It remains unclear how the isotopic signature of a large spectrum of old lithospheric fragments, possibly pelagic sediments and altered basalts and gabbros, can be preserved in the convective mantle and kept isolated from the well-mixed MORB source. Mantle tomography indicates that at least part of the oceanic lithosphere is dragged down to the core-mantle boundary (*35, 36*). At typical plate velocities, upper mantle material is transferred to the lower mantle in 10 to 100 million years, implying that the chemical and isotopic compositions should become homogeneous very rapidly in the entire mantle. The mixing time of an element, that is, the time it takes for any contrast between the chemical and isotopic inventories of two parts of the mantle to be reduced by a factor e, is $f(1-f)\,M_i/Q_i$, where M_i is the total inventory of the element i in the mantle, Q_i its flux between the two parts of the mantle, and f the fraction of the mantle mass allocated to one of the parts (*37*). For a mass flux equal to the rate of lithosphere subduction (about 300 $km^3\ year^{-1}$), the maximum mixing time of the whole mantle is on the order of 0.4 Gy. Therefore, the lower and the upper mantle should be geochemically indistinguishable. Because OIB and MORB are geochemically distinct, the elemental fluxes Q_i must be reduced and the mixing time of radiogenic isotopes increased so that heterogeneity between the OIB and MORB reservoirs is preserved. Mixing times can be increased by the delamination of the oceanic crust and its storage at the core-mantle boundary (*13, 38*). They may also be selectively increased for different elements by the extraction of continental crust material and by the hydrous stripping of the lithophile elements from the subducting slabs into the ambient mantle without substantial reduction of the total mass of the lithosphere that penetrates into the lower mantle (*37*). Such a process calls for an overall depleted deep mantle with streaks of lithospheric residues altered by subduction zone processes (such as those invoked for the U/Pb fractionation in the source of the Koolau component), but still fertile enough to produce OIBs.

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52. We thank W. Abouchami for allowing us to use her unpublished Sr, Nd, and Pb isotope data for Lanai; P. Télouk for maintaining the Plasma 54; M. Garcia and B. Leeman for providing samples for this study; M. Garcia for comments on an earlier draft of this manuscript; and two anonymous reviewers for helpful comments. Funding by the Institut National des Sciences de l'Univers, through the program Dynamique des Transferts Terrestres, is gratefully acknowledged.

9 April 1999; accepted 24 June 1999

Molecular Identification of a Eukaryotic, Stretch-Activated Nonselective Cation Channel

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Calcium-permeable, stretch-activated nonselective cation (SA Cat) channels mediate cellular responses to mechanical stimuli. However, genes encoding such channels have not been identified in eukaryotes. The yeast *MID1* gene product (Mid1) is required for calcium influx in the yeast *Saccharomyces cerevisiae*. Functional expression of Mid1 in Chinese hamster ovary cells conferred sensitivity to mechanical stress that resulted in increases in both calcium conductance and the concentration of cytosolic free calcium. These increases were dependent on the presence of extracellular calcium and were reduced by gadolinium, a blocker of SA Cat channels. Single-channel analyses with cell-attached patches revealed that Mid1 acts as a calcium-permeable, cation-selective stretch-activated channel with a conductance of 32 picosiemens at 150 millimolar cesium chloride in the pipette. Thus, Mid1 appears to be a eukaryotic, SA Cat channel.

SA Cat channels are suggested to act as mechanotransducers in various biological functions including touch sensation, hearing, and maintenance of cardiovascular tone in animals, detection of touch and gravity in plants, and sensing of osmotic changes in microorganisms (*1*). However, genes or cDNAs encoding eukaryotic SA Cat channels have not been identified, and thus, the molecular mechanism of mechanotransduction in eukaryotic cells is poorly under-

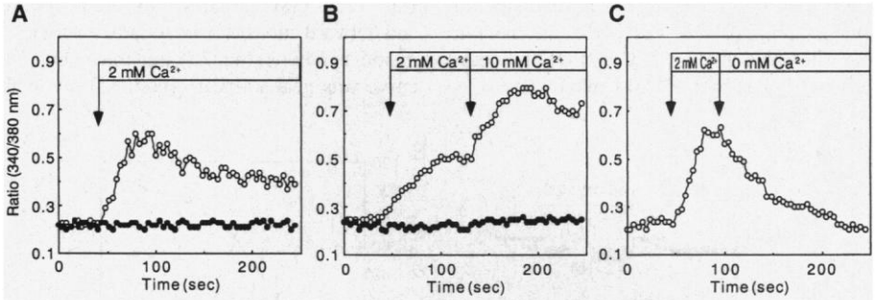
stood. By contrast, bacterial SA Cat channels are well characterized, but have no eukaryotic homolog (*2*). Several eukaryotic ion channels are claimed to be mechanosensitive (*3*).

