

AN ABSTRACT OF THE THESIS OF

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Title: LOCALIZATION OF SULFATED POLYSACCHARIDES
IN FUCUS EMBRYOS

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Previous autoradiographic and cytochemical data using Fucus distichus subsp. endentatus (Dela Pylaie) Powell zygotes indicated that an undefined fucan polysaccharide was sulfated at the time of rhizoid formation and localized in only the rhizoid area. However, this research did not (1) clarify whether sulfation of the polymer was initially random and then localized or if the sulfating system was sequestered in the presumptive rhizoid area; or (2) identify and isolate the polymer which became sulfated.

To clarify the first point, 18 and 24 hour zygotes grown on microscope slides in the presence or absence of Na_2SO_4 were pulsed five minutes with $\text{Na}_2^{35}\text{SO}_4$ and chased in Na_2SO_4 for zero, 30, and 60 minutes. The zygotes were then fixed and whole mounts subjected to autoradiography. A comparison of autoradiographic grain counts of controls to experimentals indicated that the radioactivity was selectively incorporated as a sulfate ester into the fucan polysaccharides. Bacterial contamination was not evident on these slides.

Autoradiographic grain counts for the 18 hour zygotes indicated that the sulfated polymer was first randomly distributed into the cell wall after a five minute pulse but was then localized into the rhizoid wall 30 minutes later. A significant but much smaller amount of sulfated polymer was incorporated into the wall of the thallus cell at this stage. At 24 hours, embryos were observed to have a concentration of the newly sulfated polymers in the rhizoid area after a five minute pulse. The concentration of newly sulfated polymers increased with increasing chase time. No increase in grains was observed in the thallus cell in 24 hour embryos. To determine if the sulfate was required for the localization of the sulfating sites, zygotes were grown in the absence of Na_2SO_4 . Grain count patterns comparable to zygotes grown in the presence of sulfate indicated that sulfate was not required for the localization of the sulfation sites. At the time of rhizoid formation, sulfation of the fucan was initially randomly distributed in the cytoplasm followed by transport and secretion of the sulfated fucan into the rhizoid wall. In more developmentally advanced embryos sites of sulfation also were sequestered into the rhizoid area and this localization was not dependent upon sulfate in the ASW growth medium. These results are consistent with the hypothesis that the Golgi apparatus are the sites of storage and transport of the sulfated and unsulfated fucan as well as the location of the sulfating enzymes. The mechanism by which these vesicles are

sequestered is not known.

Previous attempts to isolate the polymer which becomes sulfated and localized in the rhizoid wall have been unsuccessful. However, two fractions, an acid soluble and a calcium chloride soluble, were demonstrated to contain the major part of the fucan that was sulfated during rhizoid formation. To improve resolution of the fractions, an electrophoretic separation technique was developed utilizing cellulose acetate gels in a pH 2 buffer containing lithium chloride. Electrophoresis of the acid and calcium chloride soluble fractions in this system showed two major bands (I and II) comparable to those appearing in previous separations by diethylaminoethyl cellulose column chromatography and paper electrophoresis. However, three previously unreported minor bands in band II were now discernible. Zygotes pulsed with $\text{Na}_2^{35}\text{SO}_4$ between 16 and 18 hours, followed by extraction and separation of the fucans on cellulose acetate gels, resulted in over 90% of the radioactivity associating with band II. This polymer coincides electrophoretically with the fucan which can be isolated from the cell wall at 12 hours and could be analogous to a unique fucan localized only at the rhizoid tip. Further chemical characterization of this polymer can now be initiated.

Localization of Sulfated Polysaccharides
in Fucus Embryos

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LOCALIZATION OF SULFATED POLYSACCHARIDES IN FUCUS EMBRYOS

INTRODUCTION

Overview of Problem and Rationale of System

Aristotle contended that the "male principle" i. e. , the seminal fluid, was the molding force of the development of all living things. The seminal fluid artistically shaped the matter provided by the female into the embryo (Generation of Animals, 729a). Ever since then philosophers have sought to find the underlying principle or principles, which shaped the zygote into its complex adult form.

Since the discovery of Mendelism at the beginning of the 20th Century, it has become apparent that most if not all developmental processes are under genetic control. Evidence has been obtained that nucleo-cytoplasmic interactions exist and have a definite effect on the differentiation of cells and tissues within the developing embryo. Conklin in 1905 observed that the egg of the ascidian Styela contained at least five different areas of pigmentation. The morphological fate of a cell line depended upon which of the pigmented areas the stem cell had received upon cleavage of the zygote.

The clearest examples of segregation by cytoplasmic determinants have been shown in the formation of germ cells in Ascaris (Boveri, 1887), some insects (Mahowald, 1971) and amphibians

(Blackler, 1958). These cases show that RNA containing cytoplasmic granules are segregated into a small number of cells which later develop into the primordial germ cells. If these regions containing the granules are destroyed, removed, or displaced by centrifugation, many workers (Geyer-Duszynska, 1959; Buehr and Blackler, 1970) have shown that the development of the primordial germ cells is arrested. Smith (1968) showed that the damage caused by ultraviolet irradiation to a localized cytoplasmic area of Rana pipiens egg prevented germ cell production. However, the damage could be reversed by injection of non-irradiated egg cytoplasm from the "germ plasm" into the irradiated cell. This damage could not be reversed after the eight cell stage of cleavage.

Another very lucid example of cytoplasmic determinants is found in Drosophila melanogaster. In early embryonic development, before blastoderm formation, the posterior polar plasm of the egg becomes incorporated into several cells known as pole cells, some of which become primordial germ cells. Illmensee and Mahowald (1974) have shown the presence of germ cell determinants by transferring this type of cytoplasm to a new region of embryo. The posterior polar plasm was able to induce "pole cell" formation, and in four cases, these were functional as primordial germ cells. Their work showed that the property of the posterior polar plasm concerned with germ cell formation could be transplanted to presumptive

somatic regions of the egg and still retain its ability to produce germ cells. Because the polar grains are localized exclusively in the posterior polar plasm of the egg, they can be reasonably considered as involved in the processes of germ cell determination as postulated by Mahowald (1968).

