These $PdCl_2$ whiskers have a growth rate of about 0.1 mm/sec at a temperature between 500° and 600°C. The palladium whiskers cease to incandesce from the high temperature to which they had been subjected prior to reaction with the residual or added chlorine. The $PdCl_2$ whiskers grow in a rapidly changing, relatively sharp temperature gradient below their melting point of 683°C. This gradient may serve to limit the lengths of the whiskers produced.

The reaction between palladium whiskers and chlorine, in the absence of any initial $PdCl_2$, also produces numerous $PdCl_2$ whiskers both directly on and far removed from the dark red masses of $PdCl_2$ that are rapidly produced. Thus, vapor-phase transport of $PdCl_2$ may play a major role in the nucleation and growth of $PdCl_2$ whiskers under the two sets of conditions described here.

Figure 1 shows such a $PdCl_2$ whisker about 1 μm in diameter and 50 to 100 μm long that is being bent to about 2.5 percent strain. The variation of elastic deformation between 2.5 and 6 percent suggests that these are relatively strong crystals. Sharp kinks form (failure by way of plastic deformation) when the elastic limits are exceeded. The $PdCl_2$ whiskers dissolve readily in acetone and slowly in dilute hydrochloric acid; they are rather insoluble in water. They represent one of the few examples of halide whiskers (others include sodium chloride and cesium chloride) and the only one known to form at elevated temperatures.

E. F. RIEBLING

720 Tifft Avenue,
Horseheads, New York 14845

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Rhizoid Formation in Fucus Zygotes: Dependence on Protein and Ribonucleic Acid Syntheses

Abstract. *Ribonucleic acid required for rhizoid formation in* Fucus *zygotes is synthesized several hours before the production of proteins essential for this process. The period of synthesis of these proteins coincides with the irreversible commitment of a certain cytoplasmic region to initiate events leading to a visibly polar cell.*

Zygotes (*1*) of the brown alga *Fucus* are well suited for cellular and biochemical experimentation (*2–4*). Unlike many plants, these zygotes are relatively easy to obtain and manipulate and develop free of surrounding tissue. The spherical egg has its nucleus near the center of the cell, shows an apparent random distribution of cytoplasmic inclusions as revealed by electron microscopy, and is probably radially symmetrical. No significant increase in size of the zygote occurs until about 14 hours after fertilization when a localized protuberance (rhizoid) appears. Several hours later this polar growth is separated from other parts of the cell by the first cell division, which occurs in a plane at right angles to the long axis of the rhizoid. Derivatives of the rhizoid cell form the holdfast portion of the plant, whereas the thallus cell gives rise to the main body and reproductive structures. Thus, rhizoid formation establishes a permanent polar axis in a single cell which gives rise to a two-celled embryo, each cell of which is different in structure, function, and developmental fate (*2*, *5*).

Although the synthesis of proteins and nucleic acids during development has been studied in a number of animal embryos (*6–8*) and plant seeds (*9*), such molecular events have no specific relation to differentiation of a given cell, as has been reported for an enzyme associated with cap formation in *Acetabularia* (*10*). Macromolecular requirements for rhizoid formation will give direct information on processes involved with an intracellular differentiation, which is the basis for differences between the first two cells of the embryo and the polarity of the entire organism. This report is concerned with the following questions: Is protein or RNA synthesis (or both) required for rhizoid formation and the first cell division, and, if so, when are these molecules synthesized?

Receptacles of *Fucus vesiculosus* L. were collected, iced, and stored in the dark at 4°C. To obtain gametes, receptacles were washed thoroughly and placed individually in separate dishes containing artificial seawater (ASW) (*11*) at 15°C in diffuse light. Within several hours after immersion into ASW, either sperm or egg cells were released from each receptacle. Solutions of gametes were passed through a 102 μm nylon mesh (*12*) to remove large debris and intact oogonia or antheridia, and were then mixed. Thirty minutes later, sperm cells were removed by passage of the mixed gamete solution through a series of nylon meshes. Zygotes free of sperm were washed thoroughly with filtered (Millipore, GS type, 0.22 μm) ASW containing 25 μg of streptomycin and of penicillin (1.65 unit/μg) per milliliter to reduce bacterial contamination; they were then placed in sterile plastic petri dishes (*12*) in fresh sterile medium. Development in this medium had no adverse effects upon the temporal sequence of events or in the percentage of zygotes forming rhizoids and completing the first cell division compared to controls without antibiotics. No significant bacterial contamination was evident in such a medium plated on agar broth 3 to 4 hours after the zygotes had been washed. There was no indication of bacteria embedded in the cell wall when viewed through the light or electron microscope, nor was there incorporation of labeled amino acids in the wall when autoradiography was performed. All experiments reported here were carried out at 15°C in the dark.

