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Role of a vitronectin-like molecule in embryo adhesion of the brown alga *Fucus*

(extracellular matrix/cell wall/polarity/embryogenesis/cell attachment)

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ABSTRACT **The rhizoid cell of the two-celled embryo of the brown alga *Fucus* is structurally and functionally differentiated from the thallus cell. The rhizoid cell is highly polar and transports directionally components of the cell wall to its elongating tip, which attaches the developing embryo to the substratum. Polyclonal antibodies to human vitronectin (Vn) recognize a vitronectin-like glycoprotein (Vn-F) in extracts of zygotes and two-celled embryos of *Fucus*, with a molecular mass (≈62 kDa) similar to that of human Vn. The specificity of the immunological cross-reactivity of Vn-F to rabbit polyclonal antibodies made to human Vn is demonstrated by competition experiments using pure human Vn and monospecific antibodies generated toward Vn-F. Vn-F possesses affinities for glass and heparin that are identical to those of human Vn. Immunolocalization and subcellular fractionation results demonstrate that Vn-F is localized first in the cytoplasm of the zygote, which is followed by the polar transport of Vn-F to its exclusive localization in the cell wall of the elongating rhizoid tip. Vn does not localize to the rhizoid tip under culture conditions that prevent two-celled embryos from attaching. Furthermore, an adhesion assay demonstrates that two-celled *Fucus* embryos do not adhere to the substratum in the presence of the Vn antibody, suggesting that the Vn-F in this brown alga not only possesses structural similarity to mammalian Vn but may also have a similar functional role in adhesion. The presence of Vn-F in brown algae suggests a high degree of evolutionary conservation of its structural and functional characteristics.**

Zygotes of *Fucus* provide a model system for studying the basic mechanisms involved in the directional transport of specific cytoplasmic components to unique sites in the cell wall as well as for investigating the function of these localized components (1, 2). The polar growth of the zygote is expressed first as an outgrowth or rhizoid. This growth axis can be oriented by a number of vectors, unilateral light being the most extensively and easily utilized (3). After the first cell division, the tip of the elongating rhizoid serves as a root-like attachment filament for the developing embryo, anchoring it to the substratum. Cells derived from this and other filaments will later give rise to the "holdfast" portion of the mature plant (1).

We have previously identified a cell wall component localized only at the tip of the developing rhizoid, a highly sulfated fucan glycoprotein called fucoidan (4), which has properties similar to heparin sulfate (5). Fucoidan or F2 becomes sequestered in this highly localized region of the cell wall between 14 and 18 hr after fertilization (6–9). Prior to this localization, F2 is sulfated enzymatically and stored within Golgi-derived vesicles (4, 10) or F granules (11). After the polar axis has become fixed, F granules, which are uniformly distributed throughout the cytoplasm, are then transported directionally to the site of rhizoid growth (4). Localization of F2 into the cell wall of the rhizoid tip is dependent upon its sulfation, and a sulfated F2 is required for adhesion of the embryo to the substratum (12). Without attachment, subsequent morphogenesis of the free-floating embryo is aberrant.

We are interested in the further characterization of localized cell wall components (such as F2) that are required for adhesion and normal morphogenesis of the *Fucus* embryo. The similarities between F2 and heparin led us to determine whether molecules with functions similar to those found in the extracellular matrix (ECM) of animal cells are also found in *Fucus* embryos. Component proteins of the ECM [e.g., fibronectin and vitronectin (Vn)], which are linked structurally and functionally through the plasma membrane to the cytoskeleton via a family of transmembrane proteins—i.e., the integrins (13–15), function in several developmental processes, including cell attachment (15–17). For example, Vn has been directly implicated in cell attachment, spreading, and migration during embryogenesis (18–19). In cultured mammalian cells, Vn is an important constituent of focal adhesions or contact sites where cultured cells attach to a glass or plastic substrate (13). Vn binds to glass and shows a high affinity to the sulfated glycoproteins heparin, dextran sulfate, and F2 (5, 20). In addition to a heparin-binding region at its C-terminal end, human Vn contains a cell attachment region at its N-terminal end that contains the RGD tripeptide that interacts with the Vn receptor in the plasma membrane (15, 20). Vn is detected in a wide variety of mammalian cell types and species, ranging in molecular mass from 56 kDa to 80 kDa (21).