An ideal system to study the mechanism of localization should be one which: 1) shows a localization or polarity developing from a previous apolar stage; 2) expresses the polarity synchronously in a single cell population cultured in a defined medium; 3) produces numerous gametes/embryos per individual organism. One organism which possesses all of these qualities is the brown alga (Phaeophyta) Fucus distichus subsp. endentatus (Dela Pylaie) Powell. From a seemingly apolar egg, a zygote is formed which later forms a localized protuberance, the rhizoid. The rhizoid and the particles accumulated at that site are sequestered into the rhizoid cell after the first cell plate is formed in the embryo. Fucus thus shows cell differentiation at the two-celled stage of embryogenesis. Also, Fucus can be forced to shed innumerable zygotes in an artificial sea water (ASW) medium free of the maternal plant and can be obtained in intertidal waters in Oregon almost all year.

Purpose of this Study

Because Fucus possesses all of these qualities, it was chosen as a system for further concentration on the important developmental

problem of cytoplasmic localization. Fucus starts as an apparent apolar zygote but quickly develops particular localizations in the rhizoid. More specifically, it has been shown that at the time of the appearance of the rhizoid there is localization of organelles (Quatrano, 1972), sulfated polysaccharides (McCully, 1969, 1970), and RNA (Nakazawa and Takamura, 1966). In this study I have chosen the sulfated polysaccharides as models to study how polymers become localized in the rhizoid region.

The cytochemical data from earlier Fucus research show that a sulfated fucan can be detected about the time of rhizoid formation (Quatrano and Crayton, 1973) and is localized in the rhizoid region (McCully, 1969, 1970). However, the fucan polymer appears to be synthesized in the egg or early zygote and sulfated after synthesis (Quatrano and Crayton, 1973). What is the mechanism of localization in the embryo? There are two possibilities: the unsulfated fucan and/or sulfating enzymes pre-exist in the rhizoid region so that sulfation occurs locally; or the unsulfated fucans and sulfating enzymes are distributed throughout the cytoplasm, the sulfation process is generally distributed evenly and the sulfated fucans are then transported by some unknown mechanism to the rhizoid site.

In this study, I will attempt to answer the questions: Is sulfation random or localized? Is sulfation necessary for localization? and What is the nature of the fucan that is sulfated?

MATERIALS AND METHODS

Receptacles of Fucus distichus subsp. edentatus (Dela Pylaie) Powell were used throughout the research and were collected at Yaquina Head, Newport, Oregon. Zygotes from these receptacles were released by cold tap water washes, grown at 15 C in diffuse light and incubated in an artificial sea water (ASW) medium containing 40 µg/ml chloramphenicol (Quatrano, 1974).

Synchronously developing zygotes for autoradiography were grown on microscope slides in Petri plates containing a final volume of 10 mls ASW containing 0.1 mM Na_2SO_4 which was found to be optimal for uptake and incorporation of sulfate into polysaccharides (Hogsett and Quatrano, 1975). To achieve normal development in cultures grown in the absence of sulfate, 10 mM methionine was substituted for Na_2SO_4 (Crayton and Quatrano, 1974). The zygotes were labelled with 20 µCi/ml of $\text{Na}_2^{35}\text{SO}_4$ (specific activity 825 mCi/mM sulfur, New England Nuclear) at a concentration of 0.1 mM for 5 to 30 min. Following the labelling period, the zygotes were washed for varying chase times of 0, 30, 60, 360, and 720 min, with ASW containing 0.1 mM Na_2SO_4 . The zygotes were then fixed with formalin:ASW (1:3.7 w/v), rinsed several times with tap water, and finally air dried. The dried slides were stored in a desiccator. The slides were later dipped in Kodak NTB nuclear track emulsion

diluted 3:1 with deionized water, allowed to dry for 60 min and then sealed in light-tight boxes containing Drierite. The boxes were stored in a cold room at 4 C for up to four weeks. Development of the emulsion was performed using the following sequence: 3 min Kodak DK-19; 5 min Kodak rapid fix; 5 min distilled water wash. Scoring of the slides was done using a Nikon compound binocular microscope having a magnification of 320X. Before scoring the slides, the identity codes were masked to eliminate biased counting. One hundred zygotes per slide were counted and assigned to the following classes based on the number of grains present:

<u>Grain Count Class</u>	<u>Actual Number of Grains above Background</u>
0	-
1	1 - 10
2	11 - 35
3	36-100
4	100-200
5	greater than 200

The total area of the zygote was divided by means of an ocular micrometer into two equal portions; the one representing the thallus, and the other representing the rhizoid region. The rhizoid area was further divided into three equal regions, representing each of the two sides and of the tip. The estimate number of grain counts

assigned to each of the four regions per hundred zygotes was averaged and a mean and a standard deviation (S. D.) calculated. In no case was the S. D. greater than 0.7; usually it was between 0.25 and 0.50.

Extraction of the sulfated polysaccharide for electrophoresis was carried out according to the procedure of Hogsett and Quatrano (1975) with some modifications. After homogenization in 80% ethanol zygotes were extracted with 0.2 N HCl (pH 2) for 60 min. at 55 C and 3% (w/v) aqueous Na_2CO_3 for 60 min at 55 C, followed with 10% KOH at room temperature for 60 min. The combined extracts were dialyzed overnight against deionized water and concentrated under vacuum. The material was precipitated with cold ethanol and then centrifuged at 20,000 g in a Sorvall RC2-B. The pellet was redissolved with deionized water.

Electrophoresis was carried out using 250 μ cellulose acetate gel strips (Cellogel, Kalex Scientific) at room temperature in a buffer of 50 mM LiCl and 10 mM HCl (pH 2). Samples (10 μ l/strip) were run for 45 min at 3 mA per strip using an E-C Apparatus Corp. voltage regulator. The electrophoretograms were stained with 0.1% (w/v) toluidine blue O (TBO) in 25 mM HCl for 3 min and destained in 7% acetic acid. For scanning, pictures of the gels were taken and the negatives scanned with a Schoeffel Microdensitometer. For determinations of radioactivity, the unstained strips were cut into 5 mm lengths, placed in 5 mls of Omnifluor (New England Nuclear)

and counted in a Packard Tri-Carb liquid scintillation spectrometer (Model 2425) which operated at 87% efficiency for ^{35}S .

RESULTS AND DISCUSSION

Autoradiography

Previous autoradiographic and cytochemical evidence indicated that sulfation occurs during rhizoid formation, and the sulfated polymers become sequestered in the rhizoid cell, specifically, in the cell wall (McCully, 1970). However, because of the long pulses of $^{35}\text{SO}_4$ used by McCully, it could not be determined if sulfation was initially random and then localized or if the sulfating system was unevenly distributed initially, *i. e.*, polar. These data attempt to clarify that point.

