quantity of the ester **1** results in rapid formation of the efficient replicator **3a** as the dominant product (Fig. 3).

The superiority of **3a** as a replicator is due to its ability to base pair in both senses (**5** and **6**, $R_1 = R_2 = H$) or even a combination of the two. In addition, rapid initial reaction of **2a** with the ester **1** can take place through the Watson-Crick base pair **8** where aryl stacking interactions (*11*) position the reacting functions near each other. In contrast, initial reaction of **2b** or **2c** occurs through **9** where the functions are farther apart. Despite its efficiency, the mutant **3a** is not selfish; it provides effective catalysis for the formation of its competitors, **3b** and **3c**. The presence of 20% **3a** enhances the coupling rates of either of these more than twofold (Figs. 1 and 2).

In the present case adenine-imide base pairing in $CHCl_3$ provides the molecular recognition that leads to self-replication. Other weak intermolecular forces between other host-guest pairs in other solvents could also be used. For example, the solvophobic forces involved in cyclophane-arene complexation in water (*12*) could give rise to a synthetic replicator by way of a covalent linkage between host and guest. The variety of suitable recognition vehicles is vast. Replicating molecules are at the boundary of chemistry with biology, and such synthetic structures can be used to further model evolution at the molecular level.

REFERENCES AND NOTES

1. J. Nowick, Q. Feng, T. Tjivikua, P. Ballester, J. Rebek, Jr., *J. Am. Chem. Soc.* **113**, 8831 (1991).
2. T. Tjivikua, P. Ballester, J. Rebek, Jr., *ibid.* **112**, 1249 (1990).
3. G. von Kiedrowski, B. Wlotzka, J. Helbing, M. Matzen, S. Jordan, *Angew. Chem. Int. Ed. Engl.* **30**, 423 (1991).
4. W. S. Zielinski and L. E. Orgel, *Nature* **327**, 346 (1987).
5. V. Rotello, J. I. Hong, Q. Feng, J. Rebek, Jr., *J. Am. Chem. Soc.* **113**, 9422 (1991).
6. R. Dawkins, *The Selfish Gene* (Oxford Univ. Press, Oxford, 1976), chap. 2.
7. All new compounds were characterized by high-resolution mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectroscopy (J.-I. Hong *et al.*, in preparation).
8. G. Dodin, M. Dreyfus, J.-E. Dubois, *J. Chem. Soc. Perkin Trans. 2* **1979**, 439 (1979).
9. A. Patchornik, A. Amit, R. B. Woodward, *J. Am. Chem. Soc.* **92**, 6333 (1970).
10. Irradiation of **3c** (10 mM in $CHCl_3$) for 1.5 hours gave **3a** as the only product; for **2c** (17 mM) *p*-toluenesulfonyl hydrazide was used as a scavenger for 7 and the irradiation required 5 hours.
11. J. Rebek, Jr., K. Williams, K. Parris, P. Ballester, K. S. Jeong, *Angew. Chem. Int. Ed. Engl.* **26**, 1244 (1987).
12. S. B. Ferguson, E. M. Sanford, E. M. Seward, F. N. Diederich, *J. Am. Chem. Soc.* **113**, 5410 (1991).

13. We thank the National Science Foundation for support of this work and G. A. Berchtold for advice.

12 September 1991; accepted 18 December 1991

Gating of the Cardiac Ca^{2+} Release Channel: The Role of Na^+ Current and Na^+-Ca^{2+} Exchange

JAMES S. K. SHAM, LARS CLEEMANN, MARTIN MORAD*

In cardiac myocytes, calcium influx through the calcium channel is the primary pathway for triggering calcium release. Recently it has been suggested that the calcium-induced calcium release mechanism can also be activated indirectly by the sodium current, which elevates the sodium concentration under the cell membrane, thereby favoring the entry of "trigger" calcium via the sodium-calcium exchanger. To test this hypothesis, sodium current was suppressed by reducing the external sodium concentration or applying tetrodotoxin. At potentials positive to −30 millivolts, calcium release was unaffected. A small calcium release at more negative potentials could be attributed to partial activation of calcium channels, because it was unaltered by replacement of sodium with lithium and was blocked by cadmium. Thus, sodium influx or its accumulation does not initiate calcium release. In addition, sodium-calcium exchange–related calcium release at potentials positive to +80 millivolts has slower kinetics than calcium channel–induced release. Therefore, only the calcium channel gates the fast release of calcium from the sarcoplasmic reticulum in the range of the action potential.

