Spirochetes were visualized by incubation with monoclonal antibodies to flagellin (H9724) (*30*), Vmp7 (H9236) (*31*), or Vmp33 (H4825) (*31*), or a mouse polyclonal antibody to Vmp8 (*7*). This was followed by incubation with goat antibody to mouse immunoglobulin G conjugated with fluorescein isothiocyanate (Kirkegaard and Perry Laboratories, Gaithersburg, MD).
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Gating of CaMKII by cAMP-Regulated Protein Phosphatase Activity During LTP

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Long-term potentiation (LTP) at the Schaffer collateral–CA1 synapse involves interacting signaling components, including calcium (Ca^{2+})/calmodulin–dependent protein kinase II (CaMKII) and cyclic adenosine monophosphate (cAMP) pathways. Postsynaptic injection of thiophosphorylated inhibitor-1 protein, a specific inhibitor of protein phosphatase–1 (PP1), substituted for cAMP pathway activation in LTP. Stimulation that induced LTP triggered cAMP-dependent phosphorylation of endogenous inhibitor-1 and a decrease in PP1 activity. This stimulation also increased phosphorylation of CaMKII at Thr^{286} and Ca^{2+}-independent CaMKII activity in a cAMP-dependent manner. The blockade of LTP by a CaMKII inhibitor was not overcome by thiophosphorylated inhibitor-1. Thus, the cAMP pathway uses PP1 to gate CaMKII signaling in LTP.

Multiple signaling pathways participate in LTP in the CA1 region of the hippocampus at both presynaptic and postsynaptic sites (*1*), with the CaMKII pathway playing a central role in transmitting the postsynaptic signals required for LTP (*2*, *3*). In contrast, the role of other signaling pathways is not yet clear. The cAMP pathway is involved in LTP and memory in transgenic mice (*4*). In rats, the postsynaptic cAMP pathway is required for LTP induced by widely spaced trains of high-frequency synaptic stimulation (HFS), but activation of the pathway is not sufficient to induce LTP (*5*). Thus, the postsynaptic cAMP pathway does not transmit the signals for LTP but rather gates the transmittal pathway. It has been proposed (*5*, *6*) that the cAMP-operated gate may use PP1.

The CA3-CA1 synapse of rat hippocampal slices was stimulated with widely spaced trains of HFS (*7*). The resulting LTP was blocked by inhibiting postsynaptic cAMP-dependent protein kinase [protein kinase A (PKA)] (Fig. 1A) (*5*). This requirement for PKA can be overcome by direct inhibition of postsynaptic phosphatases (*5*), suggesting that the cAMP pathway modulates LTP by blocking phosphatases. Protein phosphatase inhibitor-1 (I-1) is a candidate for mediating cAMP inhibition of phosphatase activity. I-1, upon phosphorylation by PKA at Thr^{35}, is a specific blocker of PP1 (*8*). I-1 mRNA is expressed in CA1 neurons (*9*), and I-1 has already been implicated in plasticity at the CA3-CA1 synapse (*10*). We injected recombinant purified Thr^{35}-thiophosphorylated I-1 (*11*) into the postsynaptic neuron and tested its ability to overcome the blockade of LTP by the specific PKA inhibitor Rp–cyclic adenosine monophosphorothioate (cAMPS). Thiophosphorylated I-1 completely reversed the effect of Rp-cAMPS, yielding LTP that was indistinguishable from that of the control (Fig. 1, B and C). In contrast, mutant [Thr^{35} → Ala (T35A)] nonphosphorylatable I-1 (*11*), when injected postsynaptically, did not reverse the effect of Rp-cAMPS. Thus, PP1 appears to be the postsynaptic phosphatase that negatively regulates LTP, and I-1 activation by PKA may facilitate LTP by inhibiting PP1.

Next, we determined if LTP-inducing stimulation results in PKA phosphorylation of I-1. We examined the phosphorylation state of I-1 in the CA1 region after HFS (*12*). The same pattern of synaptic stimulation that induced cAMP-dependent LTP also raised the amount of phosphorylated I-1 in the CA1 region (Fig. 2A). The increase in phosphorylation of I-1 by HFS was dependent on PKA activity because it was blocked by the inclusion of Rp-cAMPS in the superfusate during stimulation (Fig. 2A). We then measured protein phosphatase activity in the CA1 region of stimulated and unstimulated slices (Fig. 2B). Under our assay conditions (*13*), greater than 90% of the phosphoprotein phosphatase activity in the CA1 homogenate was inhibited by thiophosphorylated I-1, defining it as PP1. HFS that activated I-1 also resulted in significant inhibition of PP1 activity. This effect was prevented by bath application of Rp-cAMPS during stimulation, indicating that PKA mediated the phosphatase inhibition.