Eighteen hour and 24 hour Fucus distichus embryos grown in the presence or absence of sulfate were subjected to 5 min pulses of $\text{Na}_2^{35}\text{SO}_4$ (0.1 mM 20 $\mu\text{Ci/ml}$) followed by chase time of 0, 30, and 60 min duration in the presence of 0.1 mM Na_2SO_4 . The embryos were then fixed and subjected to autoradiographic treatment, as described in Materials and Methods.

In order to assure that the grains occurring in the autoradiograms were derived from $^{35}\text{SO}_4$ found in polysaccharides and not found in proteins or amino acids, two sets of controls were run. Slides of 18 hour embryos having pulses of 15 and 30 min and a chase of 60 min were used. The first set of slides was placed in a solution of 0.5 N H_2SO_4 for four hours at room temperature, then washed

two times (5 min each) in distilled water. Sulfuric acid treatment removes all sulfated polysaccharides. Autoradiograms for this treatment yielded no visible grains.

The second set of controls was subjected to a treatment with methanolic-HCl (0.09 N) overnight at room temperature. After treatment, the slides were rinsed two times (5 min) in methanol and air dried. Autoradiographs of methanolic-HCl treated embryos resulted in no visible grains. Methanolic-HCl removes only ester-linked sulfates, that is, the type of linkage found in sulfated polysaccharides and not in proteins.

Embryos, grown in the presence of Na_2SO_4 , that had initiated rhizoids (18 hrs) showed an even distribution of grains throughout the embryonic wall with a zero minute chase time (Fig. 1). The ratio of grains from area II (rhizoid) over grains from area I (thallus) was 1.1 (Table I). Since the embryos appeared to be almost spherical, areas I and II represented equal halves of a sphere corresponding respectively to thallus and to rhizoid hemispheres. A 30 or 60 min chase in 18-hour embryos resulted in an increase in radioactivity associated with the rhizoid wall (Fig. 1). This latter chase time had a grain ratio of rhizoid to thallus of 2.8 (Table I).

Unlike the 18-hour embryo, the 0 chase in 24-hr embryos showed localization over the rhizoid wall (Fig. 2). A rhizoid to thallus grain count ratio of 2.6 was observed compared to 1.1 of the 18-hour

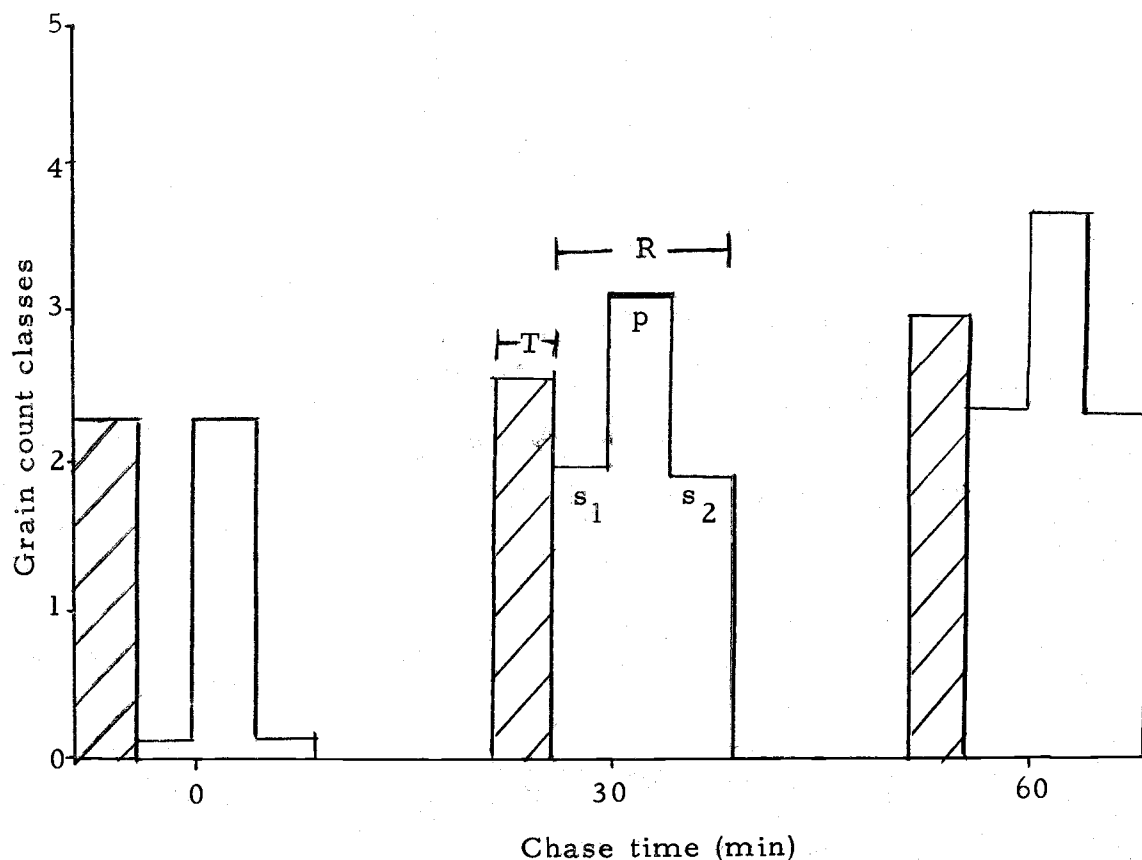


Figure 1. Radioactivity associated with various regions of the cell wall in 18 hour zygotes grown in the presence of Na_2SO_4 . A five minute pulse of $\text{Na}_2^{35}\text{SO}_4$ was given, followed by chases of 0, 30, and 60 minutes.

T (▨ striped) and R (□ clear) represent equal areas of the Thallus and Rhizoid wall. The rhizoid wall was further divided into three subareas: side₁, side₂, and tip--s₁, s₂, and p, respectively. At 0 chase time there is the same amount of grains over an equal area of the rhizoid and thallus wall. At 30 and 60 min, there are approximately three times more grains associated with the rhizoid wall.

TABLE I. Ratios of grain counts from equal areas of the thallus and rhizoid when subjected to pulses of $\text{Na}_2^{35}\text{SO}_4$.