IN MAMMALIAN CARDIAC MYOCYTES, the release of Ca^{2+} from the sarcoplasmic reticulum (SR) is controlled by Ca^{2+} influx through the Ca^{2+} channel (*1–3*) by Ca^{2+}-induced Ca^{2+} release (*4*). This mechanism is specific to Ca^{2+} because Ca^{2+} can be released by rapid elevation of Ca^{2+} in skinned (*4*) or intact cardiac myocytes (*5*), and neither Na^+ nor Ba^{2+} can substitute for Ca^{2+} in the release process when they are the charge carrier through the Ca^{2+} channel (*2*). As an extension of this scheme, it has been reported that the Na^+ current can also trigger Ca^{2+} release (*6*) through subsarcolemmal accumulation of Na^+ in quantities sufficient to reverse the Na^+-Ca^{2+} exchanger and allow Ca^{2+} to enter the cell and trigger Ca^{2+} release (*6, 7*). This could provide a theoretical basis for a beat-to-beat regulation of Ca^{2+} release and contraction by the Na^+ current via the Na^+-Ca^{2+} exchanger. In this report, we examine the role of the Na^+ channel and Na^+ accumulation in the subsarcolemmal space in Ca^{2+} release.

Rat ventricular myocytes were enzymatically isolated (*8*) and whole-cell voltage-clamped (*9*). Intracellular fura-2 (120 to 200 μM) was used to monitor the intracellular Ca^{2+} activity. Fura-2 was excited at 335 and 410 nm, and intracellular Ca^{2+} activity was determined from the ratio of the two fluorescence intensities, measured at 520 nm (*3*). The external solution bathing the experimental cell was exchanged rapidly (20 to 100 ms) for short periods (usually 1 to 5 s) with a concentration-clamp system, allowing minimal steady-state alteration of the cytosolic Na^+ and Ca^{2+} concentrations.

To examine the role of the Na^+ current (I_{Na}) in the release of Ca^{2+}, we suppressed or abolished I_{Na} by rapidly reducing extracellular Na^+ from 142 to 10 mM (for about 2 s) repeatedly at different potentials and analyzed the voltage dependence of intracellular Ca^{2+} transients in myocytes with an intracellular Na^+ concentration of 10 mM (Fig. 1). We chose 10 mM external Na^+ to set the equilibrium potential of Na^+ (E_{Na}) at about 0 mV during the experimental run. In a control solution (142 mM Na^+), depolarization of the myocyte from −80 to 0 mV activated both the Na^+ and Ca^{2+} currents and a maximal Ca^{2+} release. Reduction of the Na^+ concentration to 10 mM 0.5 to 1 s before the depolarization of the cell to 0 mV (E_{Na} in test solution) completely suppressed I_{Na} but had no effect on the rate or magnitude of Ca^{2+} release (Fig. 1, A and C). On the other hand, the smaller Ca^{2+} release in the control solution, triggered by depolarization of the myocyte to −50 mV, was abolished by the reduction of the Na^+ concentration to 10 mM (Fig. 1, A and C).

The effects of rapid reduction of Na^+ were also examined at other membrane potentials, ranging from −50 to +60 mV (Fig. 1, B and D). Despite considerable suppression of the Na^+ current, there was no significant difference in Ca^{2+} transients in high- and low-concentration Na^+ solutions except at potentials between −50 and −30 mV, suggesting a very limited range of potential for the Na^+ current–induced effect. The results were highly reproducible (n = 7 cells) with only minor variations in the degree of suppression of the inward current

Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104.

*To whom correspondence should be addressed.

