which phosphorus (and probably other nutrients) are transferred among higher plants. Although the mechanisms and dynamics of transfer are not yet known, this phenomenon has serious implications for studies of plant microhabitats, interplant competition, comparative demography and phenology, and ecosystem nutrient cycling. Because nutrient exchange potentially contributes to the differential success of individuals, attempts to relate plant success only to aboveground neighborhood structure or spatial pattern (*1*) may prove ultimately unsuccessful.

NONA CHIARIELLO*
Biological Sciences,
Stanford University,
Stanford, California 94350
JAMES C. HICKMAN
Jepson Herbarium,
Department of Botany,
University of California,
Berkeley 94720
HAROLD A. MOONEY
Biological Sciences,
Stanford University

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11. The mechanism for transfer of nutrients from host to mycobiont is not known [R. E. Beever and D. J. W. Burns, *Adv. Bot. Res.* **8**, 128 (1980)]. Root exudation of phosphorus followed by uptake by adjacent hyphae (root-soil-hypha pathway) has been proposed, but direct transfer from host to mycobiont (root-hypha pathway) is suggested by J. Whittingham and D. J. Read in (*10*). Our term "functionally connected" is intended to emphasize that we are not certain whether root exudation is involved in transfer, but we expect that hyphae will enhance transfer in either case.
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14. In an earlier experiment, an acidic solution of ^{32}P was used, and the cuticular barrier to uptake was breached. Donors retained an average of only 51 percent of the recovered ^{32}P (compared with more than 90 percent when buffered), suggesting that added phosphorus is readily mobile once it gains entry into the plant.
15. Background variation was low (standard error, 2.3 count/min), and there was no evidence of cross-contamination of the samples. We assumed that plants with fewer than 5 count/min (~ 2 standard errors) had received no ^{32}P and, to allow conservatively for errors such as aerial transport and cross-contamination, we assumed that plants with 6 to 50 count/min (up to 22 standard errors) had not received a significant amount of ^{32}P directly through their root systems.
16. Our buried-slide experiments suggest that the number of hyphal connections between roots is related to their proximity. Because there is a correlation (albeit weak) between shoot and root distributions, we do not suspect that the patterns (Fig. 2) result merely from anomalous root distribution but that there is a significant stochastic component to the establishment of the symbiosis relative to aboveground spatial patterns.
17. In a second set of experiments, we tested net flow of phosphorus by excising developing inflorescences, which should constitute strong phosphorus sinks, from half the neighbors of each of five donors. Among plants receiving significant ^{32}P transfer, there was no significant difference between experimental and control plants and no correlation between the specific activities of vegetative and reproductive portions of controls. These results may be explained by the fact that the mycorrhizal symbiosis is dependent on root exudation, which may be altered during the development of inflorescences (J. A. Menge, personal communication).
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* Present address: Department of Biology, University of Utah, Salt Lake City 84112.

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Calcium Ionophore A23187 Stimulates Cytokinin-Like Mitosis in *Funaria*

Abstract. *The plant hormone cytokinin stimulates asymmetrical division in target cells of the protonema of the moss* Funaria hygrometrica, *leading to bud formation. The initial division can be induced in the absence of cytokinin by the calcium ionophore A23187 in medium containing calcium. These findings suggest that increases in the concentration of intracellular calcium are essential to bud initiation. Therefore mitotic regulation by cytokinin may be due, at least in part, to the modulation of intracellular calcium ion concentration.*

The plant hormone cytokinin evokes a wide spectrum of responses in higher plants, including the induction of cell division (*1*). In the filamentous protonema of *Funaria hygrometrica* Hedw., exogenous cytokinin induces an asymmetrical division at the distal ends of elongate target cells (caulonema cells and the basal cell of side branches) to form a small initial cell (*2*). This initial cell continues to divide in three planes, leading to bud formation and the leafy gametophyte. Using the fluorescent Ca^{2+} chelate probe chlorotetracycline (CTC), we previously found an increase in membrane-associated calcium at the presumptive bud site in the target cell after cytokinin treatment (*3*). The CTC fluorescence remained bright in the dividing cells of the bud, suggesting that the hormone achieves at least part of its stimulatory effect through a localized modulation of the concentration of intracellular Ca^{2+}. In addition, bud formation is inhibited in Ca^{2+}-free medium and by the Ca^{2+} transport inhibitors La^{3+}, D 600, and verapamil, indicating that cytokinin is dependent on external Ca^{2+} (*4*). We have tested our hypothesis linking cytokinin and Ca^{2+} further, and report that artificially generated increases in intracellular Ca^{2+} caused by the divalent ionophore A23187 (*5*) induce bud initiation in the absence of exogenous cytokinin.