Embryo Age	ASW Growth Medium	Time of Pulse (Min.)	Time of Chase (Min.)	Rhizoid Ratio: Thallus
18 hr.	+SO ₄	5	0	1.10
			30	2.70
			60	2.80
24 hr.	+SO ₄	5	0	2.60
			30	3.10
			60	3.20
24 hr.	-SO ₄	5	0	2.50
			30	2.95
			60	2.77

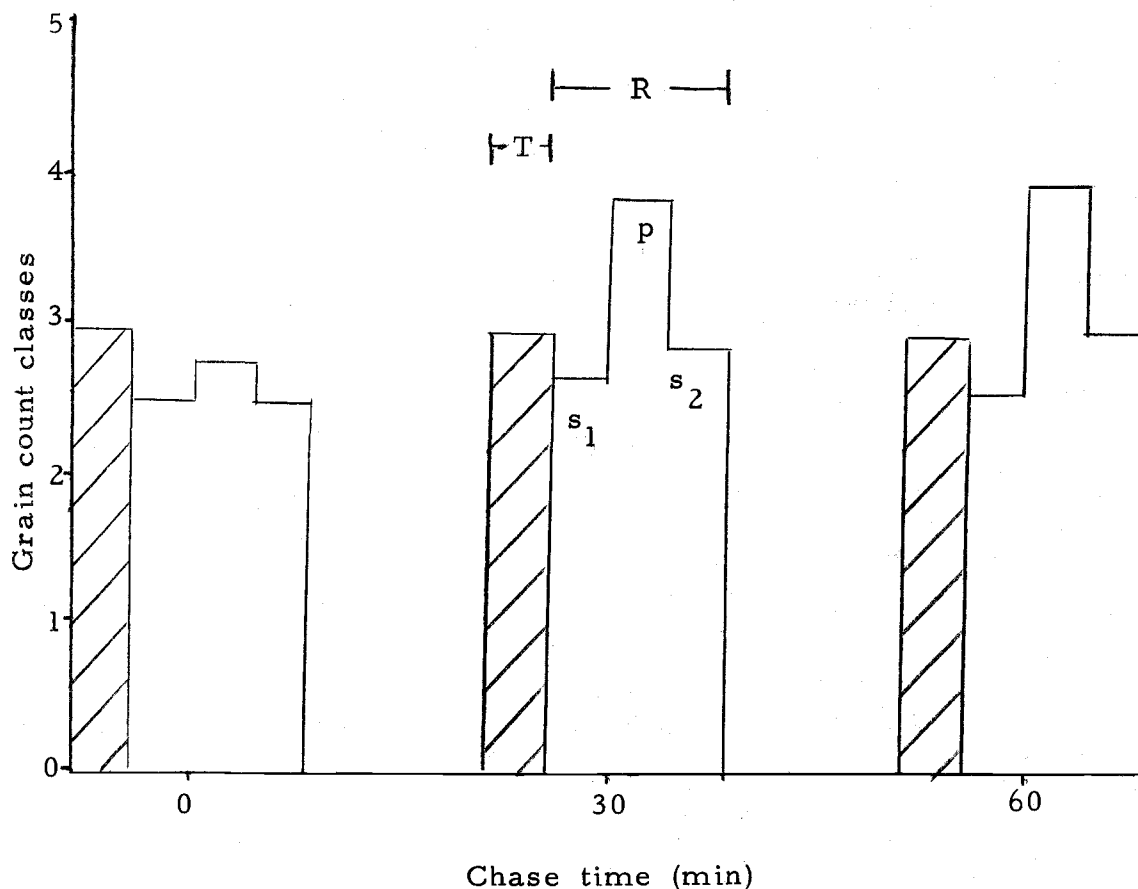


Figure 2. Radioactivity associated with various regions of the cell wall in 24 hour zygotes grown in the presence of Na_2SO_4 . A five minute pulse of $\text{Na}_2^{35}\text{SO}_4$ was given followed by chases of 0, 30, and 60 min.

T (▨ striped) and R (□ clear) represent equal areas of the Thallus and Rhizoid wall. The rhizoid wall was further divided into three subareas: side₁, side₂, and tip--s₁, s₂, and p, respectively. At 0 chase time there are three times more grains over the rhizoid wall, and with increasing chase times slightly more radioactivity is accumulated in that region.

embryo (Table I). Therefore, it appears that the sulfating sites are localized in the rhizoid region of the two-celled 24 hour stage. The embryos after 30 min chase showed only a slight increase in grain counts in the thallus area over that of the 0 time chase and the count remained constant after a 60 min chase (Table I).

Comparing the graphs of radioactivity in 18 hour and 24 hour embryos (Figs. 1 and 2), a trend becomes evident. In each longer chase time of the 18-hour stage, thallus grain counts increase; 2.26 at 0 chase, 2.59 at 30 min chase, and 2.98 at 60 min chase (Fig. 1). In the 0, 30, and 60 min chase times of the 24 hour two celled embryos, no increase in the thallus wall was observed (Fig. 2). It appears that sulfated polymers are selectively transported to or are sulfated in the tip region of the rhizoid area. It also appears that there is no appreciable increase in sulfated fucan, *i. e.*, fucoidan, in the thallus wall after the rhizoid has formed and that all of the sulfated fucan and sulfating potential is transported to the rhizoid tip. Table I summarizes the ratios of rhizoid/thallus grains and Figures 3a through e are representative photographs.

Embryos grown 24 hours in the absence of the sulfate, when pulsed for 5 min with $^{35}\text{SO}_4$ produced grain count patterns similar to those found in the embryos grown 24 hours in the presence of sulfate (Fig. 4), *i. e.*, localized in the rhizoid region with a zero chase time (compare Figs. 2 and 4). Previous research has shown that sulfation