Most of the protein components found in cell walls are unique to plants (22–25), but recently, proteins have been observed in the cell walls and plasma membranes of plants that possess structural properties similar to certain mammalian glycoproteins of the ECM and their receptors—i.e., integrins. The presence of an integrin-like Vn receptor (26) and a Vn-like protein has been detected in higher plants (27, 28) as well as in the more phylogenetically primitive brown alga *Fucus* (29). However, the emphasis of these initial reports of ECM proteins in the cell wall of plants has been on the immunological cross-reactivity and not primarily on the subcellular localization or function of these proteins. In this manuscript, we present immunological and biochemical evidence for the presence of a Vn-like molecule in *Fucus* embryos (Vn-F). Vn-F is exclusively localized in the cell wall of the rhizoid tip, at the site of contact between the *Fucus* embryo and its substrate. Furthermore, we present functional evidence for Vn-F playing a role in adhesion of *Fucus* embryos to the substratum.

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Abbreviations: ECM, extracellular matrix; Vn, vitronectin; Vn-F, vitronectin-like molecule in *Fucus*; F2, fucoidan; BSA, bovine serum albumin; ASW, artificial seawater.

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MATERIAL AND METHODS

Plant Material. Tips of reproductive fronds (receptacles) of *Fucus distichus* (L). Powell were collected at South Beach, OR (South Jetty), shipped overnight on ice to Chapel Hill, NC, and stored in the dark at 4°C for up to 3 weeks. Fertilization of eggs and synchronous development of zygotes in artificial seawater (ASW) were performed as described (30). For some experiments, two-celled "met-embryos" were obtained by culturing zygotes in ASW lacking sulfate but containing 10 mM methionine (12). Zygotes were cultured at 14°C ± 1°C in constant illumination (cool white fluorescent lamps) at 50 μmol of photons per m^2/s. At appropriate times, populations of zygotes or embryos at the desired stage of development were harvested for analysis.

Immunoblot Detection. Protein was extracted from zygotes and embryos at various stages of development (2, 7, 18, 24, and 32 hr after fertilization) by the method of Kropf *et al.* (31). The extract was resuspended in sample buffer, clarified by centrifugation, and stored at −20°C. Total protein concentration was determined by the amido black procedure (32). Purified human Vn protein (Telios Pharmaceuticals, San Diego) was reconstituted at 1 $\mu g/\mu l$ in phosphate-buffered saline (PBS) and stored at −20°C.

Fucus protein extracts and purified human Vn were electrophoresed in a 12.5% polyacrylamide vertical gel and blotted onto Hybond N (Amersham). Blotted proteins were blocked overnight in 8% (wt/vol) non-fat powdered milk in PBS at 4°C and then incubated for 1–2 hr at room temperature with primary antibody (rabbit anti-human Vn; Telios Pharmaceuticals, San Diego) at a 1:500 dilution in PBS containing 0.1% Tween 20 (Sigma) and 0.2% bovine serum albumin (BSA) (Sigma) (PBSTB). After three washes in PBSTB, the immunoblots were incubated with secondary antibody (goat anti-rabbit IgG alkaline phosphatase conjugate; Sigma) for 1 hr at a dilution of 1:1000 with PBSTB. After three additional washes, the blots were incubated with color development solution (Promega, ProtoBlot System). Nonimmune rabbit serum (gift of Alan Jones, University of North Carolina) and the secondary antibody alone were used as controls for detecting nonspecific antibody binding.

Affinity purification of monospecific antibody to *Fucus* Vn (Vn-F) was accomplished using total *Fucus* protein extracts immobilized on membrane (Hybond N), incubating the membrane with anti-human Vn, and eluting the antibody bound to Vn-F by low pH, according to the procedure of Sambrook *et al.* (33). Competition experiments were conducted by incubation of the human Vn antibody with a 10 M excess of human Vn (or BSA as a control) for 15 min before use in the immunoblot detection assay.

Vn-F Protein Purification. Embryos were harvested 32–36 hr after fertilization and frozen at −80°C until 10 ml of packed cells was collected, at which time the embryos were resuspended in 5 ml of extraction buffer (31) and homogenized on ice for 1 hr. The extract was then centrifuged at low speed (100 × *g* for 5 min) to remove large cell debris, and the supernate was applied to a 30-ml column of glass beads (Sigma) [previously washed with acid and equilibrated with 0.6 M sodium bicarbonate (pH 8.0)]. The column containing *Fucus* proteins was first washed with 200 ml of 0.6 M sodium bicarbonate (pH 8) and then eluted (at a flow rate of 1 ml/min) with a series of buffers: 150 ml of 0.6 M sodium bicarbonate/0.2 M sodium carbonate, pH 9.5; 150 ml of 0.15 M potassium bicarbonate/0.05 M potassium carbonate, pH 9.5; and, finally, 150 ml of 0.6 M potassium bicarbonate/0.2 M potassium carbonate, pH 9.7. Fractions were collected, dialyzed against water, lyophilized, and then examined by immunoblot analysis for the presence of Vn-F (34).