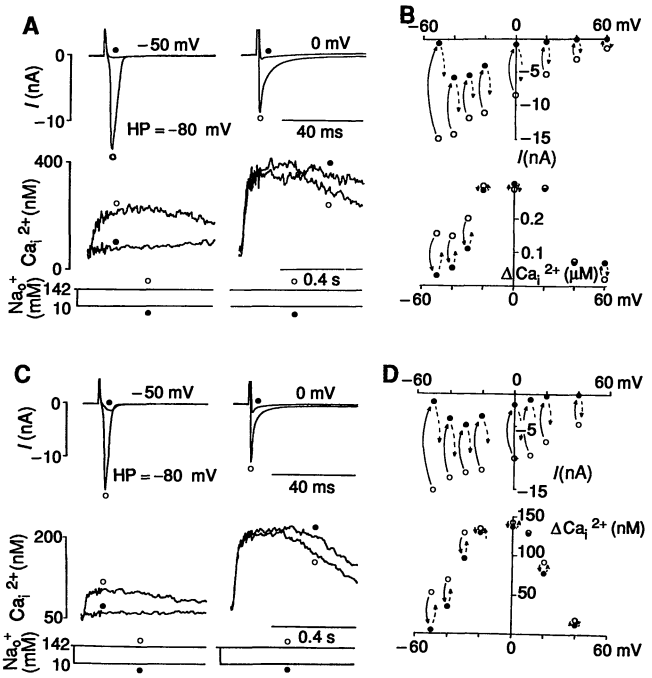

Fig. 1. The effect of extracellular Na^+ on inward current and intracellular Ca^{2+}. (**A** and **C**) The effect of rapid reduction of extracellular Na^+ on inward current and Ca^{2+} transients elicited by a 100-ms depolarizing pulse from a holding potential (HP) of −80 mV to −50 or 0 mV every 10 s. The external solution was changed 1 s before the clamp pulses from 142 to 10 mM Na^+ by equimolar substitution with Cs^+. The effect of Na^+ withdrawal was tested only after a steady state of Ca^{2+} release had been reached after three to five clamp pulses. Similar results were obtained in seven cells. (**B** and **D**) The voltage-dependence of current (upper panel) and the change in Ca^{2+}_i (lower panel) in 142 mM and 10 mM Na^+ solutions. We calculated the change in intracellular Ca^{2+} concentration by subtracting peak intracellular Ca^{2+} concentration, measured at 100 ms after the onset of the clamp pulse, from the baseline intracellular Ca^{2+} concentration. The low-concentration Na^+ solution was removed promptly after the pulse to minimize the change in SR Ca^{2+} loading. (○), 142 mM, and (●), 10 mM external Na^+; arrows indicate reversibility. (A) and (B) are from the same cell; (C) and (D) are from another cell.

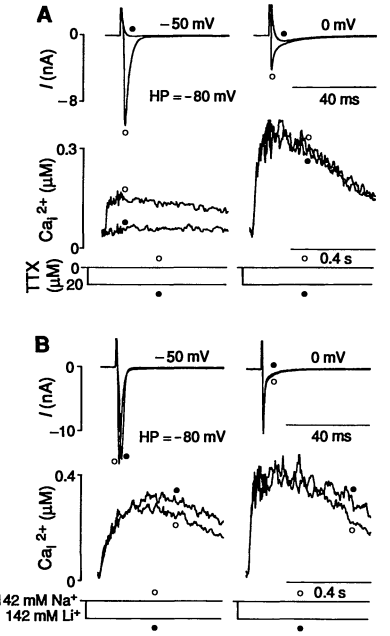

Fig. 2. The effect of TTX and Li^+ on inward current and the Ca^{2+} transient elicited at −50 and 0 mV. In (**A**), 20 μM TTX was applied 5 s before the clamp pulses (100-ms clamp). (○), absence of TTX; (●), presence of TTX. Similar results were observed in five cells. In (**B**), an Li^+ solution (completely replacing Na^+) was applied 1 s before the clamp pulses. Traces were obtained before (○) and after (●) replacement of external Na^+ with Li^+. Similar results were observed in four cells. HP = holding potential.

and Ca^{2+} transients in different myocytes (compare myocytes of Fig. 1, A and B, with myocytes of Fig. 1, C and D). These minor differences were thought to result from variations in the degree of voltage control and Ca^{2+} loading of the SR.

Similar results were obtained when tetrodotoxin (TTX) was used to inhibit the Na^+ current. Addition of 10 to 20 μM TTX 5 s before activation of the Na^+ channel caused complete suppression of the Na^+ current at both −50 and 0 mV; in contrast, the Ca^{2+} transient was suppressed by TTX only at −50 mV, not 0 mV (Fig. 2A). Taken together, these results suggest that the Na^+ influx and its possible accumulation cannot modify Ca^{2+} release when it is triggered by Ca^{2+} influx through the Ca^{2+} channel.

