nally, because the cells stimulated to divide in vitro by PHA, Con A, antigens, and allogeneic (MLR) cells are known to be predominantly T cells (*11*), selective proliferation of B (bone marrow–derived) cells in response to these mitogens cannot explain the present findings. Instead, our results raise the possibility that CRP reacts with a subpopulation of T cells which responds with proliferation to challenge with soluble or cell surface antigens but not with mitogens. Alternatively, sites capable of binding CRP may become available on the cell surface selectively upon stimulation by antigens as opposed to mitogens.

That the antigen-sensitive T cell may be a distinctive subset has received support from studies showing inactivation of the cells dividing in response to antigen without an effect on the cells capable of responding to PHA, and by separation of cells with these functions by buoyancy gradients (*12*). Thus, although the antigen-sensitive population had been considered by many to be a small proportion of the total pool of T cells (*13*), all of which are potentially responsive to T cell mitogens (*14*), this might not be the case. We do not yet know whether cells responding to blastogenic factors can bind CRP, or whether the lymphoblasts binding CRP are selectively those whose blastogenesis was induced by antigen directly; perhaps recruitment of cells not reactive with CRP explains why only one of four lymphoblasts formed in response to antigenic stimulation was able to bind CRP. A recruitment of cells that do not bind to CRP might also explain the consistently larger proportion of CRP-binding nonlymphoblasts in stimulated compared to unstimulated cultures.

In summary, we report that CRP binds preferentially to lymphoblasts stimulated by antigens as opposed to mitogens. It may thus be possible to use CRP as a marker for antigen- as opposed to mitogen-reactive T cells, or for antigen- as opposed to mitogen-reacted T cells, and to use CRP in the further definition of the surface and functional properties of such cells. While the exact biologic advantage of this interaction is yet to be defined, these experiments support the concept that CRP, by its ability to bind to certain T lymphocytes and influence the functions of T cells (*3, 4, 9*), platelets (*15*), and the C system (*6, 16*), plays an important role in the regulation of a variety of responses critical to inflammation, host defense, and tissue repair.

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Rhizoid Differentiation in Fern Spores: Experimental Manipulation

Abstract. *Germination in spores of the fern* Onoclea sensibilis *is initiated by an asymmetric division that partitions the spore into two cells of unequal size. The unequal daughter cells differentiate immediately into distinct types. When spores are germinated on the surface of solutions of methanol, the initial division is symmetrical, and the daughter cells from this equal division develop into the same type of cell. The differentiation of a rhizoid from the smaller cell in untreated spores is suppressed by methanol treatment.*

Striking examples of cellular differentiation in which two daughter cells—the products of a single mitosis—often diverge immediately and mature into different cell types are known in many plants. Bünning (*1*) reviewed many instances of this phenomenon, including root hair formation and guard cell differentiation in monocotyledons, the development of pollen grains and hyaline cell formation in the leaves of *Sphagnum*, and the germination of fern spores. One important feature shared by all of these systems is that differentiation is initiated with an asymmetric cell division, which partitions the mother cell into two daughters of unequal size. The daughter cells then develop into structurally and functionally different types.

We have discovered a simple technique with which we can almost totally control differentiation in germinating spores of a fern, *Onoclea sensibilis*. The technique is based on controlling whether the first division of the spore is asymmetric or not.

Spores were collected, stored, and sterilized prior to their use (*2*). We sterilized the liquid mediums on which spores were germinated by filtering them through a Millipore filter; aseptic conditions were maintained throughout. We initiated germination by floating spores on the surface of 4 ml of a simple inorganic medium (*2*) contained in culture tubes sealed with screw caps. The spores were exposed to 7000 ± 300 lux white fluorescent light at 27.5° ± 0.5°C. Spores were placed on a microscope slide in a solution of chloral hydrate in acetocarmine stain (*3*), and the details of germination were observed through a microscope. The staining mixture dissolves chloroplasts, which are abundant in spores of *Onoclea*, and stains the nuclei of germinated spores. It is then possible to see the positions of the nuclei and cell walls, which, in untreated spores, are obscured by the chloroplasts.