The fraction containing the Vn-F was resuspended in 10 mM sodium phosphate buffer (pH 7.7) containing 5 mM EDTA, 8 M urea, and 0.15 M NaCl and applied to a heparin-Sepharose column (Sigma) as described by Yatohgo *et al.* (35). The column was washed with 150 ml of the same buffer at a flow rate of 0.5 ml/min and the Vn-F protein was eluted with the same buffer containing 0.5 M NaCl. Each fraction was treated as above to determine the location of the Vn-F.

Immunohistochemical Localization. *Fucus* zygotes, embryos, or met-embryos at various stages of development (2, 7, 18, 24, 32, and 36 hr after fertilization) were fixed in 1% paraformaldehyde in PBS for 1 hr. The tissue was then dehydrated and embedded in methacrylate (36), sectioned at 3 μm, and mounted onto subbed slides (37). The embedding medium was removed from the sections with a xylene and then an ethanol series.

For the immunofluorescent staining, the nonspecific protein binding sites of the section were first blocked in 3% BSA in PBS. The sections were incubated for 2 hr at room temperature with the same human Vn antibody, as previously controlled in the Western blots, at a 1:50 dilution in PBS with 1% BSA. After a single washing in the above buffer for 15 min, the slides were incubated with a fluorescent secondary antibody (anti-rabbit, Polysciences) for 1 hr at room temperature in the dark, which was followed by a single rinse in buffer, immersion of the sections in buffered glycerol (38), and application of coverslips. Sections were photographed on Tri-X film using a Nikon Optiphot epifluorescence microscope.

Subcellular Localization. Cells were collected 2–4 hr or 32–36 hr after fertilization and ruptured in a glass homogenizer in an isotonic sucrose/salt homogenization medium (0.25 M sucrose in ASW). All of the manipulations were done either on ice or at 4°C and were based on the procedures of Coughlan and Evans (39). The homogenate was first centrifuged at 100 × *g* for 1 min to remove unbroken cells. The supernate was recentrifuged at 1000 × *g* for 20 min, and the resulting pellet and supernate were collected. Microscopic observation revealed that the pellet contained predominantly cell walls (later referred to as the "wall-rich" fraction). The supernate was again recentrifuged at 15,000 × *g* for 25 min, and the pellet and supernate were collected and examined. This pellet was found to contain only cytoplasmic particles (the "organelle-rich" fraction); the supernate is referred to as the "cytosol-rich" fraction. The three different fractions were later subjected to protein extraction (31), quantification (32), and immunoblot procedures as detailed above.

Cell Binding Assay. Two-hour *Fucus* zygotes were added to polystyrene Petri dishes (Corning) or a glass coverslip mounted inside the Petri dishes at a concentration of $<6 \times 10^4$ cells per ml. At this concentration the *Fucus* cells settled and coated the bottom of the dishes as a monolayer, with a space of one to three cell diameters separating the cells. Various dilutions of serum or antibodies were added directly to the cells in ASW (nonimmune, 1:25; fibronectin antibody, 1:25; Vn antibody, 1:25, 1:50, or 1:100). After 24 hr of growth, the culture dishes were flooded with ASW and sealed shut with Parafilm. The plates were inverted and centrifuged at 1500 × *g* for 15 min, providing a force that pulled cells away from the substratum. Cells on the plate were photographed before and after centrifugation with a Nikon inverted epifluorescence microscope and Tri-X film. Data for statistical analysis were obtained by counting at least 30,000 cells from micrographs from >55 photographed areas. Mean, standard deviation, and Student's *t* test were used to evaluate the percentage of cells that remained bound.