The small and often variable Ca^{2+} transients measured at −50 to −30 mV could not be triggered by the direct interaction of Na^+ with the release channel because a large influx of Na^+ through the Ca^{2+} channel in the absence of external Ca^{2+} was unable to elicit SR Ca^{2+} release (*2*). Influx of Ca^{2+} through TTX-sensitive Na^+ channels (*10*), which would in turn trigger Ca^{2+} release, was also unlikely as the Ca^{2+} selectivity of Na^+ channels is extremely low (*11*). Furthermore, the complete inhibition of the Ca^{2+} release transient at −50 mV by the reduction of the extracellular Na^+ concentration (Fig. 1) indicates that Ca^{2+} influx through the Na^+ channel could not have been mediating the Ca^{2+} release process.

To test whether transient accumulation of Na^+ regulates the magnitude of Ca^{2+} release, we substituted Li^+ for Na^+ just 0.5 s before the activation of Na^+ channels. Because Li^+ can easily permeate Na^+ channels but is not transported by the Na^+-Ca^{2+} exchanger (*12*), the possible involvement of Na^+-Ca^{2+} exchangers in the Ca^{2+} release process could be evaluated. At either −50 or 0 mV, the currents through the Na^+ channel were almost identical, whether Na^+ or Li^+ was the charge carrier (Fig. 2B). At both potentials, however, the replacement of Na^+ with Li^+ had no effect on the magnitude and the rate of activation of the Ca^{2+} transients. This finding suggests that the Ca^{2+} release at −50 mV is related to neither the influx of Na^+ nor its accumulation in a subsarcolemmal space and the activation of the Na^+-Ca^{2+} exchanger. The only noticeable effect of substitution of Na^+ with Li^+ (Fig. 2B) or Cs^+ (Fig. 1, A and C) on the Ca^{2+} transient is a slowing of Ca^{2+} removal, consistent with the well-documented inhibition of Na^+-Ca^{2+} exchange in Na^+-deficient solutions (*13*).

We examined the possibility that Ca^{2+} transients generated between −50 and −30 mV were, in part, activated by inadequate voltage-clamp control which thereby activated Ca^{2+} channels by testing whether the Ca^{2+} transients in this potential range could be suppressed by Ca^{2+} channel blockers. Cd^{2+} was used because this ion blocks the Ca^{2+} channel rapidly and reversibly without permeating it (*14*). We applied Cd^{2+} (100 μM) to a ventricular myocyte 5 s before the depolarizing clamp pulses to −40 or 0 mV (Fig. 3A). Ca^{2+} transients were completely suppressed at both potentials, even though the Na^+ current was not significantly altered in the presence of Cd^{2+}. The suppression of Ca^{2+} release at 0 mV by Cd^{2+} was consistent with previous studies and supports the idea that the Ca^{2+} channel gates the release of Ca^{2+} in cardiac myocytes (*1, 15*). On the other hand, the suppression by Cd^{2+} of Ca^{2+} release at −40 mV without a significant effect on the Na^+ current suggests that the Ca^{2+} current must have been partially activated at this potential, even though the clamp potential was set at −40 mV. Single-pipette whole-cell voltage clamping cannot adequately control the Na^+ current (*16*), so part of the sarcolemmal or t-tubular membrane may have strayed significantly from the command potential during the Na^+ spike (*17*), leading to a rapid partial activation of a Ca^{2+} current masked by the much larger Na^+ current. Although Cd^{2+} at millimolar concentration inhibits Na^+-Ca^{2+} exchange (*18*), it has no effect on the exchanger at 50 to 100 μM. Figure 3B confirms that 100 μM Cd^{2+} does not affect the entry of

Ca^{2+} through the Na^+-Ca^{2+} exchanger during a 2.5-s depolarizing pulse to +100 mV in myocytes pretreated with 5 mM caffeine to deplete their releasable Ca^{2+} pools, whereas 5 mM Ni^{2+} inhibits Ca^{2+} influx under such conditions (Fig. 3B) (*18, 19*). These findings support the idea that the Ca^{2+} transient signals observed at −50 to −30 mV were generated secondary to activation of Ca^{2+} channels rather than after activation of the Na^+-Ca^{2+} exchanger.