In ungerminated spores, the nucleus is located in the center. About 16 to 20 hours after germination begins, the nucleus migrates to one end of the spore. Two hours later, mitosis occurs (Fig. 1A), and the spore divides into a large and a small cell (Fig. 1B). The smaller cell narrows and elongates rapidly, differentiating into a rhizoid (Fig. 1, C and D). The rhizoid remains a single, elongated cell and never again divides. The larger cell retains its capacity for division and develops first a filament of two or more cells (the protonema) and later a two-dimensional plate of cells (the prothallus). In the living state, the cells of the protonema contain many chloroplasts whereas the rhi-

zoid does not. [This distinction is not shown in the photographs of fixed and stained plants (Fig. 1).] In addition to the obvious morphological difference between the rhizoid and the protonemal cell, differences between the two have been shown in ultrastructure, staining, permeability properties, and enzymatic activities (*4*).

If spores are germinated on a medium that contains methanol, nuclear movement largely ceases, yet the spores retain the ability to undergo cell division. Such spores divide into two nearly equal daughter cells (Fig. 1E). The optimum condition for germination is 3 days on the standard medium to which was added 2.5 percent methanol (by volume). At the end of this time all of the spores have germinated, but, in many, the continued growth of the cells is strongly inhibited by methanol. The methanol-treated spores are then transferred to the standard medium and grown for 2 to 3 days under 100-lux white fluorescent light at 23° ± 1°C. This procedure allows the cells to grow and reveals the developmental consequences of the equal cell division—the formation of "twin" plants. Each of the daughter cells from an equal division develops into a protonemal cell; the differentiation of one of them into a rhizoid is completely suppressed. Both cells may enlarge (Fig. 1F), undergo cell division side-by-side (Fig. 1G), and develop into twin protonemal filaments, which eventually begin two-dimensional development (Fig. 1H). Other patterns occur: sometimes the equal cells produce filaments that grow in opposite directions; in other plants, cell elongation is not extensive, and two-dimensional groups of cells are formed. In all instances, however, the cells are protonemal and no rhizoids are differentiated. Methanol treatment induces about 95 percent of a population of spores to form twins, whereas twinning in untreated spores is rare.