RESULTS

Detection. Polyclonal antibodies to human Vn recognized a polypeptide of ≈62 kDa in extracts prepared from early

stages of *Fucus* embryo formation. The amount of Vn-F remained relatively constant through the first 32 hr of embryo formation (Fig. 1). The specificity of the immunological cross-reactivity of the human Vn antibodies was evaluated by using monospecific and nonimmune antibodies and competition by purified human Vn (Fig. 2). When monospecific Vn-F rabbit antibody was prepared, it recognized the 65- and 75-kDa polypeptides in authentic human Vn protein (Fig. 2, h) and only the 62-kDa protein from *Fucus* extracts (Fig. 2, f). Nonimmune serum and the secondary antibody alone (Fig. 2, f*) failed to recognize Vn or Vn-F. Furthermore, detection of Vn-F was eliminated when the antibodies were preincubated in excess human Vn (Fig. 2, f^+).

Protein Purification. Vn-F displayed affinities to glass and heparin similar to those of authentic human Vn. Enriched preparations of Vn-F were obtained after a *Fucus* zygote extract was eluted from a glass bead column with a solution of 0.6 M potassium bicarbonate/0.2 M potassium carbonate. The predominant protein had a molecular mass of ≈62 kDa (Fig. 3*A*, gl) and was recognized by the rabbit antibody to Vn (Fig. 3*B*, gl). When this Vn-F-enriched fraction from the glass column was applied to a heparin-Sepharose column and then eluted with a salt/urea buffer, the same 62-kDa protein was detected by protein staining (Fig. 3*A*, he) and immunoblot (Fig. 3*B*, he).

Localization. Subcellular fractionation indicated that Vn-F was found predominantly in an "organellar" fraction of 2- to 4-hr zygotes, with little or none in the "wall" fraction (Fig. 4*A*). In two-celled embryos, Vn-F was detected in organellar and wall fractions (Fig. 4*B*). Vn-F was not detected in the cytosolic fraction at any later stage in development (data not shown).

Indirect immunofluorescent studies localized the Vn-F in the cytoplasm, near the nucleus, during early *Fucus* zygote development (Fig. 5 *a* and *b*). Later, in two-celled (Fig. 5*c*) and multicellular zygotes (Fig. 5*d*), Vn-F was observed only in the ECM at the tip of the rhizoid cell, the point where *Fucus* embryos attach to the substratum. Little, if any, localization of Vn-F was detected in the crosswalls of multicellular embryos (Fig. 5*d*). Treatment of sections with nonimmune rabbit serum or secondary antibody only (Fig. 5*e*) did not reveal any localized fluorescence in the *Fucus* cells. Two-celled met-embryos, which do not adhere to the substratum (12), did not exhibit the localized distribution of the Vn-F protein in the rhizoid ECM, although considerable cytoplasmic staining was observed (Fig. 5*f*).

Function in Attachment. Exposure of *Fucus* zygotes to the Vn antibodies during development reduced drastically the ability of embryos to attach to the substratum, as determined by a centrifugal-based adhesion assay (Fig. 6). The ability of the embryos to bind to the substratum was inversely proportional to the concentration of anti-human Vn serum in the medium. Statistical differences were observed between the three tested antibody concentrations ($P < 0.0004$). No statistical differences in binding were observed between untreated embryos and embryos treated with nonimmune serum or rabbit antibodies to human fibronectin ($P > 0.50$).

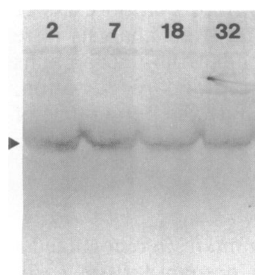

FIG. 1. Reactivity of human Vn antiserum with *Fucus* extracts: immunoblot detection of a 62-kDa vitronectin-like molecule (arrowhead) in protein extracts of *Fucus* zygotes and embryos (prepared at 2, 7, 18, and 32 hr after fertilization). Polyclonal antibodies against human Vn were used as described in the text.

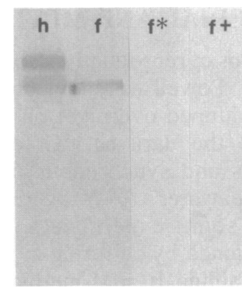

FIG. 2. Demonstration of the specificity of the immuno-cross-reactivity of Vn-F in *Fucus* extracts: immunoblot detection of human Vn (h) and *Fucus* extracts (f) by monospecific antibodies, purified from the 62-kDa protein of *Fucus* embryos. An immunoblot of *Fucus* extracts incubated with a rabbit nonimmune serum (f*), primary antibody omission (results not shown), and human Vn antiserum preincubated with purified human Vn (f^+) revealed no reaction.