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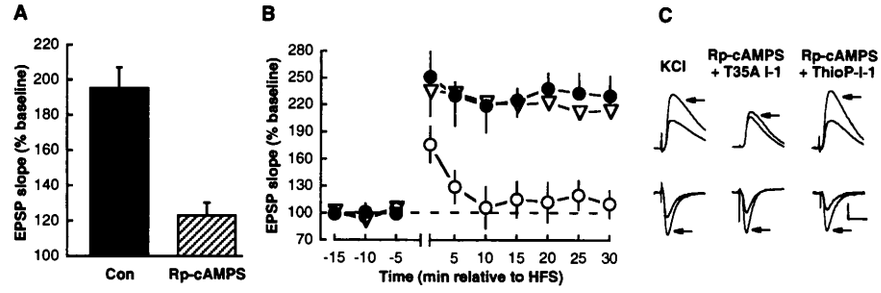

Fig. 1. Ability of Thr^{35}-thiophosphorylated I-1 to substitute for cAMP pathway activation in LTP. (**A**) Intracellular summary data showing the blockade of LTP by postsynaptic Rp-cAMPS injection. Data were taken from the period of 20 to 30 min after the final train of HFS (consisting of measurements at three time points), when LTP had stabilized. Intracellular electrodes contained either no drug [control (Con), solid column, $n = 4$] or 10 mM Rp-cAMPS (hatched column, $n = 7$). The groups differed significantly (t test, $P < 0.01$), indicating that the postsynaptic cAMP pathway is required for LTP. (**B**) Time course of intracellular LTP, showing reversal of Rp-cAMPS blockade by thiophosphorylated I-1. After HFS, stable synaptic potentiation was obtained in cells recorded with control electrodes containing only KCl (triangles, $n = 4$). LTP was blocked when the electrode contained a combination of the PKA antagonist Rp-cAMPS (10 mM) and an inactive, nonphosphorylatable (T35A) form of I-1 (10 μM) (open circles, $n = 5$). However, when inactive I-1 was replaced by constitutively active Thr^{35}-thiophosphorylated I-1 (10 μM), the blockade of LTP by Rp-cAMPS was overcome (filled circles, $n = 8$). The Rp-cAMPS + T35A I-1 group differed from the other two groups over the final three time points (Newman-Keuls test, $P < 0.05$). (**C**) Representative intracellular EPSPs (top row) and field EPSPs recorded simultaneously from the same slices (bottom row). Two superimposed traces are shown in each panel, one recorded during the baseline period and the other, indicated by the arrow, recorded 30 min after HFS. The intracellular recording electrode contained either KCl only (left traces), the combination of Rp-cAMPS and inactive T35A I-1 (middle traces), or Rp-cAMPS combined with Thr^{35}-thiophosphorylated I-1 (right traces). LTP was observed in the field recording despite the blockade of intracellular LTP by the Rp-cAMPS + T35A mutant I-1. All slices used in this experiment showed normal field LTPs, with the EPSP slope measuring at least 153% of baseline at 30 min after HFS. There were no group differences in the field LTP. Calibrations, 5 mV intracellular, 250 μV extracellular, and 10 ms.