Next, we examined whether the entry of Ca^{2+} via the Na^+-Ca^{2+} exchanger initiates Ca^{2+} release from the SR in cardiac myocytes. We tested this by either depolarizing the myocytes with long pulses to potentials positive to E_{Na} and E_{Ca} (+80 to +100 mV), at which Ca^{2+} could enter the cell via only the Na^+-Ca^{2+} exchanger, or lowering the extracellular Na^+ concentration to 10 mM to accelerate Ca^{2+} entry via the exchanger. Figure 4A illustrates the Ca^{2+} transients in a myocyte when it was depolarized from −80 to +100 mV for 1 s in the control solution. Intracellular Ca^{2+} activity increased slowly for 200 to 700 ms, then became more rapid and decayed spontaneously. The faster rise in intracellular Ca^{2+} activity was abolished by 5 mM caffeine, suggestive of the triggered release of Ca^{2+} from the SR. The slow increase, on the other hand, represented the trans-sarcolemmal Ca^{2+} influx through the Na^+-Ca^{2+} exchanger as it persisted in the presence of caffeine and was stopped only by 5 mM Ni^{2+}, which also suppressed a component of outward current related to Na^+-Ca^{2+} exchange (*18, 19*). Figure 4B compares the Ca^{2+} release activated by the slow Ca^{2+} entry via the exchanger at +80 mV and that triggered by the Ca^{2+} entry through Ca^{2+} channels activated by repolarization of the membrane from +80 to −80 mV (*1, 3*). The Na^+-Ca^{2+} exchange–triggered release was significantly slower but of equal magnitude to that triggered by the Ca^{2+} current. Rapid reduction of extracellular Na^+ from 142 to 10 mM also elicited a slow rise in intracellular Ca^{2+}, which was often followed by a faster rise in intracellular Ca^{2+} (Fig. 4C).

These findings support the idea that the Na^+-Ca^{2+} exchanger is capable of transporting sufficient Ca^{2+} to induce Ca^{2+} release but only under extreme experimental conditions. Even under favorable conditions, the slow onset (200 to 700 ms) and kinetics of release suggest a gating mechanism somewhat different from that induced by the Ca^{2+} channel. Furthermore, the ability of the Na^+-Ca^{2+} exchanger to activate Ca^{2+} release following a slow and uniform elevation of intracellular Ca^{2+} (Fig. 4) argues against the necessity for a subsarcolemmal "fuzzy" space in which Na^+ or "trigger" Ca^{2+} (or both) may accumulate (*20*).

We conclude that Na^+ influx through the Na^+ channel and its accumulation do not initiate Ca^{2+} release, and that the Ca^{2+} channel gates the release of Ca^{2+} in the range of membrane potentials spanning the cardiac action potential. Our data support previous observations that show that Ca^{2+} may be released by an increase of Ca_i^{2+}, either by influx of Ca^{2+} through the Ca^{2+} channel (*1–3*) or by its availability to the myoplasm through other pathways (*4, 5*).

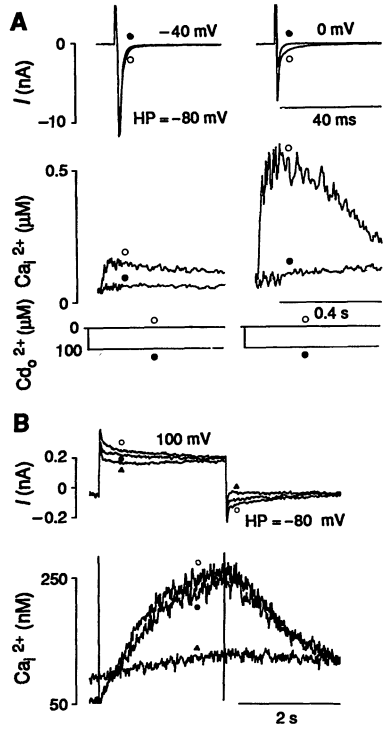

Fig. 3. (**A**) The effect of 100 μM Cd^{2+} on inward current and the Ca^{2+} transient activated by a 100-ms depolarizing pulse from −80 mV to −40 and 0 mV. At both membrane potentials, the Ca^{2+} transients activated in the control solution (○) were completely abolished by Cd^{2+} (●). Similar results were observed in four cells. (**B**) The effect of 100 μM Cd^{2+} (●) and 5 mM Ni^{2+} (▲) on Ca^{2+} influx through the Na^+-Ca^{2+} exchanger. (○) Indicates control traces. The Ca^{2+} transients were activated by a 2.5-s depolarizing pulse from −80 to +100 mV in the presence of 5 mM caffeine to abolish Ca^{2+} release from the SR. Similar results were observed in three cells. HP = holding potential.

