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31. The expression vector pCEV4 was constructed from pcDSRα (*33*). The polyoma virus origin (Bgl I–Bcl I fragment) was inserted into the Nde I site of pcDSRα and the Xba I fragment containing two Bst XI sites of CDM8 (*32*) was inserted in the Pst I site of the pcDSRα. A unique Not I site in the Xba I fragment was removed by filling in, and the Not I linker was inserted at the Cla I site of pcDSRα.
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Microinjection of a Conserved Peptide Sequence of p34^{cdc2} Induces a Ca^{2+} Transient in Oocytes

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The product of the yeast cell cycle control gene *cdc2*, and its homologs in higher eukaryotes (p34^{cdc2}), all contain a perfectly conserved sequence of 16 amino acids that has not been found in any other protein sequence. Microinjection of this peptide triggers a specific increase in the concentration of intracellular free Ca^{2+} that originates from intracellular stores in both starfish and *Xenopus* oocytes. Thus, p34^{cdc2} might interact through its conserved peptide domain with some component of the Ca^{2+}-regulatory system.

ENTRY INTO AND EXIT FROM M phase of the cell cycle are controlled by changes in the activity of maturation promoting factor (MPF) (*1*), a two-subunit mitotic protein kinase (*2*). The catalytic and regulatory subunits of MPF are encoded by homologs of the yeast cell cycle control genes *cdc2*$^+$ (*3–7*) and *cdc13*$^+$ (*2*), respectively. The yeast *cdc2* product and its homologs in higher eukaryotes (p34^{cdc2}) all contain a perfectly conserved 16-residue sequence, EGVPSTAIREISLLKE (*8*) (called PSTAIR), which has not been found in any other protein sequence (*9*). Microinjection of the PSTAIR peptide is sufficient to induce meiotic maturation in starfish oocytes (*4*) and it accelerates the action of added MPF in the *Xenopus* system (*6*).

As well as inducing MPF activation and germinal vesicle breakdown (*4*), microinjection of the PSTAIR peptide into starfish oocytes results in cortical granule exocytosis (CGE) and elevation of a fertilization membrane (Fig. 1). The threshold intracellular concentration of PSTAIR peptide required to induce CGE is the same as for triggering MPF activation (~ 0.4 m*M*). A rise in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) induces CGE after fertilization or artificial activation (*10*). Co-injection of EGTA with the PSTAIR peptide suppressed CGE (Fig. 1), suggesting that Ca^{2+} is also required for PSTAIR peptide–induced CGE. In contrast, EGTA does not suppress MPF activation and germinal vesicle breakdown, confirming that a rise in $[Ca^{2+}]_i$ is not required for the prophase to metaphase transition during meiotic maturation of oocytes (*11*). To confirm that PSTAIR peptide–induced CGE was a Ca^{2+}-dependent event, we monitored $[Ca^{2+}]_i$ in oocytes loaded with the Ca^{2+} indicator indo-1. The $[Ca^{2+}]_i$ increased from ~0.1 μ*M* to 1 μ*M* or higher within 1 min after microinjection of the PSTAIR peptide, and then decreased slowly with a half-time of ~90 s toward its resting value (Fig. 2).

The product of the budding yeast gene *PHO85* is a 34-kD protein that is homologous to the *cdc2* product and contains a 16–amino acid sequence in which 14 residues are identical to those in the PSTAIR peptide (*12*). Thus, it was possible that the PSTAIR peptide was mimicking *PHO85* rather than