Which PP1 targets are relevant for LTP? An important and well-established participant in LTP at the Schaffer collateral–CA1 synapse is CaMKII (*14*). Expression of constitutively active CaMKII increases synaptic efficiency and occludes LTP (*2*). Stimulation that induces LTP also increases Thr^{286} phosphorylation and the consequent Ca^{2+}-independent activity of CaMKII in area CA1 by 20 to 50% in a time-dependent fashion (*15*, *16*). Autophosphorylation of CaMKII is reversed by PP1 in synaptic membranes (*17*), and the Thr^{286} phosphorylation state of CaMKII plays an obligatory role in LTP and spatial memory (*18*). Thus, the cAMP pathway, through regulation of PP1, could modulate the phosphorylation state of Thr^{286} and thereby enhance Ca^{2+}-independent CaMKII activity. If such a mechanism contributes to LTP, widely spaced HFS should increase phosphorylation of CaMKII as well as Ca^{2+}-independent CaMKII activity in a cAMP-dependent manner. We determined the phosphorylation state of CaMKII by immunoblotting with an antibody that specifically recognizes Thr^{286}-phosphorylated CaMKII (*19*). HFS that induced cAMP-dependent LTP also increased Thr^{286}-phosphorylated CaMKII in the CA1 region (Fig. 3A). This effect of HFS was blocked by Rp-cAMPS, supporting the hypothesis that CaMKII phosphorylation at Thr^{286} is sustained in the presence of cAMP. Ca^{2+}-independent CaMKII activity in the CA1 region was reliably increased by HFS (Fig. 3B) (*20*), an effect that was blocked by the inclusion of Rp-cAMPS during stimulation. Thus, the increase in autonomous CaMKII activity paralleled its autophosphorylation at Thr^{286} and was mediated by a cAMP-dependent mechanism.

These results establish a locus of interaction between the cAMP and CaMKII pathways and indicate that the cAMP-operated gate in LTP, at least in part, acts at the level of CaMKII. In this model, if CaMKII activity were directly inhibited, then HFS would not induce LTP, regardless of phosphatase regulation. Thus, when LTP is blocked by a CaMKII inhibitor, the injection of activated I-1 should not restore LTP. Intracellular recordings were obtained with electrodes containing either a specific CaMKII inhibitor peptide (*21*) or a control peptide (Fig. 4). LTP was almost completely blocked by the inhibitor peptide, whereas the control peptide had no effect. The inclusion of thiophosphorylated I-1 in the recording electrode did not restore LTP to the control level, although a modest potentiation was obtained. Thus, the inhibition of PP1 has little effect on LTP in the absence of CaMKII activity, suggesting that CaMKII is a critical site for the cAMP-operated gate. The small recovery of LTP in the presence of thiophosphorylated I-1 may reflect incomplete inhibition of CaMKII by the peptide or an effect of PP1 on some other component of the LTP signal-transmitting pathway, such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (*16*). Although thiophosphorylated I-1 did not reverse the effect of the CaMKII inhibitor, it completely restored LTP in the presence of a PKA inhibitor (Fig. 1). These results are

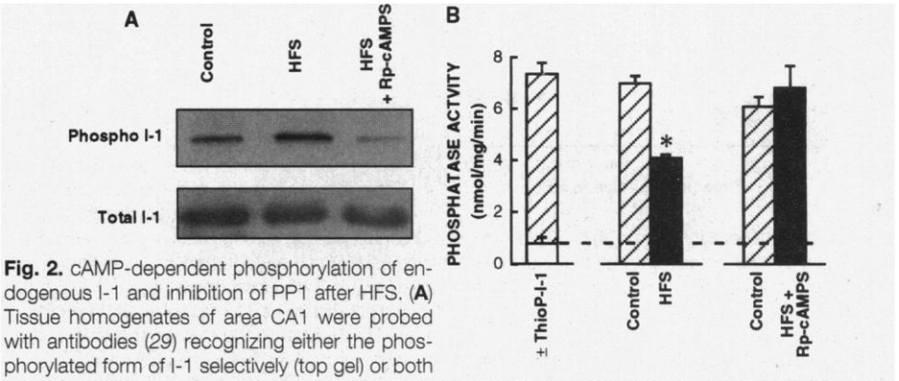

Fig. 2. cAMP-dependent phosphorylation of endogenous I-1 and inhibition of PP1 after HFS. (**A**) Tissue homogenates of area CA1 were probed with antibodies (*29*) recognizing either the phosphorylated form of I-1 selectively (top gel) or both phosphorylated and nonphosphorylated I-1 (bottom gel). HFS increased the amount of phosphorylated I-1, but this effect was prevented by inclusion of 100 μM Rp-cAMPS during stimulation. Total I-1 was not affected by either treatment. Similar results were obtained in two other experiments. (**B**) The superimposed columns on the left show that phosphatase activity measured in control CA1 tissue (open region) was predominantly due to PP1, because it was inhibited over 90% by 100 nM Thr^{35}-thiophosphorylated inhibitor-1 (hatched column and dashed line). HFS significantly reduced phosphatase activity (center columns) ($P < 0.05$, Student's t test; indicated by an asterisk), an effect prevented by the inclusion of 100 μM Rp-cAMPS during stimulation (right columns). Results are representative of three independent assays.

expected if PP1 is positioned between PKA and CaMKII.