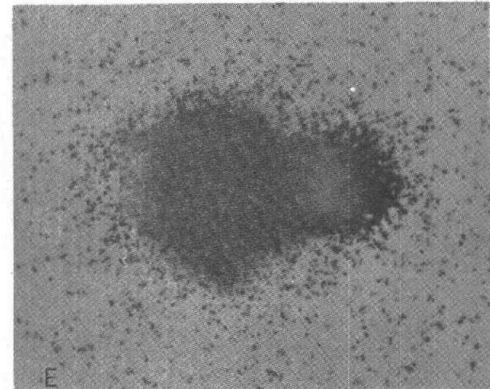
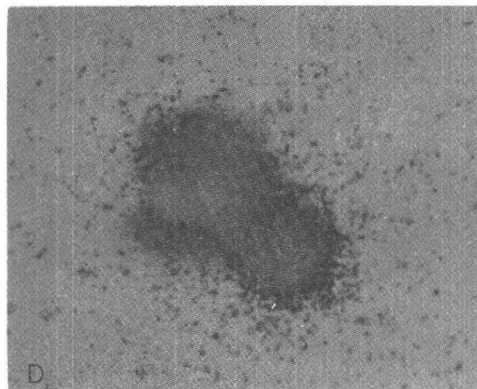
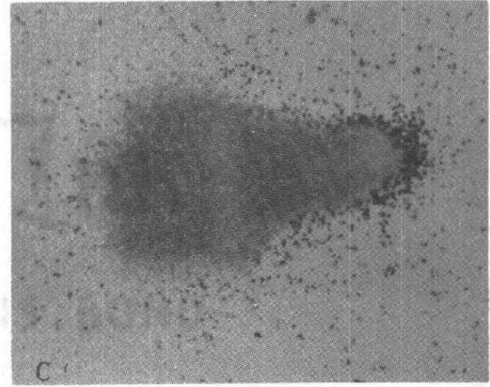
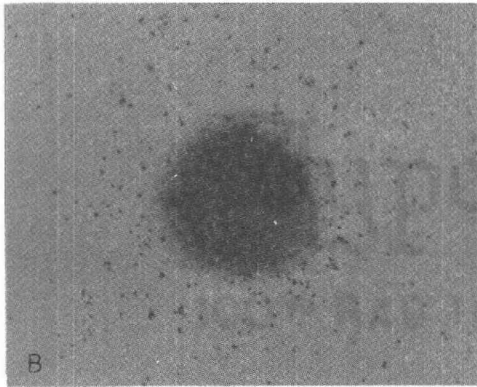
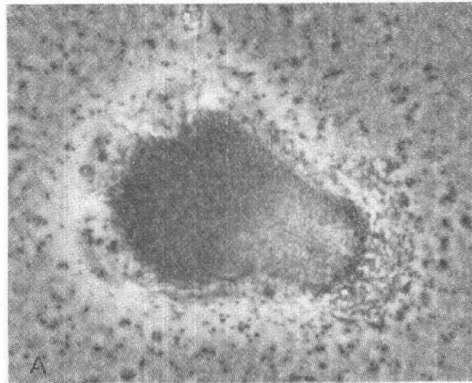
Figure 3a. A 20 hour, two celled embryo of F. distichus taken by phase contrast to show the extent of the cell wall and mucilagenous layer surrounding the embryo. The wall is represented by the white area, a layer about 5 mm thick, and is the area that accumulates the sulfated polysaccharides. The same embryo is photographed under brightfield in Fig. 3e where the grains are more easily visible. (x 400)

Autoradiographs of F. distichus embryos pulsed for 5 min with $\text{Na}_2^{35}\text{SO}_4$ (20 $\mu\text{Ci/ml}$) and then fixed.

- b. An 18 hour zygote showing an even distribution of grains in the wall area corresponding to a 5 mm layer surrounding the zygote. (x 400)
- c. A 24 hour embryo showing a localized accumulation of grains in the wall of the rhizoid tip. The wall corresponds to a 5 mm layer surrounding the embryo. (x 400)

Autoradiographs of F. distichus embryos pulsed for 5 min with $\text{Na}_2^{35}\text{SO}_4$ (20 $\mu\text{Ci/ml}$) and then chased 60 min before fixation.

- d. An 18 hour zygote showing an accumulation of grains in the rhizoid wall area corresponding to a 5 mm layer. (x 400)
- e. A 24 hour embryo showing an accumulation of grains in the rhizoid wall area corresponding to a 5 mm layer. (x 400)