Sterile protonemata were grown from single spores on nutrient agar for 8 to 10 days (*6*). Production of the target caulonemata (which are characterized by small chloroplasts and oblique end walls) is enhanced by transfer to liquid medium for 4 days. Two protonemata (mean fresh weight, 14 mg) were transferred to 15 ml of fresh medium containing 0.3 m*M* Ca^{2+} and 0.1 m*M* Mg^{2+} (*p*H 6.4), and 1 to 60 μl of A23187 stock solution (15 m*M* dissolved in 100 percent methanol) (*7*) was pipetted over the tissue and quickly stirred. Much of the ionophore was visible as a milky precipitate (*8*). Control media included one with equivalent amounts of methanol, one with no additives, and one treated with 1 μ*M* benzyladenine, a synthetic cytokinin. Bud production was monitored for several days (*9*).

Filamentous tip growth and side branch formation continued in both the methanol-treated and no-additive media (Fig. 1A). In protonemata grown in liquid culture initiation of side branches occurred at the seventh or eighth cell from the tip, whereas on agar initiation took place at the third cell. Two successive transfers to fresh media ensured that naturally formed cytokinins did not build up in the medium to cause bud forma-

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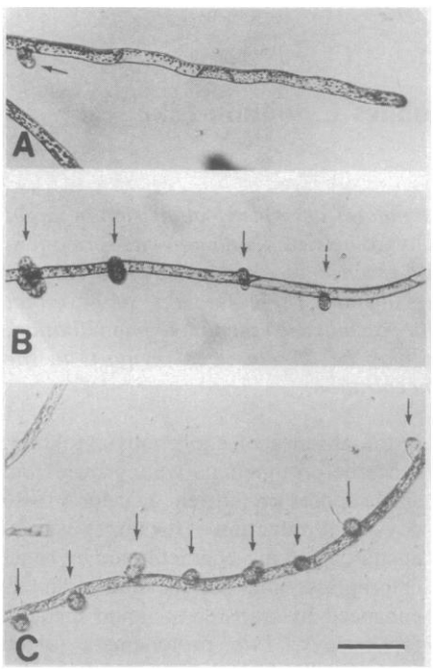
tion. However, in the benzyladenine-treated medium initial cells were visible on target cells after 24 hours (Fig. 1B).

In 40 μM A23187 (0.3 mM Ca^{2+}), virtually every target cell divided in a manner analogous to that of cytokinin-induced division (Fig. 1C). Although the cells appeared healthy overall, there was some localized cell death, with tip cells the most susceptible to damage (*10*). A higher concentration of A23187 (60 μM) did not increase the response, and more cells died. Lower concentrations of A23187 (1 to 10 μM) had little or no effect on bud production; however, in 20 μM A23187 some initial cells were visible, usually clustered on one portion of the protonemal mat. This patchy distribution may result from the topical application of the ionophore leading to its uneven distribution in the tissue. We determined the distribution of A23187 with fluorescence microscopy (*11, 12*). Cells responding to the ionophore exhibited blue fluorescence (Fig. 2).

Because there are similarities in the initial developmental events leading to the formation of buds and side branches, attention has been given to characterizing the A23187-induced initial cell (Fig. 3). The following observations support the conclusion that the divisions induced by A23187 are similar to hormone-induced divisions. First, both cytokinin- and A23187-stimulated divisions occur closer to the distal end walls than do divisions that produce branches. Second, the new wall defining the first cell of a side branch lies in the same plane as the original wall of the caulonema cell—parallel to the long axis—whereas the new wall of a cytokinin-induced initial cell often curves into the caulonema cell (forming a lens-shaped cell) and occasionally fuses with the original cross wall of the target cell. The A23187-induced initial cell follows the latter pattern. Third, the initial cell appearing after the application of cytokinin or A23187 is spherical in shape, as opposed to the more cylindrical shape of branch initial cells. Fourth, the CTC fluorescence of the ionophore-induced initial cell is identical to that of benzyladenine-induced buds and is distinct from that of branches. Finally, in some instances A23187-induced initial cells continue to divide and form a bud with a typical tetrahedral apical cell. Normally though, the A23187-induced initial cells fail to divide in three planes, and some revert to filamentous growth.