Complex physiological functions are often regulated by cooperative interactions between multiple signaling pathways. We have proposed that the cAMP pathway uses protein phosphatases to gate signal flow through a transmittal pathway (5, 6). Here, we explicitly established that synaptic stimulation results in cAMP-dependent activation of I-1 and the concomitant inhibition of PP1, thus protecting the phosphorylation of CaMKII on Thr^{286} and maintaining increased CaMKII activity. Our data, in agreement with those of others (2, 3), indicate that CaMKII may be the transmittal pathway for LTP. Like other protein kinases in diverse physiological systems (22), CaMKII converts extracellular signals into physiological events upon sustained activation. Our data suggest that phosphatase regulation may be an important mechanism contributing to such persistent kinase activation.

Three phases of LTP have been proposed (23, 24). The coordinated actions of phosphatases, calcineurin and others, have been implicated in the transition between these phases (24, 25). Of particular interest is calcineurin, which may negatively regulate the transition between early and late phases of LTP in a cAMP-dependent manner. Because calcineurin is a likely I-1 phosphatase in hippocampal neurons (26), cross-talk between calcium and cAMP at this point may be pivotal in synaptic plasticity. In any case, the cAMP pathway may bridge the early and late phases of LTP and provide an explanation for the involvement of cAMP signaling in memory in mice (4) and men (27).

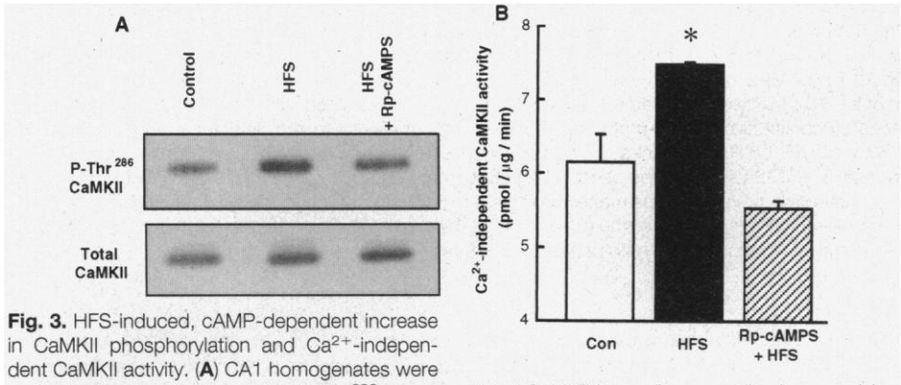

Fig. 3. HFS-induced, cAMP-dependent increase in CaMKII phosphorylation and Ca^{2+}-independent CaMKII activity. (**A**) CA1 homogenates were probed with an antibody specific for Thr^{286}-phosphorylated CaMKII (top gel) or an antibody recognizing total CaMKII (bottom gel). HFS increased the concentration of Thr^{286}-phosphorylated CaMKII by 38% as determined by densitometry, with little or no effect on total CaMKII (center lanes). In tissue stimulated in the presence of 100 µM Rp-cAMPS, the increase in Thr^{286}-phosphorylated CaMKII was blocked (right lane). Results are representative of two independent assays. (**B**) HFS increased Ca^{2+}-independent CaMKII activity. A 22% increase in CaMKII activity was observed, which was blocked in slices stimulated in the presence of 100 µM Rp-cAMPS. The asterisk indicates significant difference from other groups (Newman-Keuls test, $P < 0.05$). No significant group differences in total CaMKII activity were observed (control, 37.4 ± 11.3 pmol μg^{-1} min^{-1}; HFS, 43.7 ± 2.9; HFS + Rp-cAMPS, 55.5 ± 15.2; $P > 0.05$). Results are based on data from two independent experiments.