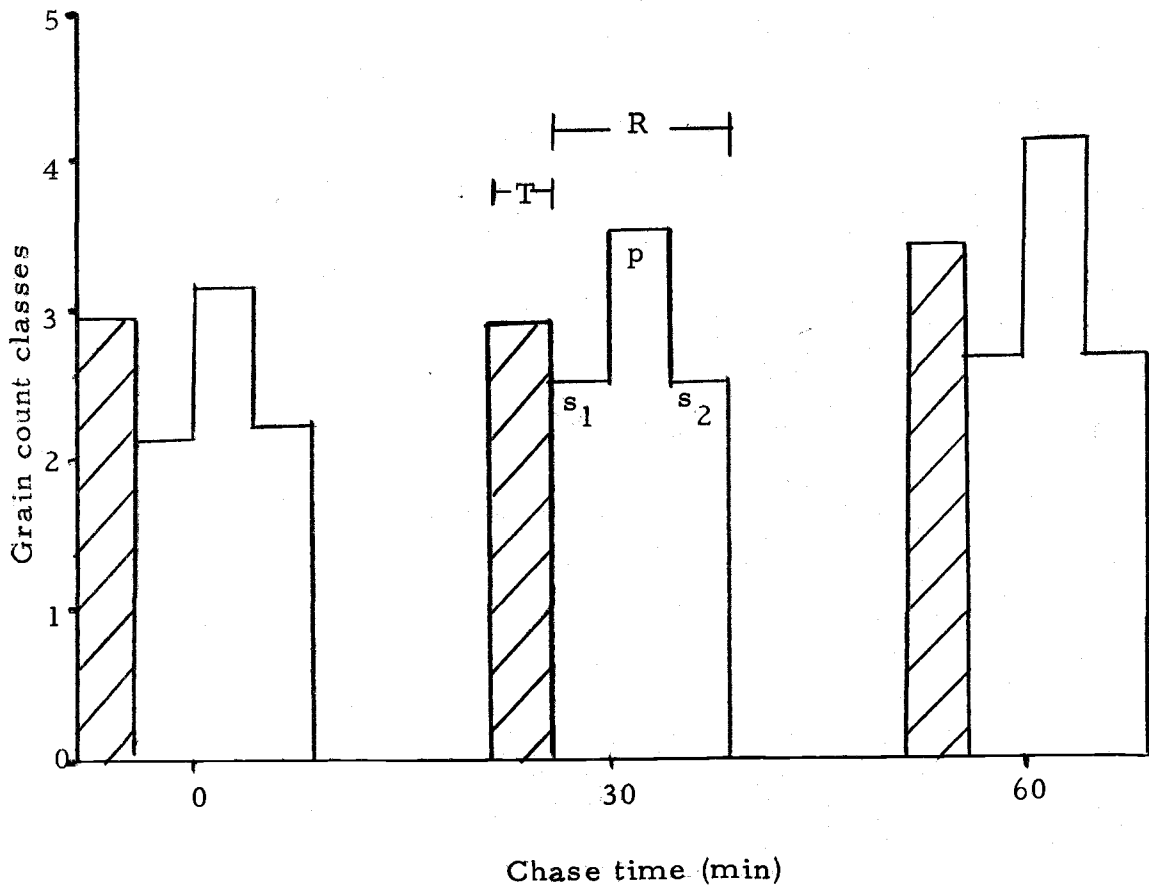


Figure 4. Radioactivity associated with various regions of the cell wall in 24 hour zygotes grown in the absence of Na_2SO_4 . They were grown in 10 mM methionine. A 5 min pulse of $\text{Na}_2^{35}\text{SO}_4$ was given followed by chases of 0, 30, and 60 min.

T (▨ striped) and R (□ clear) represent equal areas of the Thallus and Rhizoid wall. The rhizoid wall was further divided into three subareas: side₁, side₂, and tip--s₁, s₂, and p, respectively. At 0, 30, and 60 min there appears to be about three times more grains associated with the rhizoid wall area.

is not necessary for rhizoid formation (Crayton, 1974). Sulfation does not appear to be required for the segregation of the sulfation sites, since the rhizoid to thallus ratio of grains in the wall of embryos in the absence of sulfate is comparable to that of embryos grown in the presence of sulfate (Table I).

From the autoradiographic data, it is evident that the sites of sulfation are randomly distributed in the 18 hour embryo. These sites very quickly become localized in the rhizoid area. This localization is not dependent on exogenous sulfate or sulfation of the fucan since the same labeling pattern is observed in embryos grown in the presence or in absence of sulfate.

From these data and previous cytological and biophysical data, a working hypothesis about the sites of sulfation and how they become localized can be made. There is strong cytological evidence that the cytoplasmic storage site of sulfated fucan, *i. e.*, fucoidan, is in Golgi-derived vesicles (McCully, 1969, 1970; Quatrano, 1972; and Evans, 1973). There is also a sequestering of Golgi bodies and Golgi derived vesicles in the presumptive rhizoid area as well as in the rhizoid cell (Quatrano, 1972, 1974). This distribution of Golgi apparatus follows the timing and localization of the sulfated fucan by autoradiographic and cytochemical staining. Unpublished observation by Quatrano indicate that a particulate fraction contains the sulfating enzyme when assayed in a cell-free system. Many studies

using animal systems have also reported the association of mucopolysaccharide sulfation with Golgi (Sugiyama, 1972; Kosher and Searls, 1973; Kinoshita, 1971; Aoki and Koshihara, 1972). If the sulfating sites are directly shown to be associated with the Golgi apparatus in Fucus their migration to the rhizoid occurs in embryos either grown in the presence or absence of sulfate. When embryos are pulsed with sulfate at the 24 hour stage the Golgi bodies are localized and therefore sulfation appears localized by the autoradiographic data.

What is the mechanism of this localization? One hypothesis states that the pattern of localization could be established in a cell by self-generated cataphoresis (Spek, 1934). L. F. Jaffe (1966) supported the hypothesis in the Fucus system. He postulated that if a single cell were a developing system, the membrane potential differences would be enough to be able to drive a current across the cytoplasm and back through the medium. He found, using longitudinally arranged Fucus embryos, that an electric current of at least 60 picoamps began to flow through each zygote just prior to rhizoid formation. The current generated was sizeable enough to localize relatively large, negatively charged particles to the rhizoid region. The Golgi apparatus and their vesicles could also be localized this way.

Electrophoresis

The autoradiographic data has shown that exogenously supplied

sulfate is rapidly incorporated as an ester into a fucan polymer.

The polymer is evenly distributed in the 18 hour embryo wall and, by 24 hours, is localized primarily in the rhizoid cell wall. What is the nature of the polymer? Some facts about the polymer are already known. First, the sulfate is incorporated into a polymer as a sulfate-ester which is characteristic of a sulfated fucan rather than a protein (Quatrano and Crayton, 1973). Incorporation of the $^{35}\text{SO}_4$ into the unsulfated fucan occurs at about the same time as the polymer can be visualized in the rhizoidal area and ultimately in the rhizoid wall by TBO staining. Finally, this sulfation of a fucan, which is believed to be already present in the zygote cytoplasm at the time of sulfation, increases the net negative charge on the polysaccharide. Hogsett and Quatrano (1975) found that the $^{35}\text{SO}_4$ which is incorporated at the time of localization is primarily associated with two fractions; an acid soluble and a calcium chloride soluble fraction. Using DEAE column chromatography and high voltage paper electrophoresis, each fraction appeared to be similar in that both fractions contained varying amounts of two polymers. From the chromatographic and electrophoretic profiles, these polymers in each fraction appeared to be identical (Hogsett and Quatrano, 1975). However, these techniques appeared to offer little resolution of the sulfated fucans. An improvement was reported using cellulose acetate (Quatrano and Stevens, 1976) and in this present study further resolution was obtained using

cellulose acetate gels in a pH 2 LiCl/HCl buffer.

Using this modification, I wished to determine if the polymers in the two fractions that become sulfated are similar upon electrophoresis, and, if all of the polymers equally incorporate exogenously supplied sulfate.

An acid and calcium chloride extract of 18 hour embryos was electrophoresed on a cellulose acetate gel (Cellogel) in the pH 2 LiCl/HCl buffer. Two major bands (I and II) were evident in both extracts, but it was also evident in the acid fraction that there were three minor bands (a, b, c) delineated in II (Fig. 5). The two major bands probably correspond to the polymers separated by DEAE chromatography and paper electrophoresis, but the minor heterogeneity in band II has never been reported. Whether these are different sugar polymers, or the same polymers with different amounts of sulfate has not been determined. It is also interesting to note that IIa is the only minor component found in the CaCl_2 extract whereas all three (a, b, c) are observed in the acid extract (Fig. 5).