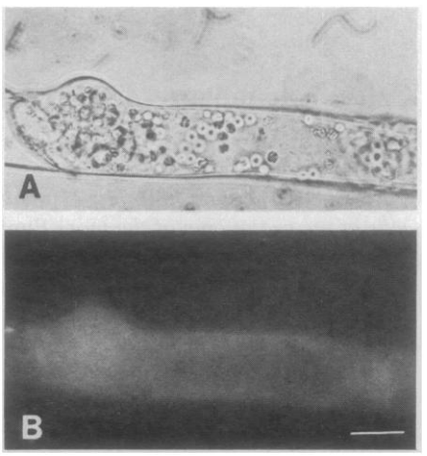


Fig. 1 (left). (A) Caulonemata 24 hours after treatment with 0.2 percent methanol. The initial cell of a side branch (arrow) is visible seven cells from the tip. (B) Initial cells (arrows) induced by treatment with 1 μM benzyladenine. (C) Result of treatment with 40 μM A23187. Initial cells (arrows) are present on every target cell. Scale bar, 500 μm. Fig. 2 (right). Phase-contrast (A) and fluorescence (B) micrographs of a caulonema cell treated with 20 μM A23187. Chloroplast fluorescence was blocked with a 560-nm barrier filter. Scale bar, 50 μm.

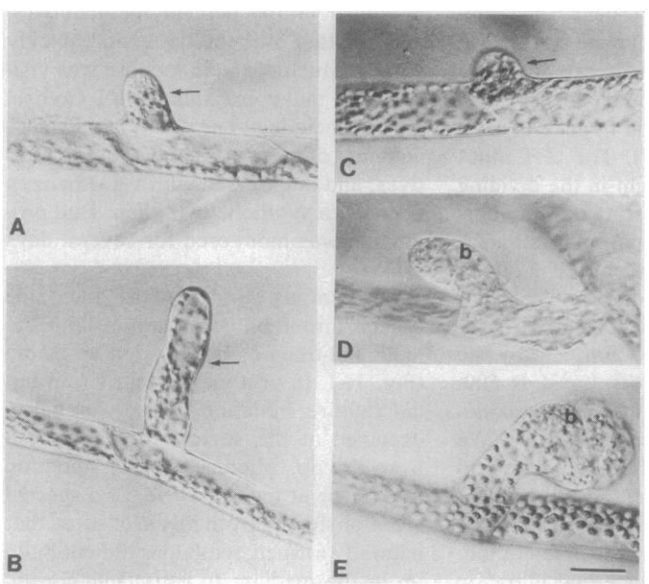


Fig. 3. (A and B) Side branch (arrow) in an early stage (A) and later in its development (B). (C to E) Bud formation induced by 40 μM A23187. (C) Initial cell (arrow) 24 hours after treatment; note placement of the new cross wall. (D) Bud (*b*) 36 hours after treatment. (E) Bud 72 hours after treatment. Scale bar, 50 μm.

The effectiveness of A23187 is highly dependent on the presence of extracellular Ca^{2+} and much less dependent on extracellular Mg^{2+}. Removal of Ca^{2+} from the medium eliminates the response, whereas removal of Mg^{2+} has no effect; raising the concentration of Mg^{2+} to 0.2 or 2 mM cannot substitute for Ca^{2+} in inducing this division. High external Ca^{2+} alone (0.1M) can stimulate an asymmetrical division without inducing an accompanying outgrowth (*13*). A similar division is sometimes seen in older caulonema cells treated with A23187.

Attempts were made to force the A23187-induced initial cells farther along their developmental pathway by a variety of treatments, including a second application of A23187 and raising or lowering the concentration of Ca^{2+} and the *p*H of the bathing medium. We were unable to increase the number of initial cells that eventually formed buds; however, the addition of 1 μM benzyladenine to the A23187-induced initial cells stimulated complete bud development. This indicates that, although these cells are capable of forming buds, other cytokinin-induced factors are necessary which an elevation in intracellular Ca^{2+} alone cannot trigger.