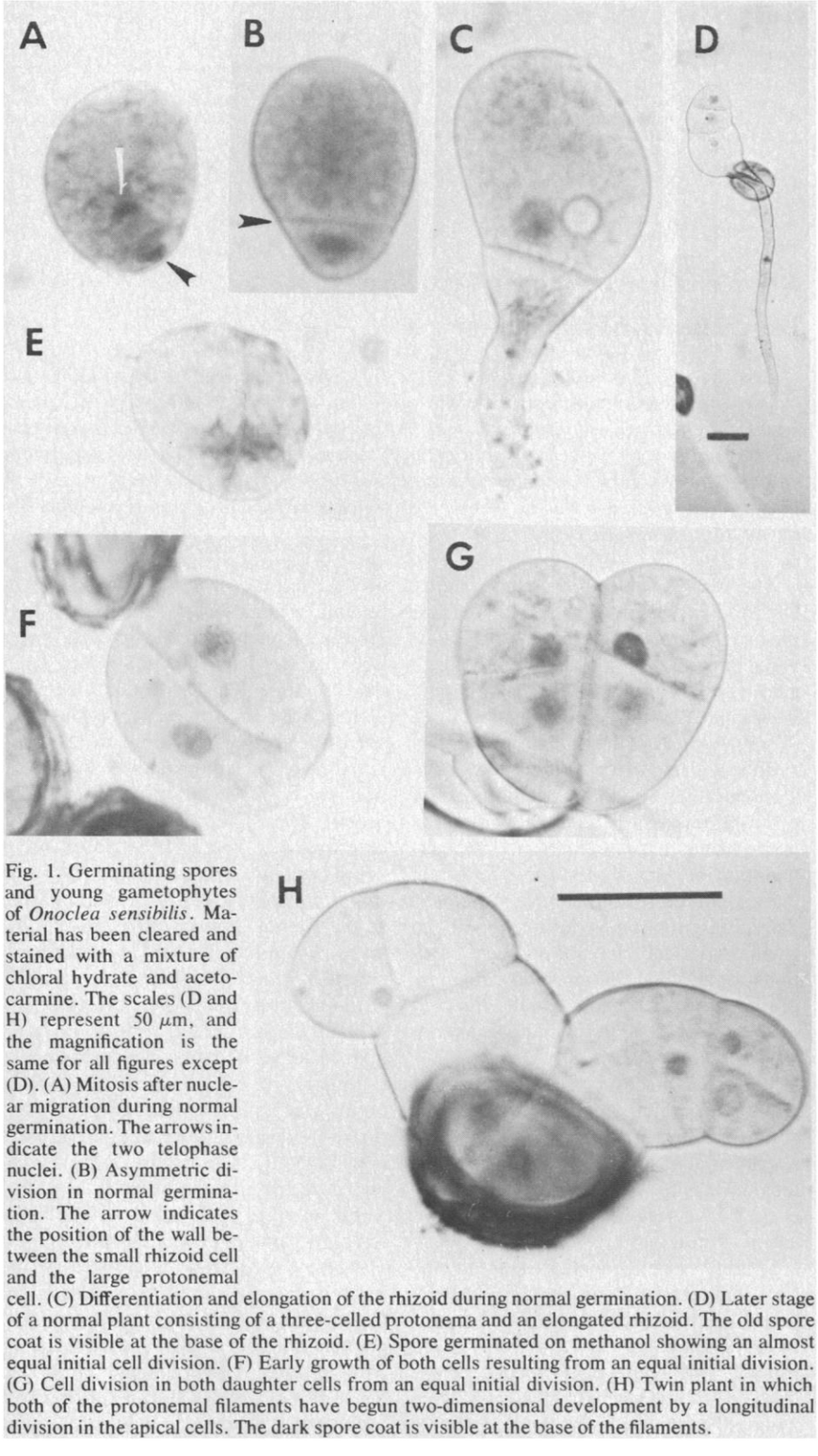

Fig. 1. Germinating spores and young gametophytes of *Onoclea sensibilis*. Material has been cleared and stained with a mixture of chloral hydrate and acetocarmine. The scales (D and H) represent 50 μm, and the magnification is the same for all figures except (D). (A) Mitosis after nuclear migration during normal germination. The arrows indicate the two telophase nuclei. (B) Asymmetric division in normal germination. The arrow indicates the position of the wall between the small rhizoid cell and the large protonemal cell. (C) Differentiation and elongation of the rhizoid during normal germination. (D) Later stage of a normal plant consisting of a three-celled protonema and an elongated rhizoid. The old spore coat is visible at the base of the rhizoid. (E) Spore germinated on methanol showing an almost equal initial cell division. (F) Early growth of both cells resulting from an equal initial division. (G) Cell division in both daughter cells from an equal initial division. (H) Twin plant in which both of the protonemal filaments have begun two-dimensional development by a longitudinal division in the apical cells. The dark spore coat is visible at the base of the filaments.

Methanol concentrations of 3, 4, and 5 percent produce increasingly severe inhibitions of mitosis as well as of nuclear migration, and germination is reduced. Even 5 percent methanol, which almost totally blocks germination, is surprisingly nontoxic since germination occurs soon after spores are transferred from methanol to the standard medium. In these circumstances germination begins with a normal asymmetric division, and a rhizoid differentiates from the smaller cell. Nuclear migration seems to be more sensitive to methanol inhibition than is mitosis; a concentration of 2.5 percent methanol blocks nuclear movement but does not stop division. In this difference rests our ability to induce symmetrical germination. All germination activities are suspended in spores continuously exposed to 5 percent methanol. In effect, germination does not begin until the spores are transferred to a medium without methanol, on which the ensuing germination is normal.

Methanol treatment does not permanently suppress the ability to form rhizoids in twin plants. When such plants are transferred from an inorganic medium containing methanol, rhizoids begin to appear after a few days of growth. This delayed differentiation of rhizoids occurs when one or more cells in a twin plant undergo asymmetric division; the smaller cells develop immediately into

rhizoids. The pattern is identical to that described for normal secondary rhizoid formation in fern gametophytes (*5*).

These observations support the hypothesis that asymmetry of cell division is a key factor in cellular differentiation (*1*). In our example, the differentiation of a cell into a rhizoid is associated with the size of the cell in which the nucleus resides. A treatment that replaces the normal, unequal division of a fern spore with an equal division drastically changes the differentiation of one of the daughter cells. In an other instance, extensive twinning seems to be induced in spores of the fern *Anemia phyllitidis* by treatment with allogibberellic acid (*6*). Spore germination in *Anemia*, however, normally occurs with a symmetrical first division that produces equal cells; the rhizoid does not originate until one of the equal cells divides asymmetrically (*7*). The effect of allogibberellic acid could be the result of the suppression of the second cell division combined with some enlargement of each of the initially equal cells.

The most interesting feature of the system is an apparent coupling between cytokinetic asymmetry and cellular differentiation, which can be controlled by a simple technique. One may produce for comparative purposes populations of spores in which either (i) rhizoid differentiation occurs normally or (ii) essentially no rhizoids are produced and all cells are protonemal.