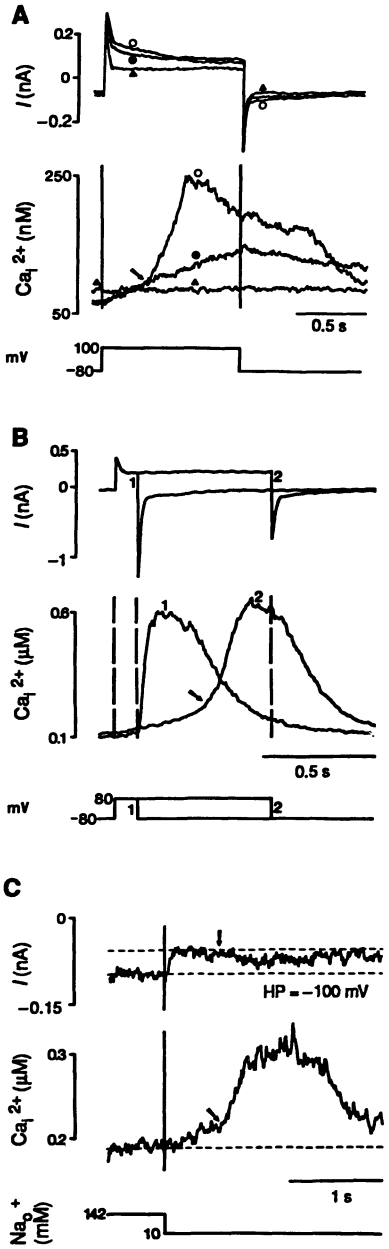

Fig. 4. (**A**) Membrane currents and Ca^{2+} transients activated by a 1-s depolarizing pulse from −80 to +100 mV in the absence (○) or in the presence of 5 mM caffeine without (●) or with (▲) 5 mM Ni^{2+}. (**B**) The Ca^{2+} transients elicited by the Ca^{2+} current activated by repolarization from +80 to −80 mV (curve 1) and by a 0.7-s depolarizing pulse from −80 to +80 mV (curve 2). (**C**) The effect of rapid reduction of external Na^+ from 142 to 10 mM on the membrane current and the Ca^{2+} transient in a cell held at −100 mV. HP = holding potential. The Na^+ in the 10 mM solution was replaced with equimolar Cs^+. Arrows indicate triggered release of SR Ca^{2+}.

REFERENCES AND NOTES

1. D. J. Beuckelmann and W. G. Wier, *J. Physiol. (London)* **405**, 233 (1988); G. Callewaert, L. Cleemann, M. Morad, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2009 (1988).
2. M. Näbauer, G. Callewaert, L. Cleemann, M. Morad, *Science* **244**, 800 (1989).
3. L. Cleemann and M. Morad, *J. Physiol. (London)* **432**, 283 (1991).
4. M. Endo, *Physiol. Rev.* **57**, 71 (1977); A. Fabiato, *Am. J. Physiol.* **245**, C1 (1983); *J. Gen. Physiol.* **85**, 247 (1985); *ibid.*, p. 291.
5. M. Valdeolmillos, S. C. O'Neill, G. L. Smith, D. A. Eisner, *Pfluegers Arch.* **413**, 676 (1989); M. Näbauer and M. Morad, *Am. J. Physiol.* **258**, C189 (1990); E. Niggli and W. J. Lederer, *Science* **250**, 565 (1990).
6. N. Leblanc and J. R. Hume, *Science* **248**, 372 (1990).
7. W. J. Lederer, E. Niggli, R. W. Hadley, *ibid.*, p. 283.
8. R. Mitra and M. Morad, *Am. J. Physiol.* **249**, H1056 (1985).