DISCUSSION

Vn-F, which is immunologically related to human Vn, was detected in zygotes and embryos of *Fucus*. The specificity of the immuno-cross-reactivity of Vn-F to rabbit polyclonal antibodies made to human Vn was demonstrated by competition experiments using pure human Vn, monospecific antibodies generated toward Vn-F, and controls of primary antibody omission and nonimmune serum. Information obtained from cDNA analysis of the human Vn gene indicates a protein of 52 kDa (40, 41), with a carbohydrate component accounting for the observed mass of the 75-kDa Vn glycoprotein. Furthermore, a cDNA has recently been characterized from the mouse with properties similar to those of the human gene (42). Proteolytic cleavage is likely responsible for the additional 65-kDa component found on PAGE analysis of human Vn (20). Only one major protein species is found in *Fucus*, with a mass of 62 kDa. This includes an uncharacterized carbohydrate component (data not shown), which might account for the differences in mass between Vn-F and Vn from other mammals [i.e., 56–80 kDa (21)] and the Vn-like protein from higher plants [i.e., 55 kDa (27)]. The monospecific antibody to Vn-F recognized the 65- and 75-kDa species of human Vn.

Not only did Vn-F have immunological similarities to Vn but it also possessed similar biochemical properties. When extracts of *Fucus* embryos were subjected to the same affinity column procedures as used for mammalian Vn, a protein was purified with the same molecular mass and immunological properties as those of Vn-F. The affinity properties of Vn-F for glass and heparin and its elution profile from these columns were identical to those of human Vn. The demonstration by Tomasini and Mosher (5, 20) that human Vn binds to heparin and the heparin-like F2 molecule from brown algae and our results that Vn-F binds to heparin present a strong argument for the existence of heparin/F2 binding sites in Vn-F, which may be the protein epitope recognized by the antibody to Vn (43).

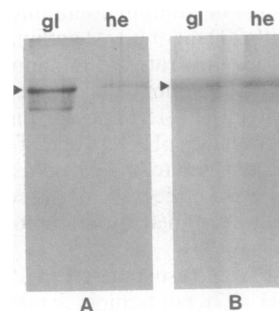

FIG. 3. Affinity of Vn-F to glass and heparin. (*A*) Gel stained with Coomassie brilliant blue to illustrate the affinity of the 62-kDa protein (arrowhead) to a glass bead (gl) column and a heparin (he) column. (*B*) Duplicate gel blotted and immunostained with human Vn antiserum for verification that the 62-kDa protein was Vn-F (arrowhead).

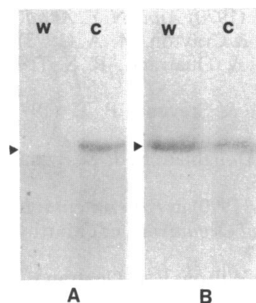

FIG. 4. Subcellular fractionation of Vn-F from 2- to 4-hr zygotes (*A*) and 32- to 36-hr embryos (*B*). Equal amounts of protein from the wall-rich (w) and cytoplasm-rich (c) fractions were subjected to immunoblot analysis to detect the presence and relative abundance of the 62-kDa Vn-F (arrowheads).

Further evidence that supports such an association between Vn-F and F2 comes from subcellular fractionation and immunolocalization results presented in this paper. Vn-F is first detected in young zygotes and throughout early embryo development by immunoblot techniques. Subcellular fractionation and immunolocalization demonstrated that Vn-F is localized at first intracellularly, in particles surrounding the nucleus of young zygotes that do not possess a rhizoid. When a rhizoid forms, Vn-F is localized predominantly in the cell wall, only at the tip of the elongating rhizoid. This pattern of distribution of Vn-F is identical to that of F2, which we have previously localized in the cell wall of the elongating rhizoid between 14 and 18 hr after fertilization. Although F2 was located in the cell wall when rhizoids were formed, it was earlier stored, sulfated, and selectively transported within F granules surrounding the nucleus of young zygotes (4, 10).