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[^{3}H]Morphine Localization in Myenteric Plexus

Abstract. *Preferential binding of ^{3}H-labeled morphine to satellite cells, but not to large neurons in the myenteric plexus, is demonstrated autoradiographically. Microfluorometric spectra of the plexus show nerve fibers that contain norepinephrine and impinge on satellite cells. Cells containing serotonin occur occasionally on longitudinal muscle outside the myenteric plexus.*

To demonstrate that narcotic analgesic drugs act at a specific anatomical site, it is important that their localization be correlated with various pharmacological, physiological, and biochemical parameters. There is considerable evidence suggesting interactions of morphine with one or more neurotransmitters, but such evidence regarding specific neurotransmitters is often confusing, contradictory, and incomplete. Acetylcholine, catecholamines, and indolealkylamines have all been implicated in the pharmacological effects of morphine (*1*). While a major site of action of narcotic analgesics is considered to be within the central nervous system, the complexity of the brain and neurotransmitters has made the elucidation of the mode of action of such analgesics a formidable task. To circumvent some of the problems associated with the study of brain a simpler peripheral system was proposed as a model (*2*). This system, consisting of isolated guinea pig ileum with the myenteric plexus attached to the longitudinal muscle layer, is useful for the study of narcotic drugs and their antagonists.

The myenteric plexus preparation serves as a model of the central nervous system because it contains neurons, satellite cells, glial elements, and nerve fibers. It has a large surface area, which facilitates the diffusion of drugs into the tissue from incubation media and, because it is stripped from the circular muscle of the ileum, it is sufficiently thin for use in cellular autoradiography and fluorescence microscopy. Experiments with isolated myenteric plexus preparations have demonstrated that the agonist and antagonist actions of narcotic analgesic agents are dose-dependent and stereospecific. Morphine and other narcotic agonists inhibit the contraction of ilia smooth muscle, and such inhibition is reversed by narcotic antagonists (*3*).

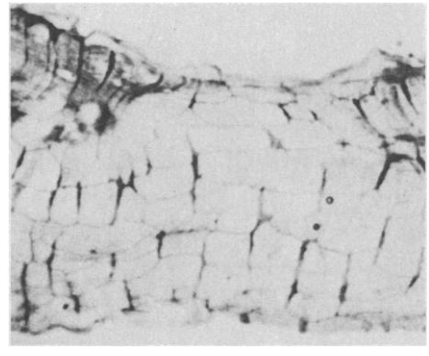

Fig. 1. Guinea pig ileum preparation. The longitudinal muscle strip contains the myenteric plexus which has a honeycomb appearance. Stained with 0.01 percent toludine blue (×16).

Using combined autoradiography, fluorescence microscopy, and microspectrofluorometry we have identified those cells within the myenteric plexus preparation that became labeled with [^{3}H]morphine; we have characterized the biogenic amines by their excitation and emission spectra, and have correlated the localization of morphine and putative neurotransmitters within the same preparation.

Female guinea pigs (150 to 200 g) are starved for 24 hours prior to decapitation. The ilia are cut 2 cm from the cecum to a length of 20 to 30 cm, flushed with ice-cold Tyrode's solution, and divided into segments of 4 to 5 cm in length. A flushed segment is then pulled over a 2-ml pipette for isolation of the longitudinal muscle layer with the attached myenteric plexus. The external longitudinal muscle layer is separated from the internal circular muscle at one end of the segment by gentle rubbing with a Q-tip wetted with Tyrode's solution. The separated longitudinal muscle is then grasped with a small tweezer and stripped free from the circular muscle along the entire length of the intestinal segment. The isolated longitudinal muscle strip with the myenteric plexus attached is placed in a petri dish containing ice-cold Tyrode's solution for dissection of 4- to 6-mm sections, and examined for the presence of plexus under the dissecting microscope. The attached plexus on the longitudinal muscle is identified by its honeycomb appearance (Fig. 1). These sections are then incubated in Tyrode's solution containing 0.1 μg/ml of [^{3}H]morphine (1 μc/ml) for 1 minute at 25°C, washed three times for 1 minute in nonradioactive Tyrode's solution to remove the unbound [^{3}H]morphine, and mounted directly on Formvar-coated microscope slides without adhesive or media. The sections are dried in air and then stored over Drierite for 24 hours. Sections prepared in this manner may be coated directly with NTB-3 Kodak liquid emulsion for autoradiography. For the combined cytopharmacological method, a Formvar-coated slide is cemented at one end to a cover slip previously coated with NTB-3 emulsion. Both slide and cover slip are of the same optical glass