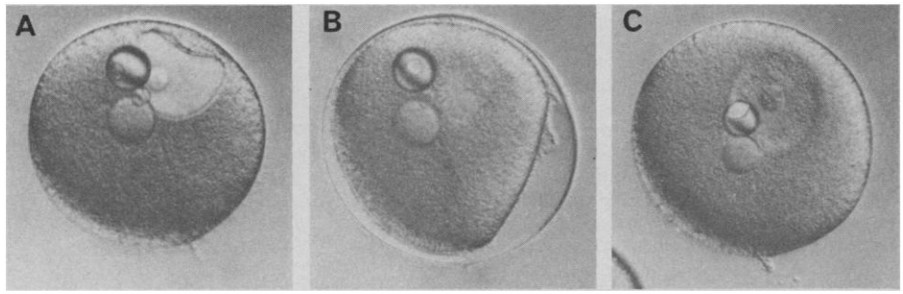

Fig. 1. Microinjection of the PSTAIR peptide induces germinal vesicle breakdown and elevation of the fertilization membrane in starfish oocytes. Oocytes of the starfish *Marthasterias glacialis* were prepared free of follicle cells (*4*) and then transferred to natural sea water. The PSTAIR peptide was solubilized in distilled water (20 mg/ml; ~12.5 m*M*) and microinjected (*30*), to give a final intracellular concentration of 1 m*M*, together with two oil droplets (preventing contact with sea water). About 50 oocytes were microinjected, giving identical results. (**A**) Micrograph taken 2 min after microinjection. The germinal vesicle (oocyte nucleus) is still limited by an intact envelope and CGE has not occurred. (**B**) The same oocyte as in (A), 25 min after peptide microinjection. The germinal vesicle has broken down and a fertilization membrane has elevated as a consequence of CGE. (**C**) Another oocyte injected first with 1 m*M* EGTA (*p*H 7.0), and then with 1 m*M* PSTAIR (both concentrations are intracellular). The micrograph was taken 17 min after peptide microinjection. The envelope limiting the germinal vesicle has disappeared, but the fertilization membrane did not elevate. It did not elevate and CGE did not occur even 1 hour later.

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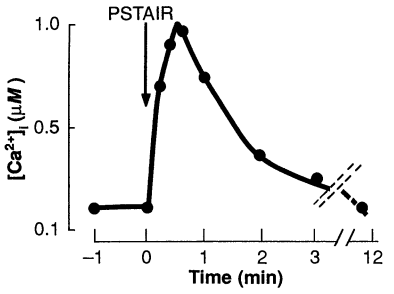

Fig. 2. A typical example of the transient rise in $[Ca^{2+}]_i$ after microinjection of the PSTAIR peptide into oocytes of the starfish *M. glacialis*. Oocytes were microinjected first with 5 μ*M* indo-1, then with 0.4 m*M* PSTAIR peptide (both final concentrations). We monitored indo-1 fluorescence at two wavelengths (405 and 470 nm) with a dual photomultiplier detection system adapted to an inverted microscope. The $[Ca^{2+}]_i$ values were obtained by computation of the fluorescence ratio at the two wavelengths as described (*31*).

$p34^{cdc2}$. We synthesized the 16-residue peptide corresponding to the PSTAIR region of *PHO85*, as well as two peptides that differ from PSTAIR by only a single residue (Table 1). None of these peptides (up to an intracellular concentration of 1 m*M*) induced CGE or MPF activation in starfish oocytes. Likewise, two peptide fragments of the PSTAIR peptide, EGVPSTAIRE and ISLLKE, either individually or in combination, failed to elicit CGE or MPF activation after microinjection into oocytes. Thus, the entire PSTAIR peptide structure was required to induce specifically a transient increase of $[Ca^{2+}]_i$ and MPF activation in starfish oocytes.

An alternative way to monitor an increase in $[Ca^{2+}]_i$ in *Xenopus* oocytes is to monitor the activity of Ca^{2+}-dependent Cl^- channels under voltage-clamp conditions (*13–16*).

Table 1. Biological activities of the synthetic PSTAIR peptide and its analogs. Solid phase synthesis of the peptides was performed with a 9050 peptide synthesizer (Milligen) with polyacrylic resin (*29*), and 9-fluorenylmethyloxycarbonyl for temporary α-amino group protection. Peptides were microinjected into starfish oocytes at intracellular concentrations in the range 0.2 to 1 m*M*. Biological activities were monitored by phase-contrast microscopy (Fig. 1). Underlined residues highlight differences to PSTAIR sequence.

Peptide	Biological activities	
	MPF	CGE
EGVPSTAIREISLL KE*	Yes	Yes
EGTPSTAIREISLM KE†	No	No
EGTPSTAIREISLL KE	No	No
EGVPSTAIREISLM KE	No	No
EGVPSTAIRE	No	No
ISLL KE	No	No

*PSTAIR peptide sequence. †PSTAIR homolog found in *PHO85* product.