Which of the polymers corresponds to the grains found in the autoradiograms? Quatrano and Stevens (1976) showed that by 12 hours following fertilization, a fucan appears in the cell wall that is highly sulfated. This fucan is rich in fucose compared to an electrophoretically distinct fucan which is assembled into the wall at about four hours after fertilization. Upon electrophoresis in cellulose acetate

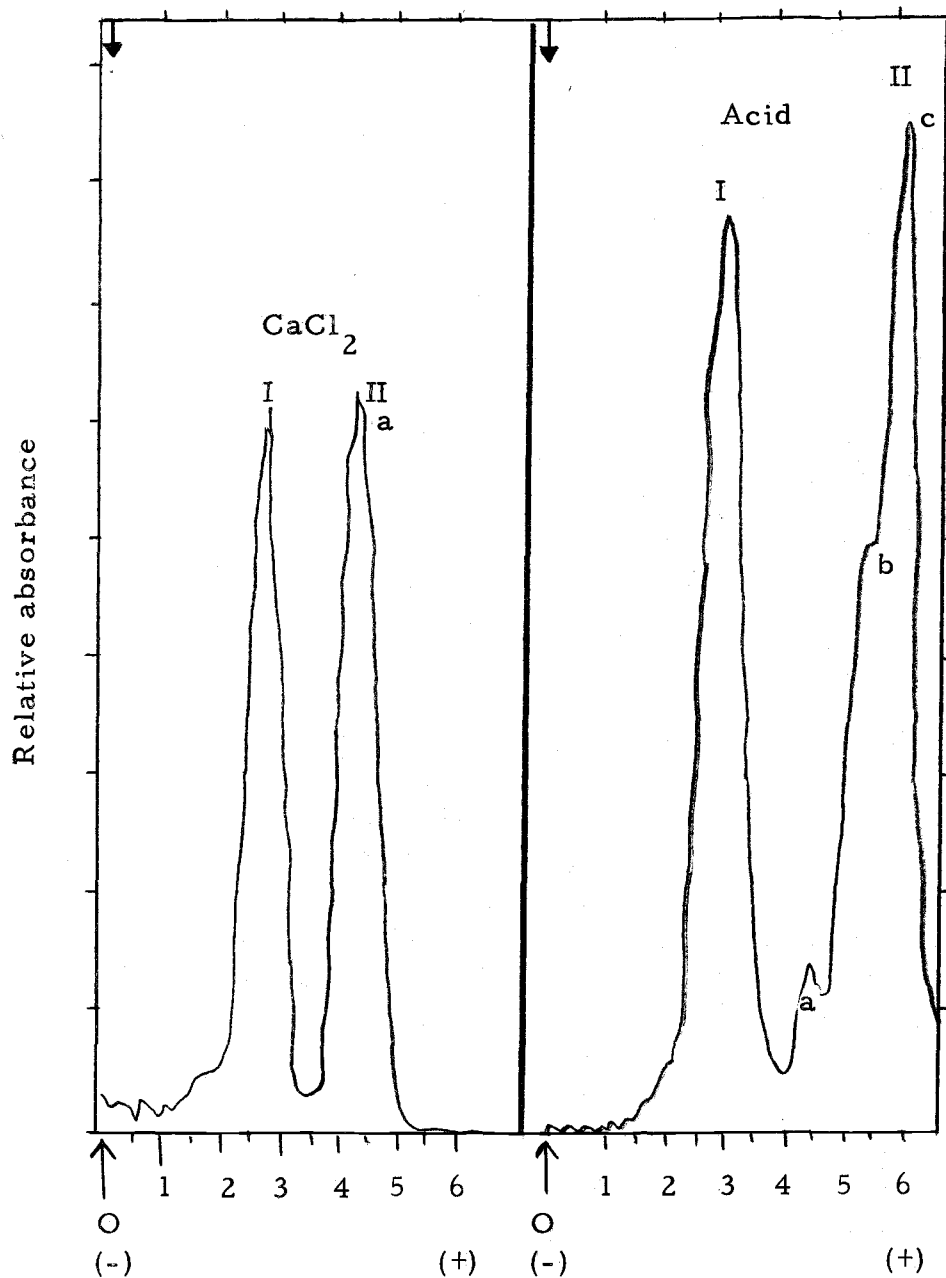


Figure 5. The content of fucan polymers present in the CaCl_2 and acid soluble fractions when electrophoresed in a buffer Li/HCl pH 2 at room temperature.

Major bands are designated I and II with the subgroups in band II shown as a, b, c. The extracts were spotted on cellulose acetate gels at 0.

gels at pH 2 these two fucan polymers in the cell wall correspond to I and II. I is assembled into the wall at four hours and II is present in the wall by 12 hours. Both are different in their sugar composition (Quatrano and Stevens, 1976). From these data, it was suggested that II is the fucan that is sulfated and is incorporated into the rhizoid wall as detected by autoradiography.

To test this, embryos were pulsed with $^{35}\text{SO}_4$ between 16-18 hours and the total fucan components were isolated and electrophoresed. In Figure 6, it is clear that II, the polymer that appears in the cell wall after 12 hours is the one being actively sulfated. Apparently, the fucan is present prior to 12 hours in the cytoplasm (Quatrano and Crayton, 1973) and upon sulfation can be electrophoretically isolated as II.