The pattern of intracellular and cell wall colocalization of F2 and Vn-F during early *Fucus* embryo development and their directed transport to the cell wall site of attachment for the embryo to the substratum prompted us to determine if Vn-F played a role in adhesion, similar to its function in mammalian cells. The results of Crayton *et al.* (12) suggested a role for F2 in the attachment of the two-celled *Fucus* embryo to the substratum. By using embryos grown to the two-celled stage in the absence of extracellular SO_4^{-2} (met-embryos), they found that localization of F2 in the cell wall of the rhizoid tip was dependent upon F2 sulfation and, furthermore, that a sulfated F2 was required for adhesion of the embryo to the substratum (12). Although cell divisions continued, subsequent morphogenesis of the met-embryo did not produce an elongated rhizoid and typical embryo form but, rather, a free-floating globular embryo resembling a blastula (unpublished observations). Hence, attachment of the embryo was essential for further normal morphogenesis. Similarly, Vn-F, like F2, was not localized in the cell wall of the elongating rhizoids from met-embryos. These met-embryos did not adhere to the substratum, as reported earlier (12). By preventing sulfation, perhaps the association of F2 with Vn-F was prevented and the colocalization was not observed. Regardless of the mechanism, attachment of two-celled *Fucus* embryos is prevented in met-embryos when F2 and Vn-F are absent from the ECM at the rhizoid tip.

Further evidence for the role of Vn-F in adhesion was obtained from the attachment assay developed in this study. The same polyclonal antibodies made to human Vn that detected Vn-F in embryos also disrupted attachment of the normal two-celled embryo to a glass or plastic substrate in a concentration-dependent manner. No interference of attachment was observed using nonimmune serum or polyclonal antibodies made to human fibronectin. These results cannot yet distinguish the site on Vn-F at which the Vn antibody has the observed effect on adhesion or whether the site of Vn antibody binding is a component of the attachment complex. The Vn antibody could directly interfere with the true attachment complex of the embryo through its specific binding to exposed Vn-F in the cell wall. The attachment complex may also include F2, and the antibody could interfere with the Vn-F/F2 linkage. This appears a likely possibility not only because of the strong affinity of Vn to F2 (5, 20) but also because most antibodies raised to Vn recognize the heparin binding domain (43). Alternatively, the interference of attachment by Vn antibodies could be due to steric hindrance

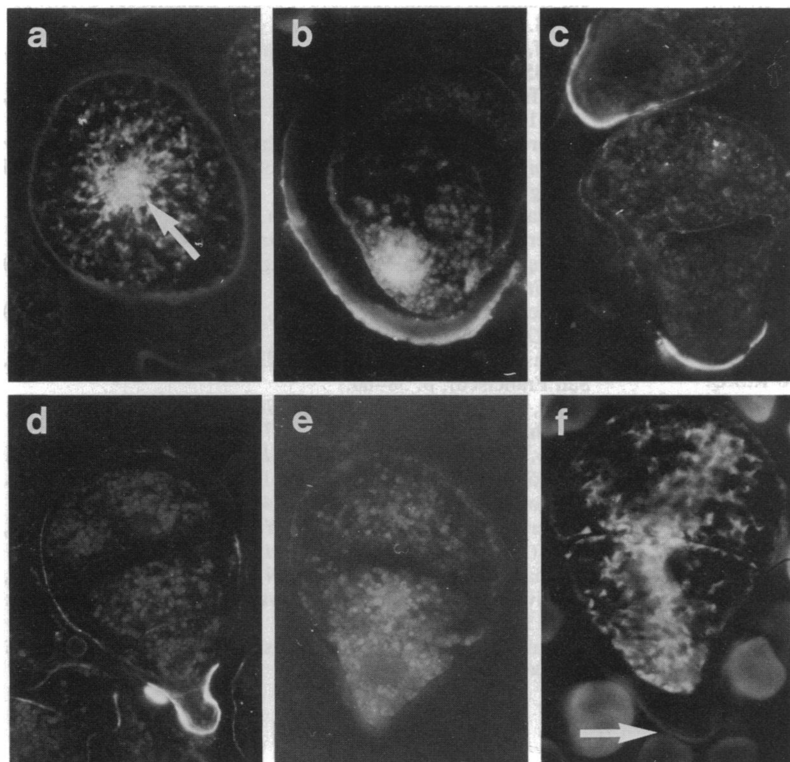

FIG. 5. Indirect immunofluorescent localization of Vn-F in *Fucus* zygotes and embryos. Vn (arrow) is localized in the cytoplasm near the nucleus in the one-celled zygote (*a*, 2 hr after fertilization; *b*, 1 day after fertilization; ×560). Later it is localized in the cell wall of the rhizoid tips of the two-celled (*c*, 2 days after fertilization; ×420) and multicelled embryos (*d*, >2 days after fertilization; ×340). The controls of rabbit nonimmune serum (*e*) and primary antibody omission (results not shown) showed no localization. Met-embryos (*f*) grown in the absence of SO_4^{-2} revealed a lack of Vn localization in the cell wall (arrow) and did not adhere to the substratum. (*e* and *f*, ×560.)