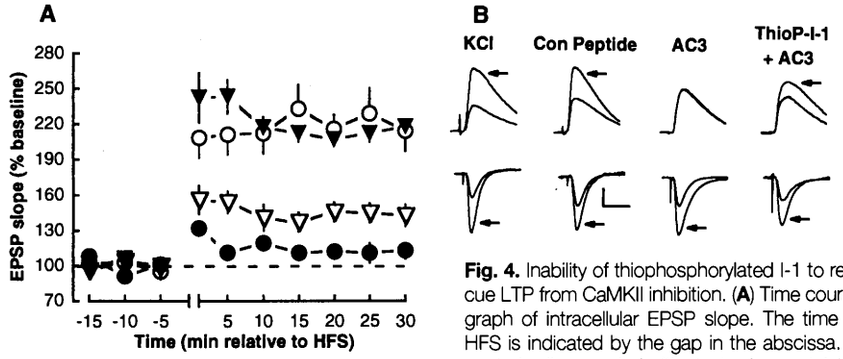

Fig. 4. Inability of thiophosphorylated I-1 to rescue LTP from CaMKII inhibition. (**A**) Time course graph of intracellular EPSP slope. The time of HFS is indicated by the gap in the abscissa. In control cells recorded with electrodes containing either KCl alone (filled triangles, $n = 4$) or 2.5 mM of inactive control peptide (open circles, $n = 5$), stable LTP was induced by HFS. However, when the electrode contained 2.5 mM of the CaMKII inhibitor autocamtide-3 (filled circles, $n = 8$), HFS did not induce LTP. The addition of Thr^{35}-thiophosphorylated I-1 (10 µM) to electrodes containing autocamtide-3 (open triangles, $n = 7$) did not restore LTP to control levels, in contrast to its effectiveness in reversing the effect of a PKA inhibitor (see Fig. 1). Statistical analyses performed on the final three time points indicated that the modest reversal of the autocamtide-3 effect by thiophosphorylated I-1 was statistically reliable, whereas both autocamtide-3–treated groups differed significantly from the two control groups (Newman-Keuls tests, all $P < 0.05$). (**B**) Representative intracellular (top) and field (bottom) traces from slices in which the intracellular electrode contained, from left to right, KCl alone, inactive peptide, autocamtide-3, or autocamtide-3 combined with Thr^{35}-thiophosphorylated I-1. Two superimposed traces are shown in each panel, one recorded during the baseline period and the other, indicated by the arrow, recorded 30 min after HFS. Intracellular and field traces were recorded simultaneously. Normal potentiation of the field EPSP was obtained in each case. For all slices used in this experiment, the field EPSP was greater than 145% of baseline at 30 min, and there were no group differences in the field LTP over the final three time points. Calibrations, 5 mV intracellular, 250 µV extracellular, and 10 ms.

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7. Dissection and recording methods were similar to those previously described (5). Intracellular and field recordings were obtained from area CA1 of hippocampal slices from male Sprague-Dawley rats (125 to 200 g), with a submersion recording chamber. Slices were superfused at 31°C with a solution containing NaCl (118 mM), KCl (3.5 mM), $MgSO_4$ (1.3 mM), $CaCl_2$ (3.5 mM), NaH_2PO_4 (1.25 mM), $NaHCO_3$ (24 mM), and glucose (15 mM), bubbled with 95% O_2 and 5% CO_2. Cells in stratum pyramidale were impaled with sharp electrodes containing 3 M KCl [electrode resistance (*Re*) = 60 to 90 megohms], and field recordings were made with electrodes (2 M NaCl; *Re* = 2 to 5 megohms) placed in stratum radiatum. Thr^{35}-thiophosphorylated I-1, T35A nonphosphorylatable I-1, autocamtide-3 and a related inactive control peptide (KKALHRQEAVDAL and KKALHAQERVDAL, respectively; gifts of A. P. Braun and H. Schulman) (28), and Rp-cAMPS were applied in the intracellular electrode and allowed to diffuse into the cell. In these experiments, HFS was delivered 40 to 60 min after impalement. For experiments in which Rp-cAMPS was applied in the superfusate, slices were exposed to 100 µM Rp-cAMPS in a maintenance chamber for 2 to 6 hours before recording. Synaptic stimulation consisted of monophasic, constant-current pulses of 100-µs duration delivered to the Schaffer collaterals (stratum radiatum in area CA3). A series of test pulses (four pulses at 0.2 Hz) was given every 5 or 10 min, and the excitatory postsynaptic potentials (EPSPs) within each series were averaged. EPSP amplitude and maximum initial slope (defined as the greatest slope within any 1-ms interval between the stimulus artifact and the EPSP peak) were measured offline for the averaged waveforms. LTP was induced by three trains of 100 pulses delivered at 100 Hz, separated by 10 min. The stimulus intensity used for HFS was adjusted to produce a 20- to 25-mV intracellular EPSP when measured from a membrane voltage of −80 mV; during HFS, the holding current was removed. Data were analyzed with either Student's *t* test or analyses of variance followed by Newman-