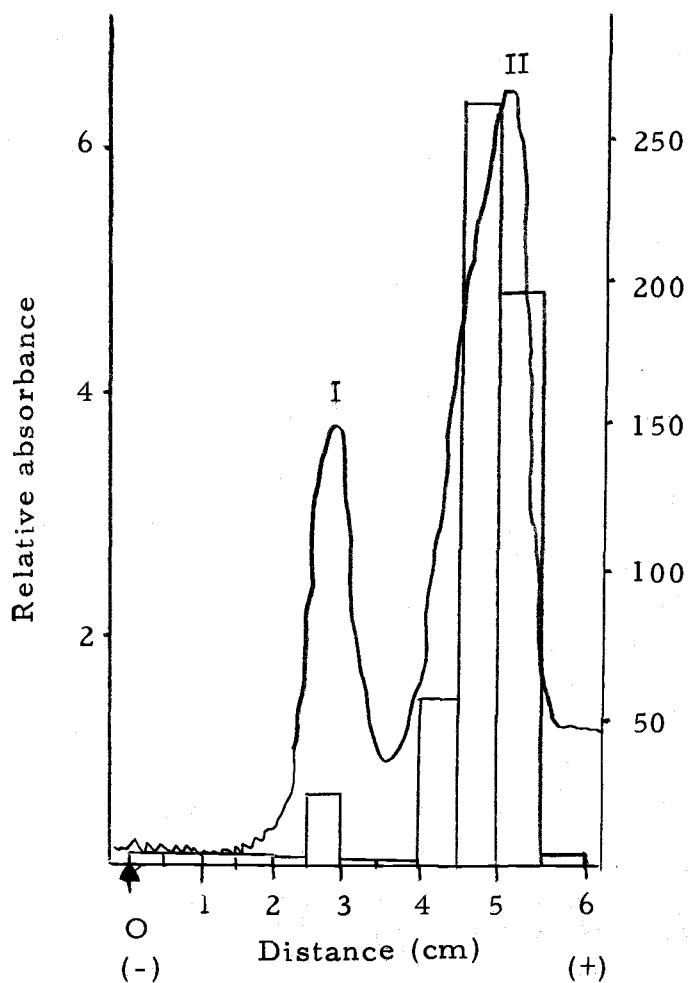


Figure 6. The relationship between the relative absorbance (—) and the radioactivity (□) of the sulfated polysaccharides in an extract from 18 hours zygotes. A two hour pulse of $\text{Na}_2^{35}\text{SO}_4$ was given at 16 hours after fertilization. Major bands are designated I and II. Extracts were spotted at 0 on cellulose acetate gels and electrophoresed in a buffer of Li/HCl pH 2 at room temperature.

BIBLIOGRAPHY

- Aoki, Y., and H. Koshihara. 1972. Inhibitory effects of acid polysaccharides from sea urchin embryos on RNA polymerase activity. *Biochem. Biophys. Acta* 272:33-43.
- Aristotle. 1952. *Generation of Animals*. Encyclopedia Britannica. Chicago. 729a.
- Blackler, A. W. 1958. Contribution to the study of germ-cells in the Anura. *J. Embryol. Exp. Morphol.* 6:491-496.
- Boveri, T. 1887. *Über Differenzierung der Zellkerne Während der Furchung des Eies von Ascaris megalocephala*. *Anat. Anz.* 2: 688-91.
- Buehr, M. L. and A. W. Blackler. 1970. Sterility and partial sterility in the South African clawed toad following pricking of the egg. *J. Embryol. Exp. Morphol.* 23:375-84.
- Conklin, E. G. 1905. The organization and cell lineage of the ascidian egg. *J. Acad. Natl. Sci. (Philadelphia)*. 13:1.
- Crayton, M. A., E. Wilson, and R. S. Quatrano. 1974. Sulfation of fucoidan in Fucus embryos. II. Separation from polar growth. *Devel. Biol.* 39:164-167.
- Evans, L. V., M. Simpson, and M. E. Callow. 1973. Sulfated polysaccharide synthesis in brown algae. *Planta (Berlin)*. 110:237-252.
- Evans, L. V., and M. Callow. 1974. Polysaccharide sulfation in Laminaria. *Planta (Berlin)*. 117:93-95.
- Evans, L. V., and M. Callow. 1974. Studies on the synthesis and composition of extracellular mucilage in the unicellular red alga Rhodella. *J. Cell Science.* 16:1-24.
- Geyer-Duszynska, I. 1959. Experimental research on chromosome elimination in *Cedidomyidae* (Diptera). *J. Exp. Zool.* 141:391-448.
- Hogsett, W. E. and R. S. Quatrano. 1975. Isolation of polysaccharides sulfated during early embryogenesis in Fucus. *Plant Physiol.* 55:25-29.

- Illmensee, K. and A. P. Mahowald. 1974. Transplantation of posterior polar plasm in Drosophila: Induction of germ cells at the anterior pole of the egg. *Proc. Natl. Acad. Sci.* 71: 1016-1020.
- Jaffe, L. R. 1966. Electrical currents through the developing Fucus egg. *Proc. Natl. Acad. Sci. U. S.* 56:1102-1109.
- Kinoshita, S. 1971. Heparin as a possible initiator of genomic RNA synthesis in early development of sea urchin embryos. *Exp. Cell. Res.* 64:403-411.
- Kosher, R. A. and R. L. Searls. 1973. Sulfated mucopolysaccharide synthesis during the development of Rana pipiens. *Devel. Biol.* 32:50-68.
- Mahowald, A. P. 1968. Polar granules of Drosophila: II. Ultrastructural changes during early embryogenesis. *J. Exp. Zool.* 167:237.
- _____. 1971. Origin and continuity of cell organelles. eds Reinert, J. and H. Ursprung. Springer-Verlag. N. Y., Heidelberg, Berlin. pp. 158-169.
- McCully, M. E. 1969. The synthesis and secretion of polysaccharides during early development of the Fucus embryo. (Abstr.) *Int. Bot. Congr.* 11th, Seattle, Wash., 134.
- _____. 1970. The historical localization of the structural polysaccharides of seaweeds. *Ann. N. Y. Acad. Sci.* 175:702-711.
- Nakazawa, S. and K. Takamura. 1966. An analysis of rhizoid differentiation in Fucus eggs. *Cytologia* 32:408-415.
- Quatrano, R. S. 1972. An ultrastructural study of the determined site of rhizoid formation in Fucus zygotes. *Exp. Cell Res.* 70: 1-12.
- _____. 1974. *Developmental Biology: development in marine organisms.* In *Experimental Mar. Biol.* Academic Press, N. Y., San Francisco, London. pp. 303-338.

- Quatrano, R. S. and M. A. Crayton. 1973. Sulfation of fucoidan in Fucus embryos. I. Possible role in localization. *Devel. Biol.* 30:29-41.
- Quatrano, R. S. and P. Stevens. 1976. Cell wall assembly in Fucus zygotes. I. Characterization of the polysaccharide components. *Plant Physiol.* In Press.
- Smith, L. D. 1966. Role of a 'germinal plasm' in the formation of primordial germ cells in Rana pipiens. *Devel. Biol.* 14: 330-343.
- Spek, J. 1934. Über die bipolare Differenzierung der Eizellen von Neries limbata und Chaetopterus pergamentaceus. *Proto-plasma* 21:394-405.
- Sugiyama, K. 1972. Occurrence of mucopolysaccharides in the early development of the sea urchin embryo and its role in gastrulation. *Devel. Growth. Diff.* 14:63-73.