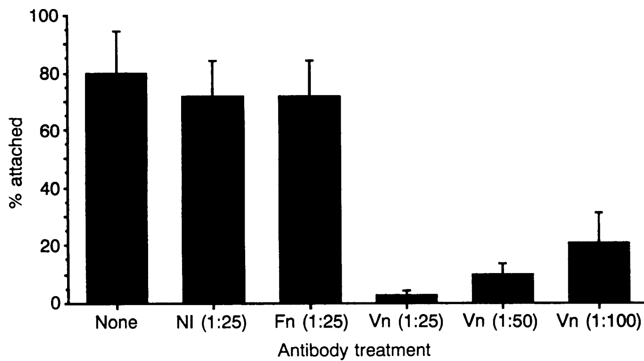

FIG. 6. Effect of Vn antibody on adhesion of intact *Fucus* embryos. Results are reported as a percentage of the embryos remaining attached after 24 hr of growth. Untreated embryos (None) and embryos treated with a 1:25 dilution of rabbit nonimmune (NI) or anti-human fibronectin (Fn) serum showed maximum adhesion in a centrifugal-based adhesion assay. Various dilutions of the human Vn antiserum (1:25, 1:50, or 1:100) were shown to quantitatively reduce the ability of the *Fucus* embryos to attach.

of the true attachment complex with the substratum by the Vn antibodies binding to Vn-F or the Vn-F/F2 complex.

The molecules responsible for the adhesion of organisms to various structures have not been characterized nor has the mechanism of attachment been elucidated. For example, many algae and fungi must adhere to a living or nonliving substratum via spores or other attachment structures to complete their life cycles. These structures provide the mechanisms by which some of these organisms inflict costly detrimental effects, such as biofouling and crop diseases. Since our data suggest evolutionary conservation of structure and function of Vn between brown algae and mammals, determination of the presence of Vn-like molecules in attachment structures of other algae and fungi may lead to the identification of molecules contributing to their adhesion and new approaches for their control.

In conclusion, we present evidence for a Vn-like protein in a brown alga and demonstrate that it functions in a manner similar to mammalian Vn by being associated with attachment of the *Fucus* embryo to the substratum. The presence of integrin-like proteins in *Fucus* embryos (29) may also lead to a possible role of Vn-F in the polar axis stabilizing complex proposed by Quatrano (2).

Note Added in Proof: A 70-kDa vitronectin-like protein was dectected and isolated from the acellular slime mold *Physarum*. This protein exhbitied an (Arg-Gly-Asp)-mediated cell-spreading activity but an NH_2-terminal sequence different than mammalian Vn (44).