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11. cDNAs encoding wild-type and nonphosphorylatable (T35A) human I-1 were subcloned into the pT7-7 and pT7-5 vectors, respectively. The vectors were transformed into *Escherichia coli* BL21 (DE3). The recombinant proteins were expressed and purified by sequential trichloracetic acid (TCA) precipitation with I-1 purified from rabbit skeletal muscle as a marker. Homogeneously purified I-1 was obtained with preparative SDS–polyacrylamide gel electrophoresis (PAGE), and the purified proteins were phosphorylated with PKA and adenosine triphosphate (ATP)-γ-S [S. Endo, X. Zhou, J. Connor, B. Wang, S. Shenolikar, *Biochemistry* **35**, 5220 (1996)].
12. Field potentials were monitored and HFS delivered as described (*7*); 2 to 3 min after, the final train slices were removed from the recording chamber and placed on a cold plate. The CA1 region was rapidly dissected out and frozen at −70°C. Individual CA1 samples were lysed in 100 μl of lysis buffer [50 mM tris, 4 mM EGTA, 10 mM EDTA, 15 mM Na phosphate, 100 mM β-glycerophosphate, 10 mM NaF, 0.1 mM pepstatin, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM benzamidine (pH 7.5)] and ground three times for 30 s with a pellet pestle (Kontes glassware, Vineland, NJ). The resulting lysate was spun down at 15,000*g* at 4°C for 10 min, and the supernatant was then assayed for total protein concentration. Twenty micrograms of protein from each sample was run on a 15% SDS-PAGE gel. The samples were transferred onto a polyvinylidene difluoride membrane overnight (25-mA constant current) and then blotted with an antibody to either phosphorylated DARPP-32/I-1 or recombinant human I-1. The monoclonal antibody (mAb) to phosphorylated DARPP-32/I-1 (provided by G. L. Snyder and P. Greengard) (*29*) was blotted at 1/1000 dilution into tris-buffered saline (TBS) + 0.3% Tween 20. It was probed with a horseradish peroxidase (HRP)–conjugated antibody to mouse, diluted 1/2500 in TBS + 0.3% Tween. The polyclonal antibody to human I-1 was blotted at 1/2500 dilution in TBS and probed with 1/2500 dilution of antibody to rabbit. Both blots were developed with enhanced chemiluminescence (ECL), revealing bands at 28 kD, which correspond to native I-1 in these gels.
13. Hippocampal slices were treated and dissected as described above (*7*, *12*). Individual CA1 regions were homogenized in 50 mM tris (pH 7.5), 0.2 mM EDTA, 0.2 mM EGTA, leupeptin (2 μg/ml), aprotinin (2 μg/ml), and 10 nM okadaic acid. Phosphatase activity of 20 ng of protein of CA1 homogenate was measured with the Protein Phosphatase Assay System (GIBCO BRL) in a final concentration of 3.3 nM okadaic acid. With this method, inclusion of 100 nM thiophosphorylated I-1 inhibited >90% of phosphatase activity, confirming the selectivity of the system for PP1.
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19. Hippocampal slices were stimulated and field potentials measured, and the CA1 region was dissected out as described (*7*, *12*). CA1 (2.5 μg) extract from a single CA1 sample was run on a 10% SDS-PAGE, transferred to nitrocellulose, and then blotted with a mAb to Thr^{286}-phosphorylated CaMKII α subunit (Affinity Bioreagents, Golden, CO) [B. L. Patton, S. S. Molloy, M. B. Kennedy, *Mol. Biol. Cell* **4**, 159 (1993)] or a polyclonal antibody to CaMKII (Upstate Biotechnology, Lake Placid, NY). The mAb to autophosphorylated CaMKII was blotted at 1/2000 dilution into 5% nonfat dried milk, 0.1% Tween, and 1 μM microcystin-LR in phosphate-buffered saline (PBS). It was probed with an HRP-conjugated antibody to mouse at 1/3000 dilution into 5% nonfat dried milk and 0.1% Tween in PBS. The polyclonal antibody to CaMKII was blotted at 1 μg/ml in 3% nonfat dried milk in PBS and probed with a 1/3000 dilution of antibody to rabbit. Both blots were developed with ECL, revealing a band at ~50 kD (corresponding to the α subunit of CaMKII).
20. Homogenates from individual CA1 regions were assayed for CaMKII activity essentially as described [M. Mayford, J. Wang, E. R. Kandel, T. J. O'Dell, *Cell* **81**, 891 (1995)]. Tissue was homogenized in a buffer containing 50 mM Hepes (pH 7.5), 0.5 mM EDTA, 0.5 mM EGTA, 100 mM sodium pyrophosphate, 25 mM NaF, 10 mM sodium-β-glycerophosphate, 1 μM okadaic acid, leupeptin (60 μg/ml), aprotinin (60 μg/ml), 0.4 mM dithiothreitol (DTT), and 0.1 mM PMSF. Protein concentrations were assayed by Bradford assay (Bio-Rad) with bovine serum albumin (BSA) as the standard. The enzyme reaction mix consisted of 50 mM Hepes (pH 7.5), 10 mM $MgCl_2$, BSA (100 μg/ml), leupeptin (200 μg/ml), 0.4 mM DTT, 0.6 mM EGTA, 0.2 mM EDTA, 2 μM Wiptide (PKA inhibitor; American Peptide), 2 μM protein kinase C (PKC) (19-36) peptide (PKC inhibitor; American Peptide, Sunnyvale, CA), 200 μM ATP, [γ-^{32}P]ATP (100 μCi/ml), and 20 μM autocamtide-2 (CalBiochem), with either 1 mM $CaCl_2$ and calmodulin (5 μg/ml) (total activity) or 2 mM EGTA (Ca^{2+}-independent activity). Enzyme reactions were carried out at 30°C for 1 min in a final volume of 50 μl. Assays were run in quadruplicate. The reaction was initiated by the addition of 2 μg of CA1 homogenate and terminated by the addition of an equal volume of 10% ice-cold TCA. The protein was pelleted, and the supernatant was spotted onto Whatman P81 filter paper and washed three times for 10 min with water. The amount of ^{32}P incorporated into substrate peptide was determined by liquid scintillation counting.
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28. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; H, His; K, Lys; L, Leu; Q, Gln; R, Arg; and V, Val.
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A Calcium Sensor Homolog Required for Plant Salt Tolerance