For experiments with inhibitors and radioactive labeling, solutions of actinomycin D (20 μg/ml) and cycloheximide (0.05 μg/ml) (*12*) were made up fresh in sterile ASW. Incorporation of a mixture of amino acids uniformly labeled with C^{14} (specific activity, 1 mc/mg) into proteins, and of uridine-5-H^3 (specific activity, 2.5 mc/mmole) into RNA (*12*) was then analyzed.

For protein determinations washed zygotes were homogenized in 10 percent trichloroacetic acid (TCA) at 2° to 4°C. The precipitate was washed once with 5 percent TCA and then incubated with fresh 5 percent TCA at 90°C for 30 minutes. The material insoluble in hot TCA was washed at room temperature as follows: 5 percent TCA, one time; 95 percent ethanol, two times; ethanol and chloroform (3 : 1), two times; ethanol and

ether (1:1), two times; and ether, two times. The dried powder was dissolved in 1.0 ml of 1.0*N* NaOH overnight at room temperature and centrifuged; and the undissolved pellet was washed twice with distilled water. The washings and NaOH supernatant were combined and brought to 4°C before cold TCA was added to bring the final concentration of TCA to approximately 20 percent. The reprecipitated protein powder was dissolved in 1.0 ml of 0.5*N* NaOH, and samples were taken for protein determination (*13*) and liquid scintillation counting in Bray's solution (*14*).

For determination of RNA, washed zygotes were processed exactly according to the method of Holdgate and Goodwin (*15*) to obtain a powder free of lipid and insoluble in cold trichloroacetic acid. The dried powder was then hydrolyzed with 0.3*N* KOH at 37°C for 16 hours. The soluble RNA nucleotide fraction, after precipitation of DNA and protein with a mixture of perchloric acid, ethanol, and Mg^{2+} (*15*, *16*), was neutralized with KOH, and samples were taken for counting in Bray's solution and for ultraviolet spectrophotometric determination of RNA (*17*).

Continuous incubation of zygotes in solutions containing known inhibitors of RNA (actinomycin) and protein (cycloheximide) synthesis in plants (*18*), added immediately after fertilization, inhibit rhizoid formation and cell division in the entire population when examined after 36 hours. Cycloheximide introduced at any time up to 8 hours after fertilization had the same effect, indicating that proteins essential for these events are formed subsequent to this period. However, rhizoid formation and cell division occur at their normal times if cycloheximide is given at 13 hours. If protein synthesis is inhibited after 10 hours, about two-thirds of the population unexpectedly undergo one division but no rhizoid formation, giving rise to spherical two-celled embryos. Proteins required for completion of the first division seem to be synthesized just before those proteins required for the formation of the rhizoid. Actinomycin exhibits its effects much earlier. If it is added 1 hour after fertilization, no rhizoids or cell divisions are observed, whereas if added at 5 hours, most zygotes form a rhizoid but undergo only one division (control populations have three to four divisions by this time). No clear separation of these two events was obtained in the RNA synthesis period with actinomycin. The same results were obtained when these inhibitors were used with ASW without streptomycin and penicillin.