The PSTAIR peptide induced a transient inward current, starting ~10 to 20 s after microinjection in oocytes clamped at −80 mV (Fig. 3A). The reversal potential of the PSTAIR peptide–induced current was found to be approximately −29 mV, which is similar to the reversal potential reported for Cl^- current in *Xenopus* oocytes (*15*). Moreover, the PSTAIR peptide–induced current was not detected in oocytes microinjected with EGTA. The threshold concentration of PSTAIR peptide required to elicit the Ca^{2+}-dependent Cl^- current was ~20 μ*M*, and the maximal response was obtained at 50 μ*M*. This is consistent with the optimal peptide concentration (25 μ*M*) for acceleration of MPF-induced nuclear envelope breakdown and chromosome condensation in cell-free extracts prepared from *Xenopus* eggs (*6*). Microinjection of the *PHO85* 16-residue PSTAIR peptide homolog had no effect, even at a 50-fold higher concentration (Fig. 3B).

The PSTAIR peptide–induced increase in $[Ca^{2+}]_i$ was not dependent on extracellular Ca^{2+}; it occurred even in the presence of external Cd^{2+} (0.1 m*M*) or Mn^{2+} (5 m*M*) (*17*), which block Ca^{2+} currents in oocytes (*13, 18*). Thus the PSTAIR peptide must induce the release of Ca^{2+} from intracellular stores in both starfish and amphibian oocytes. Although the peptide induces both the release of intracellular Ca^{2+} and the activation of MPF in starfish oocytes, the increase of $[Ca^{2+}]_i$ was not dependent on activation of the mitotic kinase. Microinjection of $p13^{suc1}$, a yeast protein that interacts physically with the *cdc2* kinase (*19, 20*), suppressed the 30-fold increase in activity of the mitotic kinase in response to the PSTAIR peptide without affecting the PSTAIR peptide–dependent increase in $[Ca^{2+}]_i$ (*21*). Moreover, histone H1 kinase activity did not increase at all in *Xenopus* oocytes after microinjection of the PSTAIR peptide (up to 100 μ*M*). Therefore, the catalytic activity of the MPF kinase did not appear to be necessary for the transient increase in $[Ca^{2+}]_i$.

Although not required for meiotic maturation, Ca^{2+} has long been considered to be a necessary signal for the initiation of mitosis (*22*). A transient elevation of $[Ca^{2+}]_i$ is necessary for regulating the onset of mitosis (*23–25*). Also, sea urchin embryos can be released from the arrest after the first mitotic cleavage caused by inhibition of protein synthesis simply by increasing $[Ca^{2+}]_i$ (*25*). This has led to the suggestion that preventing cyclin synthesis prevents the increase in $[Ca^{2+}]_i$ responsible for nuclear envelope breakdown and chromosome condensation. In other words, it was suggested that the clock controlling the timing of the transient changes of $[Ca^{2+}]_i$ during the cell cycle was coupled to the MPF oscillator. Our results support this view and suggest that the PSTAIR region of $p34^{cdc2}$ interacts in a specific reaction with a cell component to regulate $[Ca^{2+}]_i$. This component could be part of, or interact with, the calsequestrin-containing organelles that have been shown to regulate $[Ca^{2+}]_i$ in nonmuscle cells, including eggs (*26*). When antibodies to