9. Myocytes were whole-cell voltage-clamped with 1.5- to 2.5-megohm pipettes containing 110 mM CsCl, 10 mM NaCl, 5 mM magnesium adenosine triphosphate, 10 mM Hepes buffer, 20 mM tetraethylammonium chloride, 0.01 mM adenosine 3',5'-monophosphate, and 0.2 mM potassium salt of fura-2. External Tyrode's solution contained 137 mM NaCl, 5.4 mM KCl, 1 mM $MgCl_2$, 10 mM Hepes, 10 mM glucose, and 2 mM $CaCl_2$ at pH 7.4. K^+ in the external solution was replaced with Na^+ after the cells were perfused and equilibrated for 5 min. Cells were perfused for at least 10 min before test protocols were performed. All experiments were performed at room temperature (20° to 23°C).
10. E. A. Johnson and R. D. Lemieux, *Science* **251**, 1370 (1991).
11. B. Nilius, *J. Physiol. (London)* **399**, 537 (1988).
12. B. Hille, *J. Gen. Physiol.* **59**, 637 (1972); P. F. Baker, *Prog. Biophys. Mol. Biol.* **24**, 177 (1972).
13. D. M. Bers, W. J. Lederer, J. R. Berlin, *Am. J. Physiol.* **258**, C944 (1990).
14. J. B. Lansman, P. Hess, R. W. Tsien, *J. Gen. Physiol.* **88**, 321 (1986).
15. M. Morad, Y. E. Goldman, D. R. Trentham, *Nature* **304**, 635 (1983).
16. A. M. Brown, K. S. Lee, T. Powell, *J. Physiol. (London)* **318**, 455 (1981).
17. R. H. Adrian and L. D. Peachey, *ibid.* **235**, 103 (1973); L. D. Peachey and R. H. Adrian, in *Structure and Function of Muscle*, G. Bourne, Ed. (Academic Press, New York, 1973), vol. 3, pp. 1–30.
18. J. Kimura, S. Miyamae, A. Noma, *J. Physiol. (London)* **384**, 199 (1987).
19. D. J. Beuckelmann and W. G. Wier, *ibid.* **414**, 499 (1989).
20. The fuzzy space model is based on several assumptions: (i) the Na^+ current has to be large—for example, 50 nA as suggested previously (7), (ii) a diffusion barrier for Na^+ and Ca^{2+} has to exist to delimit a subsarcolemmal space, (iii) the Na^+-Ca^{2+} exchanger has to operate at a sufficiently fast rate to bring enough Ca^{2+} into the space, and (iv) all molecular entities including Na^+ channels, Ca^{2+} channels, Na^+-Ca^{2+} exchanger, and SR release channels have to be located close together to interact intimately. Some of these assumptions remain untested.
21. Supported by NIH research grants HL-16152; J.S.K.S. was supported by HL-07400 training grant.

11 October 1991; accepted 12 December 1991

Tyrosyl Phosphorylation and Activation of MAP Kinases by p56^{lck}

ELHAM ETTEHADIEH, JASBINDER S. SANGHERA, STEVEN L. PELECH, DENISE HESS-BIENZ, JULIAN WATTS, NILABH SHASTRI, RUEDI AEBERSOLD*

T cell signaling via the CD4 surface antigen is mediated by the associated tyrosyl protein kinase p56^{lck}. The 42-kilodalton mitogen-activated protein (MAP) kinase (p42^{mapk}) was tyrosyl-phosphorylated and activated after treatment of the murine T lymphoma cell line 171CD4+, which expresses CD4, with antibody to CD3. Treatment of the CD4-deficient cell line 171 with the same antibody did not result in phosphorylation or activation of p42^{mapk}. Purified p56^{lck} both tyrosyl-phosphorylated and stimulated the seryl-threonyl phosphotransferase activity of purified p44^{mpk}, a MAP kinase isoform from sea star oocytes. A synthetic peptide modeled after the putative regulatory phosphorylation site in murine p42^{mapk} (Tyr^{185}) was phosphorylated by p56^{lck} with a similar V_{max}, but a fivefold lower Michaelis constant (K_m) than a peptide containing the Tyr^{394} autophosphorylation site from p56^{lck}. MAP kinases may participate in protein kinase cascades that link Src family protein-tyrosyl kinases to seryl-threonyl kinases such as those encoded by *rsk* and *raf*, which are putative substrates of MAP kinases.