Actinomycin added to developing zygotes at 4 and 7 hours effectively blocks incorporation of uridine into RNA nucleotides within 1 hour after its addition (Fig. 1A). Differences in amounts of incorporation in controls at these times are similar to results of Koehler and Linskens (*3*), who found a decreasing rate of uridine incorporation into RNA after fertilization. Since they did not use antibiotics, this result most likely represents zygote incorporation of RNA precursors and not a decreasing amount of bacterial incorporation due to the presence of streptomycin and penicillin. The fact that actinomycin inhibits incorporation at 7 hours but has no apparent effect on subsequent rhizoid formation and cell division suggests that the lack of an observable morphogenetic effect of actinomycin at this time cannot be accounted for by decreased permeability to the antibiotic. A general, nonspecific inhibition of general metabolism by actinomycin also seems unlikely since it can be present continuously from 5 hours with a delay in rhizoid formation and cell division of no more than a few hours. The accumulation of labeled amino acid mixture into a hot trichloroacetic acid insoluble fraction of zygotes is inhibited within 1 hour after the addition of cycloheximide and can resume after a 4-hour block with cycloheximide (Fig. 1B). In other experiments, the accumulation of labeled amino acids into proteins occurring at this time is inhibited by actinomycin if it is given at times early enough to prevent rhizoid formation. The resumption of amino acid incorporation after cycloheximide treatment, and the fact that cycloheximide added at 10 to 11 hours inhibits rhizoid formation (14 to 18 hours) but does not cause a delay of more than a few hours in cell division (20 to 24 hours), demonstrates that the inhibi-

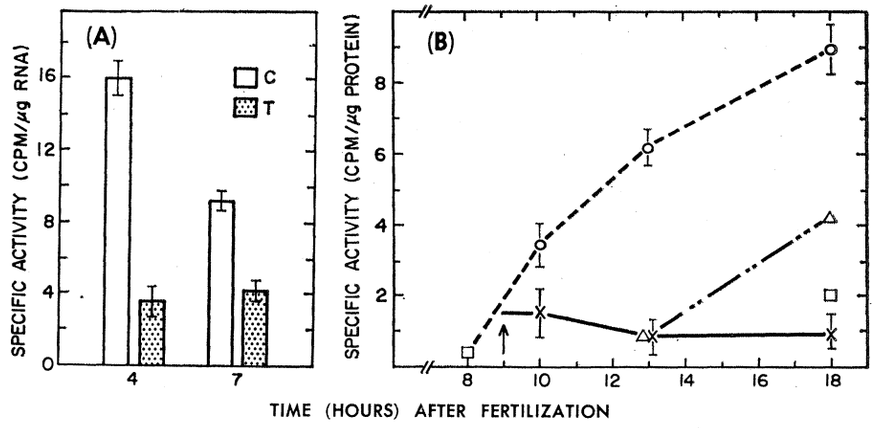

Fig. 1. (A) Bar graph comparing uridine incorporation into RNA at 4 and 7 hours after fertilization, with (*T*) and without (*C*) the presence of actinomycin. Standard deviations of replicate samples are given for control and treated populations. At the stated times, equal volumes of aseptic developing zygotes were exposed for 45 minutes to uridine-H^3 (30 μc/ml), then for 15 minutes to unlabeled ASW medium (three washes, 25 ml per wash). In treated populations, actinomycin and label were added simultaneously and treated exactly as above except actinomycin was present in the unlabeled medium. No differences were found in radioactivity recovered from cold trichloroacetic acid washes, indicating that actinomycin had no effect on uridine uptake by zygotes. Samples of 4- and 7-hour control zygotes were placed in ASW immediately after the wash, and at 36 hours, slightly greater than 90 percent of both groups had formed rhizoids and had undergone at least one division. Similar samples from 4- and 7-hour treated cultures were placed in ASW and actinomycin after addition of unlabeled medium. At 36 hours, 63 percent of the zygotes in actinomycin since 4 hours had a rhizoid and one cell division, whereas 87 percent of the zygotes had undergone these events when actinomycin was present since 7 hours. (B) Comparison of the accumulation of a mixture of amino acids labeled with C^{14} (2.5 μc/ml) in ASW medium into material insoluble in hot trichloroacetic acid between control and cycloheximide-treated populations of *Fucus* zygotes; (○) control, (×) cycloheximide-treated at 9 hours, (△) treated zygotes removed from cycloheximide at 13 hours. Standard deviations of replicate samples are given for control and treated cells. Square (□) at 8 hours represents a 0 time control and the square at 18 hours represents unfertilized eggs left in radioactive solution between 8 and 18 hours as a bacterial control. Control zygotes began forming rhizoids by 13.5 hours, 95 percent of the population had rhizoids and more than one division. Populations incubated with cycloheximide beginning at 9 hours had less than 8 percent of the zygotes with rhizoids or a cell division (or both). About 78 percent of the zygotes released from the cycloheximide block at 13 hours had rhizoids and one division at 36 hours.