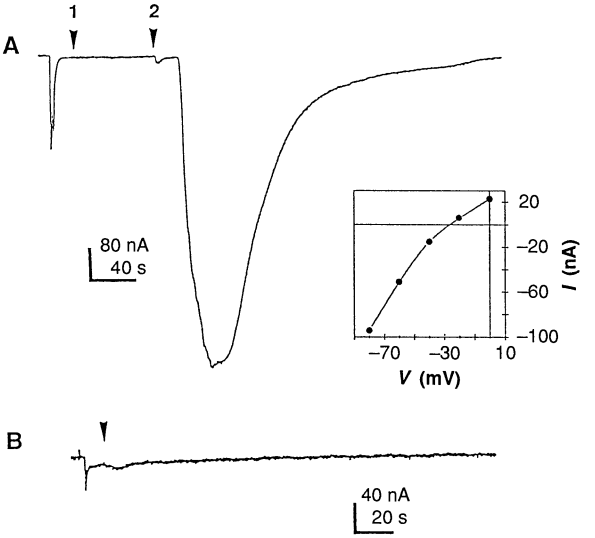

Fig. 3. The PSTAIR peptide (**A**), but not the homologous peptide from the *PHO85* product (**B**), induces a Cl^- current in *Xenopus* oocytes. The peptides, diluted in sterile water, were microinjected (arrowhead) with a micrometer-driven 10-μl micropipette (Drummond Scientific, Broomall, Pennsylvania). The initial transient downward deflection on each trace represents penetration of the micropipette. Inward current is denoted by a downward deflection of the trace. The final intracellular concentrations of peptides were 15 μ*M* and 30 μ*M* [(A) arrowheads 1 and 2, respectively] and 2 m*M* (B). The current-voltage (*I-V*) curve of the PSTAIR peptide–induced current (A) is shown in the inset. For these experiments, stage 6 oocytes were defolliculated by collagenase treatment [Type 1A (Sigma); 1000 units/ml] in Ca^{2+}-free ND96 solution [96 m*M* NaCl, 2 m*M* KCl, 1 m*M* $MgCl_2$, 5 m*M* Hepes-NaOH (*p*H 7.4)]. The oocytes were washed several times, stored, and tested in normal ND96 solution (containing 1.8 m*M* $CaCl_2$). Whole-cell current was recorded from single oocytes with a two-microelectrode voltage-clamp amplifier. The microelectrodes were filled with 3*M* KCl and voltage-clamp experiments were performed from a holding potential of −80 mV. Stimulation of the preparation, data acquisition, and analyses were monitored with an IBM PC using pCLAMP software (Axon Instruments, Burlingame, California). Each experiment was repeated at least five times with oocytes from different females.

p34^{cdc2} are used to precipitate p34^{cdc2}-containing complexes from cell extracts, a number of bands are detected (*27*). Moreover, it has been proposed that p34^{cdc2} might participate in a number of different complexes (*28*). One of them might include the unknown Ca^{2+}-regulatory component.

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Far-Red Radiation Reflected from Adjacent Leaves: An Early Signal of Competition in Plant Canopies

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When individual seedlings of *Datura ferox* and *Sinapis alba* were transferred to populations formed by plants of similar stature, they responded with an increase in the rate of stem elongation. The reaction was detected within 3 days after transplanting and occurred well before shading among neighbors became important. This rapid response, which may be crucial for success in the competition for light, was reduced or abolished when individual internodes were "blinded" to the far-red radiation scattered by the surrounding seedlings. These results show the operation of a localized, photomorphogenetic control of stem elongation that may play a central role in the plastic adjustment of plants during the early stages of canopy development.

ONE OF THE MOST STRIKING CHARacteristics of higher plants is their capacity to react to the presence of neighbors with changes in the pattern of morphological development. This is documented by a large number of descriptive studies, but few ecological experiments have been conducted at a sufficiently reductionist level to establish the precise nature of the morphogenetic signals (*1, 2*).

A classic example of morphological plasticity is the redistribution of growth toward stem elongation exhibited by plants of many species when growing in dense populations (*1*). Several factors of the aboveground environment that may influence elongation, such as light intensity and spectral distribution, air movement, air humidity and temperature, are substantially altered when the number of plants per unit area increases (*3*). The spectral distribution of radiation, particularly the ratio of red (R) to far-red (FR) wavelength bands (R:FR), is now widely accepted to be a powerful environmental signal for plants growing in the lower strata of established canopies (*4*). Perception of a low R:FR balance by phytochrome, a photochromic plant pigment, may trigger mechanisms whereby these plants react to shading, accelerating stem elongation (shade-avoidance reactions). This idea is supported by (i) spectroradiometric studies showing that light filtered through a leaf canopy has a low R:FR ratio due to preferential absorption of R light by chlorophyll (*5*) and (ii) physiological studies with isolated plants in which light conditions simulated those experienced by heavily shaded individuals (*6*).

Recent analysis of the growth of plants in even-aged populations revealed, however, that the stems start exhibiting increased rates of elongation well before the leaves become shaded, and thus before they experience the R-impoverished light that prevails under dense vegetation (*7, 8*). This behavior may be crucial for success in the competition for light since, in fast-developing stands, individuals are likely to be rapidly suppressed if their mechanisms of "shade avoidance" begin to operate only after light availability has been severely reduced (*8*). We show that this early reaction to the presence of neighbors is triggered by low R:FR ratios received at the stem level and that this localized drop in the R:FR balance is mainly a consequence of the FR light reflected from nearby leaves.

The possibility that FR radiation reflected from neighboring plants may initiate shade-avoidance reactions before canopy closure was suggested by previous experiments in canopies of low leaf area index (LAI = leaf area per unit of soil area) (*7*). The strongest evidence for this hypothesis came from fiber optic studies showing large changes in the fluence rate of FR light inside the internodes in response to relatively small changes in canopy density (*9*). In the present experiments, seedlings of *Datura ferox* L. and *Sinapis alba* L. were grown in stands of different

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