MEMBERS OF A FAMILY OF TYrosyl-phosphorylated 42- to 45-kD seryl-threonyl kinases, known both as mitogen-activated protein (MAP) kinases and extracellular signal-regulated kinases (ERKs), have been implicated in a variety of cytokine signal transduction pathways and in cell cycle control (*1*). Both p42^{mapk} (*2*) and p43^{erk1} (*3*) exhibit increased myelin basic protein (MBP) phosphotransferase activity after stimulation of quiescent mammalian cells with insulin and other mitogens. The p44^{mpk} is activated near the onset of germinal vesicle breakdown at M phase in maturing sea star oocytes (*4*). Enzymological and immunological characterization of p42^{mapk}, p43^{erk1}, and p44^{mpk} supports their relatedness (*3, 5*), and primary sequence analysis has revealed at least 80% amino acid identity between these isoforms (*6*). Tyrosyl phosphorylation is required for activation of each of these MAP kinases (*3, 7, 8*), but the responsible tyrosyl kinases have not been identified.

Stimulation of Jurkat cells and normal human T cells with antibodies to CD3 and phorbol ester tumor promoters such as phorbol myristate acetate (PMA) results in tyrosyl phosphorylation of two proteins of 42 and 43 kD, which were tentatively identified as MAP kinases (*9*). CD4 is a cell surface glycoprotein that recognizes nonpolymorphic regions of class II antigens of the major histocompatibility complex and potentiates the signal induced by stimulation of the CD3–T cell antigen receptor complex. A fraction of CD4 protein directly interacts with the CD3–T cell receptor complex (*10*). The functional importance of CD4-CD3–T cell receptor interaction is further supported by the observation that coaggregation of CD3 and CD4 with monoclonal antibodies enhances CD3-mediated activation of MAP kinase in Jurkat cells, although antibodies to CD4 alone failed to elicit stimulation of MAP kinase activity (*9*). The tyrosyl kinase p56^{lck} is physically associated with the cytoplasmic tail of CD4 via cysteine motifs (*11*) and is activated when CD4 is cross-linked with monoclonal antibodies (*12*). T cell lines expressing CD4 protein with altered or truncated cytoplasmic tails are defective in CD4 signal transduction (*13, 14*).

Mono Q ion-exchange chromatography of cytosol from the murine T lymphoma cell line 171CD4+ (*13*) resolved a peak of MBP phosphotransferase activity that was selectively stimulated after treatment of the cells for 5 min with monoclonal antibody 2C11 to CD3 (anti-CD3) (Fig. 1A). When the Mono Q fractions surrounding this peak were probed on protein immunoblots with monoclonal antibody 4G10 to phosphotyrosine, an immunoreactive protein of 42 kD that cofractionated with the activated MBP kinase was evident only with extracts from cells treated with anti-CD3 (Fig. 1, B and C). A 44-kD phosphotyrosine-containing protein was detected in extracts from both untreated cells and those exposed to anti-CD3. Identification of the 42- and 44-kD phosphoproteins as MAP kinases was supported by their immunoreactivity with affinity-purified rabbit polyclonal antibodies (anti-Erk1-CT) to a synthetic peptide patterned after the COOH-terminal 35 residues of rat p43^{erk1} (*6*). Anti-Erk1-CT cross-reacted with purified preparations of sea star p44^{mpk}, murine p42^{mapk}, and human p43^{erk1} (*15*). In Mono Q fractions from both control and anti-CD3–stimulated cells, anti-Erk1-CT reacted intensely with protein bands of 41 and 44 kD (Fig. 1, D and E). However, after treatment of cells with anti-CD3, proteins of 42 and 45 kD were also detected. Such band shifts on SDS-polyacrylamide gels are typical consequences of protein phosphorylation. The simplest interpretation of these findings is that CD3-mediated stimulation of the T cells induced tyrosyl phosphorylation and activation of p42^{mapk}, but a 44-kD MAP kinase was already tyrosyl-phosphorylated in un-

E. Ettehadieh, J. S. Sanghera, S. L. Pelech, D. Hess-Bienz, J. Watts, R. Aebersold, The Biomedical Research Centre and the Departments of Biochemistry and Medicine, University of British Columbia, Vancouver, B.C. V6T 1Z3 Canada.
N. Shastri, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

*To whom correspondence should be addressed.