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Excessive sodium (Na^+) in salinized soils inhibits plant growth and development. A mutation in the *SOS3* gene renders *Arabidopsis thaliana* plants hypersensitive to Na^+-induced growth inhibition. *SOS3* encodes a protein that shares significant sequence similarity with the calcineurin B subunit from yeast and neuronal calcium sensors from animals. The results suggest that intracellular calcium signaling through a calcineurin-like pathway mediates the beneficial effect of calcium on plant salt tolerance.

Soil salinity stresses plant growth and agricultural productivity (*1*). For many salt-sensitive plants, glycophytes, which include most crop plants, a major part of the growth inhibition is caused by excess Na^+ (*2*). High Na^+ disrupts potassium (K^+) nutrition and, when accumulated in the cytoplasm, inhibits many enzymes (*3*–*5*). Adding calcium (Ca^{2+}) to root growth medium enhances salt tolerance in glycophytic plants (6–8). Ca^{2+} sustains K^+ transport and K^+-Na^+ selectivity in Na^+-challenged plants (*8*).

In *Arabidopsis thaliana*, the recessive *sos3* mutant is hypersensitive to Na^+ and another alkali ion, Li^+ (*9*). Under salt stress, *sos3* plants accumulate more Na^+ and retain less K^+ than the wild type. *sos3* mutant plants are also incapable of growing under low-K^+ conditions. Increased Ca^{2+} in the culture medium can partially suppress the Na^+ hypersensitivity of *sos3* plants and completely suppress the defect in K^+ nutrition (*9*). These phenotypes suggest that the *SOS3* gene product is part of a crucial pathway for mediating the beneficial effect of Ca^{2+} during salt stress (*9*).

The *SOS3* locus is on chromosome V between the molecular markers *nga139* and *CDPK9* (*9*). Mapping of yeast artificial chromosome (YAC) clones containing *nga139* or *CDPK9* (or both) (*10*) placed *SOS3* between the left end of YAC EG20H2 (20H2L) and the left end of YAC CIC12F2 (12F2L) (Fig. 1A). Bacterial artificial chromosome (BAC) clones hybridizing to 20H2L or 12F2L (or to both) were isolat-

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