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1. Quatrano, R. S., Griffing, L. R., Huber-Walchli, V. & Doubet, S. (1985) *J. Cell Sci.* **2**, Suppl., 129–141.
2. Quatrano, R. S. (1990) in *Genetics of Pattern Formation and Growth Control*, ed. Mahowald, A. (Wiley, New York), pp. 31–46.
3. Jaffe, L. F. (1968) *Adv. Morphog.* **7**, 295–328.
4. Brawley, S. H. & Quatrano, R. S. (1979) *Dev. Biol.* **73**, 193–205.
5. Tomasini, B. R. & Mosher, D. F. (1988) *Blood* **72**, 903–912.
6. McCully, M. E. (1970) *Ann. N.Y. Acad. Sci.* **175**, 702–711.
7. Quatrano, R. S. & Crayton, M. A. (1973) *Dev. Biol.* **30**, 29–41.
8. Hogsett, W. E. & Quatrano, R. S. (1978) *J. Cell Biol.* **78**, 866–873.
9. Quatrano, R. S. & Stevens, P. T. (1976) *Plant Physiol.* **58**, 224–231.
10. Callow, M. E., Doughlan, S. J. & Evans, L. V. (1978) *J. Cell Sci.* **32**, 337–356.
11. Quatrano, R. S. (1990) in *Experimental Embryology in Aquatic Plant and Animal Organisms*, ed. Marthy, H.-J. (Plenum, New York), pp. 41–57.
12. Crayton, M. A., Wilson, E. & Quatrano, R. S. (1974) *Dev. Biol.* **39**, 164–167.
13. Burridge, K., Fath, K., Kelly, T., Nuckolls, G. & Turner, C. (1988) *Annu. Rev. Cell Biol.* **4**, 487–525.
14. Marcantonio, E. E. & Hynes, R. D. (1988) *J. Cell Biol.* **106**, 1765–1772.
15. Preissner, K. T. (1991) *Annu. Rev. Cell Biol.* **7**, 275–310.
16. Hynes, R. O. (1981) in *Cell Biology of Extracellular Matrix.*, ed. Hay, E. D. (Plenum, New York), pp. 295–334.
17. Edelman, G. (1986) *Annu. Rev. Cell Biol.* **2**, 81–116.
18. Hayman, E. G., Pierschbacher, M. D., Suzuki, S. & Ruoslahti, E. (1985) *Exp. Cell Res.* **160**, 245–258.
19. Underwood, P. A. & Bennett, F. A. (1989) *J. Cell Sci.* **93**, 641–649.
20. Tomasini, B. R. & Mosher, D. F. (1990) *Prog. Hemostasis. Thromb.* **10**, 269–305.
21. Kitagaki-Ogawa, H., Yatohgo, T., Izumi, M., Hayashi, M., Kashiwagi, H., Matsumoto, I. & Seno, N. (1990) *Biochim. Biophys. Acta* **1033**, 49–56.
22. Cassab, G. I. & Varner, J. E. (1988) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 321–353.
23. Varner, J. E. & Hood, E. E. (1988) in *Self-Assembling Architecture*, ed. Varner, J. E. (Liss, New York), pp. 97–103.
24. Roberts, K. (1989) *Curr. Opinion Cell Biol.* **2**, 920–928.
25. Knox, P. (1990) *J. Cell Sci.* **96**, 557–561.
26. Schindler, M., Meiners, S. & Cheresh, D. A. (1989) *J. Cell Biol.* **108**, 1955–1965.
27. Sanders, L. C., Wang, C.-S., Walling, L. L. & Lord, E. M. (1991) *Plant Cell* **3**, 629–635.
28. Zhu, J.-K., Shi, I., Singh, U. & Carpita, N. C. (1991) *Plant Physiol.* **96** Suppl., 10 (abstr).
29. Quatrano, R. S., Brian, L., Aldridge, J. & Schultz, T. (1991) *Development (Suppl.)* **1**, 11–16.
30. Quatrano, R. S. (1980) in *Handbook of Phycological Methods*, ed. Gantt, E. (Cambridge Univ. Press, Cambridge, U.K.), pp. 59–68.
31. Kropf, D. L., Hopkins, R. & Quatrano, R. S. (1989) *Dev. Biol.* **134**, 452–461.
32. Schaffner, W. & Weissmann, C. (1973) *Anal. Biochem.* **56**, 502–514.
33. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.).
34. Ruoslahti, E., Suzuki, S., Hayman, E. G., Ill, C. R. & Pierschbacher, M. D. (1986) *Methods Enzymol.* **144**, 430–437.
35. Yatohgo, T., Izumi, M., Kashiwagi, H. & Hayashi, M. (1988) *Cell Struct. Funct.* **13**, 281–292.
36. Jamrich, M., Mahon, K., Garvis, E. R. & Gall, J. G. (1984) *EMBO J.* **3**, 1939–1943.
37. Humason, G. L. (1979) in *Animal Tissue Techniques* (Freeman, San Francisco), p. 58–60.
38. Giloh, H. & Sedat, J. W. (1982) *Science* **217**, 1252–1255.
39. Coughlan, S. & Evans, L. V. (1978) *J. Exp. Bot.* **29**, 55–68.
40. Jenne, D. & Stanley, K. K. (1987) *Biochemistry* **26**, 6735–6742.
41. Suzuki, S., Oldberg, A., Hayman, E. G., Pierschbacher, M. D. & Ruoslahti, E. (1985) *EMBO J.* **4**, 2519–2524.
42. Sieffert, D., Keeton, M., Eguchi, Y., Sawdey, M. & Loskutoff, D. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9402–9406.
43. Akama, T., Yamada, M., Seno, N., Matsumoto, I., Kono, I., Kashiwagi, H., Funaki, T. & Hayashi, M. (1986) *J. Biochem.* **100**, 1343–1351.
44. Miyazaki, K., Hamano, T. & Hayashi, M. (1992) *Exp. Cell Res.* **199**, 106–110.