In cytokinin-induced buds the hor-

mone must remain in the medium for continued bud development. Removal of cytokinin causes the cells to revert to filamentous growth, indicating that cytokinin does not act as a trigger but must be present throughout development (*2*). Removal of A23187 from the cells by washing after 5 hours also eliminates the response, suggesting that elevated intracellular Ca^{2+} must be maintained for development to proceed. This finding agrees with our CTC results, which suggest that a long-term change in Ca^{2+} is associated with bud formation (*3*).

Our findings are consistent with the calcium hypothesis of Metcalfe *et al.* (*14*) that the transition of cells from the resting state (G_0) is regulated by an increase in the concentration of free calcium in the cytoplasm. The increase in intracellular Ca^{2+} must be of long duration. It is necessary to expose human lymphocytes to A23187 for more than 20 hours (over a wide range of Ca^{2+} concentrations) to stimulate mitosis (*15*); a short exposure to a high concentration of Ca^{2+} does not generate a signal that commits the cell to divide (*14, 16*). Similarly, in *Funaria* stimulation of mitosis by A23187 appears to require the maintenance of high intracellular Ca^{2+} for several hours. There is evidence that target cells in *Funaria* are arrested in G_2 whereas nontarget cells are maintained in G_1 (*17*). Indeed, Fosket (*18*) postulated that cytokinin stimulates essential events in the transition from G_2 to mitosis in the plant cell cycle. We believe that an essential step may be the establishment of a long-term increase in intracellular Ca^{2+}. These results, together with earlier reports linking cytokinin and stimulation of Ca^{2+} uptake in other systems (*19–21*), suggest that cytokinin exerts its effect on *Funaria* by elevating intracellular Ca^{2+}. The increase is spatially controlled in the target cell and therefore may determine the location of the initial asymmetrical division leading to bud formation.

MARY JANE SAUNDERS
PETER K. HEPLER
Botany Department, University of Massachusetts, Amherst 01003

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Subperiosteal Expansion and Cortical Remodeling of the Human Femur and Tibia with Aging

Abstract. *Increases with aging in subperiosteal dimensions and second moments of area (measures of bending and torsional rigidity) in femoral and tibial cross sections are documented in an archeological sample from the American Southwest. Significant differences between cross-sectional sites and between sexes in the pattern of cortical remodeling with age are also present. These differences appear to be related to variations in the stress or strain levels in different regions of the femur and tibia which result from in vivo mechanical loadings of the lower limb.*

Subperiosteal expansion of long bones with aging was first demonstrated radiographically for the femur by Smith and Walker (*1*) and has since been shown to occur in several other areas of the skeleton (*2, 3*). These observations are clinically significant in that apposition of bone on the subperiosteal surface may mechanically compensate for endosteal resorption and cortical thinning with aging (*1, 2*), thus reducing the risk of fracture among older adults (*4*). We present here evidence that supports a model of general subperiosteal expansion of long bones with aging but also indicates that this remodeling process is modified by localized bone site differences in mechanical loadings, some of which are apparently sex-specific. The results suggest that exercise-related stress and strain is an important stimulus to subperiosteal appositional growth and skeletal remodeling in adults, and consequently that relatively less mechanically stressed areas may be more subject to fracture in later life.

A major problem common to most studies of cortical bone remodeling with aging is that they rely on extrapolations from one-dimensional radiographic breadths, a procedure that can cause significant errors in the estimation of two-dimensional cross-sectional parameters (*5*). There is at present no practical noninvasive technique available for accurately measuring the cross-sectional geometries of internal body segments in vivo in large samples (*6*). For this reason we chose direct measurement of cut bone sections in vitro as a means for studying geometrical changes with age.

A sample of femora and tibiae from 119 individuals was selected from the Pecos Pueblo archeological collection, excavated from a large late prehistoric and protohistoric site in New Mexico (*7*). Archeological samples have the advantage of being generally more genetically and environmentally homogeneous than modern dissection room collections; thus, intrapopulational differences in skeletal structure due to factors such as sex and age may be defined more clearly (*8*). Pelvic and cranial morphology were used to determine sex and age estimates were based upon pubic symphyseal form, functional dental wear, and endocranial suture closure (*9*). The use of multiple skeletal indicators has been shown to yield sex estimates with an accuracy of better than 95 percent; age estimation is adequate for placing individuals into 5-year categories in the third and fourth decades and into 10-year cate-

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