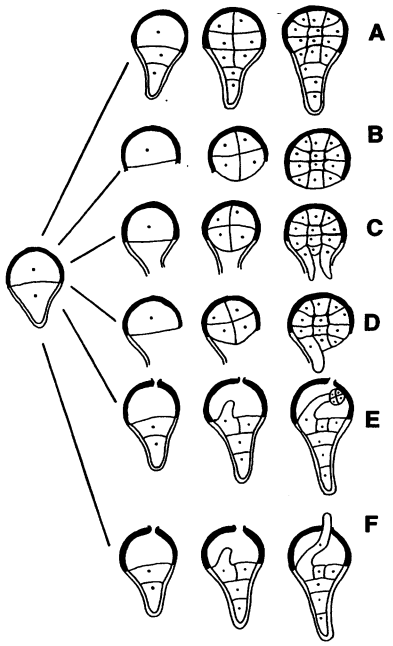

Fig. 4. Schematic representation of the development of (**A**) intact embryos and after laser ablation at (**B**) the two-cell stage of the rhizoid cell and wall, (**C**) rhizoid cell contents, (**D**) half of the rhizoid cell wall, and (**E** and **F**) thallus cell contents. (E and F) Also shown is the fate of a rhizoid (E) produced within the thallus cell compartment after prolonged contact with the thallus cell wall or (F) growing through a hole previously cut in the cell wall. Bold lines represent original thallus wall and double lines represent original rhizoid wall.

with the inner thallus cell wall can alter the fate of rhizoid cells.

Experiments have demonstrated the relative importance of position-dependent factors rather than cell lineage in the direction of plant embryogenesis (*8*, *9*), although inductive effects have not been demonstrated directly. Plant embryos are known to dedifferentiate and redifferentiate (*8–13*). Somatic embryos of *Daucus* respond to surgical tissue removal by reorganization of the cut region and tissue regeneration (*9*, *12*). In addition to the postulated roles of diffusible morphogens (*14*), indirect evidence has implicated cell surface– and wall-related molecules as fate determinants (*15*). We have demonstrated positionally regulated induction in a plant embryo and shown that fate-determining information is associated with the cell wall, most likely the inner face of the mature wall. If the original rhizoid-thallus dividing wall does not possess fate-determining properties, the mature rhizoid or thallus wall should have an overriding effect on the fate of recently divided cells bounded predominantly by primary, immature wall. The factors involved in these inductive processes must be long-lived and not readily diffusible because isolated cell wall retains its inductive properties for several days. The factors must act through the wall of the induced cell and must therefore be rendered diffusible by cell contact, possibly in response to secretions from the cell itself. The evidence presented here implicates the plant cell wall both in maintaining the differentiated state and in directing the pattern and cell fate in plant development.

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Recombination Between Viral RNA and Transgenic Plant Transcripts

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Transformed plants expressing the 3′ two-thirds of the cowpea chlorotic mottle virus (CCMV) capsid gene were inoculated with a CCMV deletion mutant lacking the 3′ one-third of the capsid gene. Although the deletion inoculum replicates in inoculated cells, systemic infections occur only if recombination restores a functional capsid gene. Four of 125 inoculated transgenic plants, representing three different transgenic lines, became systemically infected. Analysis of viral RNA confirmed that RNA recombination had united the transgenic messenger RNA and the challenging virus through aberrant homologous recombination.

The evolution of plus sense RNA viruses proceeds by natural mechanisms including errors by viral RNA polymerase, which lacks proofreading capabilities, and by homologous and heterologous RNA recombination (*1*). Recombination has generated mosaic-type defective interfering RNAs in cymbidium ringspot tombusvirus (*2*) and variants of tobacco rattle tobravirus (*3*). Recombination has been reported in the 3′ untranslated and intercistronic sequences of bromoviruses (*4–6*). The mechanism of plant viral RNA recombination has been addressed experimentally in both brome mosaic virus and turnip crinkle virus subviral RNAs (*7*, *8*).

There are indications that plant RNAs have recombined with replicating viruses. Several potato leafroll virus isolates contain sequences homologous to an exon of tobacco chloroplast RNA (*9*). Additionally, a deletion mutant of red clover necrotic mosaic virus was restored by recombination with transgenically expressed viral RNA (*10*). The rarity of reported recombination events between viral RNA and host mRNA may reflect their infrequency or the failure of products to be viable.

Virus resistance can be conferred on transgenic plants by expression of segments of viral genome, such as capsid genes (*11*). Transgenic plants expressing a viral capsid protein exhibit resistance to that virus and closely related strains (*12*) but remain susceptible to other viruses.

Plants frequently resist viral attack by restricting virus movement rather than inhibiting replication (*13*). Therefore, plants challenged by viruses that are not patho-

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