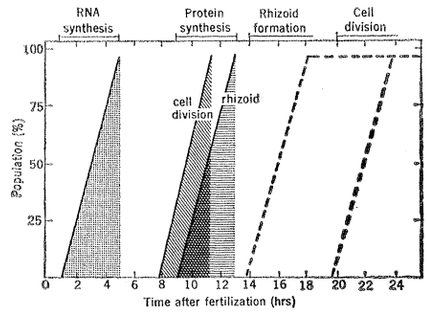

Fig. 2. Illustration of the timing of RNA and protein synthesis required for rhizoid initiation and cell division. Each shaded triangle represents the period during which a given percent of the population has synthesized an RNA or protein fraction essential for rhizoid initiation or cell division. Dashed lines indicate the normal time course of the zygote population passing through these two events.

tion of the cell's capacity to form rhizoids by cycloheximide is a result of a relatively specific block in protein synthesis (Fig. 2).

Experimentation with a number of diverse organisms has demonstrated that inhibitors exhibiting their effect at the level of transcription of RNA from DNA (actinomycin) must be given earlier than inhibitors acting at the translational level of protein synthesis (cycloheximide) to prevent a given growth or differentiative event (or both) (*7*, *19*). Such experiments provide evidence for the existence of control of protein synthesis at translation. Rhizoid formation in *Fucus*, like cap regeneration in *Acetabularia* (*10*), seems to be an example of a unicellular system demonstrating control of differentiation at the translation level of protein synthesis.

On the basis of similar experiments during animal embryonic development, Tyler (*7*) has proposed that the production of "masked forms" of RNA (*6*) occurs roughly at the stage when the eventual fate of certain multicellular regions becomes "determined." Tissue differentiation, then, would be equivalent to the eventual translation of this masked form of RNA into tissue-specific proteins.

In *Fucus* zygotes the cytoplasmic area destined to be the site of rhizoid formation is irreversibly fixed only 1 to 3 hours before rhizoid emergence (*2*). This corresponds more closely to the time when proteins, not RNA, required for rhizoid formation are synthesized. Therefore, in *Fucus*, it seems that the production of an essential RNA fraction for a given intracellular differentiative event does not correspond to "fixing" a cytoplasmic area to its future developmental fate. It is the synthesis of proteins, the timing of which seems to be controlled at the translation level, which irreversibly determines a specific region of the cytoplasm to initiate events quickly leading to a macroscopic polar cell. It is not known whether essential proteins are synthesized at the site of subsequent rhizoid initiation, or synthesized at random and transported to this site.

RALPH S. QUATRANO*
Department of Biology, Yale University, New Haven, Connecticut

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* Present address: Department of Botany, Oregon State University, Corvallis 97331.

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DNA Biosynthesis by Isolated Mitochondria: A Replicative Rather Than a Repair Process

Abstract. *The previously observed incorporation of deoxynucleoside triphosphate precursors into DNA by isolated rat liver mitochondria could reflect either replication of DNA or a repair process. Density labeling experiments in cesium chloride demonstrate that DNA synthesized in the presence of 5-bromodeoxyuridine triphosphate instead of thymidine triphosphate shows an appreciable increase in density. In some of the molecules undergoing synthesis, the amount of the density increase indicated a replacement of thymine by bromouracil to the extent of 33 percent. This extensive replacement, which would compute to twice this amount if only a single strand of the duplex is labeled, provides evidence for the synthesis of fairly long pieces of DNA. Such synthesis is characteristic of replication rather than repair, and the results thus suggest that mitochondria are able to replicate their own DNA.*

Recent studies lend support to the view that mitochondria possess a remarkable degree of autonomy; they appear to contain some of the information and apparatus for macromolecular biosynthesis and possibly for their own biogenesis. They contain DNA (*1*), aminoacyl tRNA synthetases and tRNA (*2*), and ribosomes (*3*), all of which appear to be distinct from their nuclear or cytoplasmic counterparts; they have also been demonstrated to carry out the biosynthesis of protein (*1*). Although mitochondria are under the control of nuclear genes (*4*), they are under the control of cytoplasmic genetic factors as well (*5*). Recent evidence indicates that these genes are located in the mitochondrion (*6*), suggesting that they are identical with mitochondrial DNA. The