The *MID1* gene of the yeast *Saccharomyces cerevisiae* encodes an integral plasma membrane protein required for Ca^{2+} influx stimulated by mating pheromone (*4*). When exposed to the pheromone, cells lacking *MID1* die because of the restricted Ca^{2+} influx. Thus, the Mid1 protein has a crucial role in supplying Ca^{2+} during the mating process. Although Mid1 has no overall amino acid sequence similarity to those of known ion channels, the amino acid sequence of its putative transmembrane segment is similar to that of the S3 or H3 segment of a superfamily of ion channels (*4*). We therefore investigated the possibility that Mid1 is an ion channel.

The *MID1* gene was placed under the control of the Zn-inducible human metallothionein IIa promoter in the vector pMEP4; the resulting plasmid, pMEP4-MID1, was then transfected into Chinese hamster ovary (CHO) cells (*5*). Immunoblot analysis revealed that pMEP4-MID1–transfected cells specifically produced a protein of 95 kD size when incubated for 24 hours in medium containing 80 μM $ZnCl_2$, but not in medium without $ZnCl_2$ (Fig. 1D). Cells transfected with the vector did not produce the 95-kD protein in response to $ZnCl_2$ (*6*). Because the molecular size of Mid1 deduced from its amino acid sequence is 61.5 kD, it appears that Mid1 might be modified by *N*-glycosylation in CHO cells, as it is in yeast cells (*4*).

To examine whether the expression of Mid1 could alter Ca^{2+} permeability across the plasma membrane, we monitored changes in $[Ca^{2+}]_c$ (cytosolic calcium concentration) in response to an increase in the extracellular Ca^{2+} concentration, using the Ca^{2+} indicator fura-2 (*7*). An increase in the extracellular Ca^{2+} concentration from nominally 0 to 2 mM resulted in an increase in $[Ca^{2+}]_c$ in Mid1-expressing cells (Fig. 1A). When extracellular Ca^{2+} concentration was further increased from 2 to 10 mM, an additional increase in $[Ca^{2+}]_c$ was observed (Fig. 1B). Removal of extracellular Ca^{2+} (Fig. 1C), addition of lanthanum (*8*), or an increase in osmolarity in the medium (from 320 to 350 mosM) (*8*) caused a decrease in $[Ca^{2+}]_c$. The last result suggests that Mid1-expressing cells are exposed to a turgor pressure. Under the same conditions, essentially no change in $[Ca^{2+}]_c$ was observed in $ZnCl_2$-treated, mock-transfected cells (Fig. 1, A and B) or *MID1*-transfected cells not treated with $ZnCl_2$ (*9*).

To examine the possibility that Mid1 functions as a Ca^{2+}-permeable channel, we employed the whole-cell voltage-clamp technique (*10*). The steady-state current-voltage (*I-V*) relationship showed that control cells not treated with Zn produced little Ca^{2+} cur-



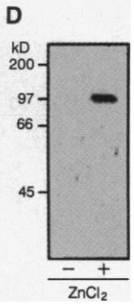


Fig. 1. Detection of Mid1 and changes in $[Ca^{2+}]_c$ in CHO cells. [(A) to (C)] CHO cells were treated with $ZnCl_2$ for 24 hours and then loaded with fura-2. Extracellular Ca^{2+} concentration was changed from 0 to 2 mM (**A**), further to 10 mM (**B**) or from 2 mM to 0 mM (**C**) as indicated by the arrows. Open and solid circles represent Zn-treated, pMEP4-MID1–transfected cells and Zn-treated, mock-transfected cells, respectively. (**D**) Immunoblot analysis showing Zn-dependent expression of the Mid1 protein. CHO cells transfected with pMEP4-MID1 were incubated for 36 hours in the presence (+) or absence (−) of 80 μM $ZnCl_2$. Cell extracts were prepared and analyzed by immunoblotting with affinity-purified rabbit antibodies to a synthetic oligopeptide corresponding to the COOH-terminus of Mid1 (^{530}TCNYIGNSSLMVIHPLDDT^{548}) (*27*).

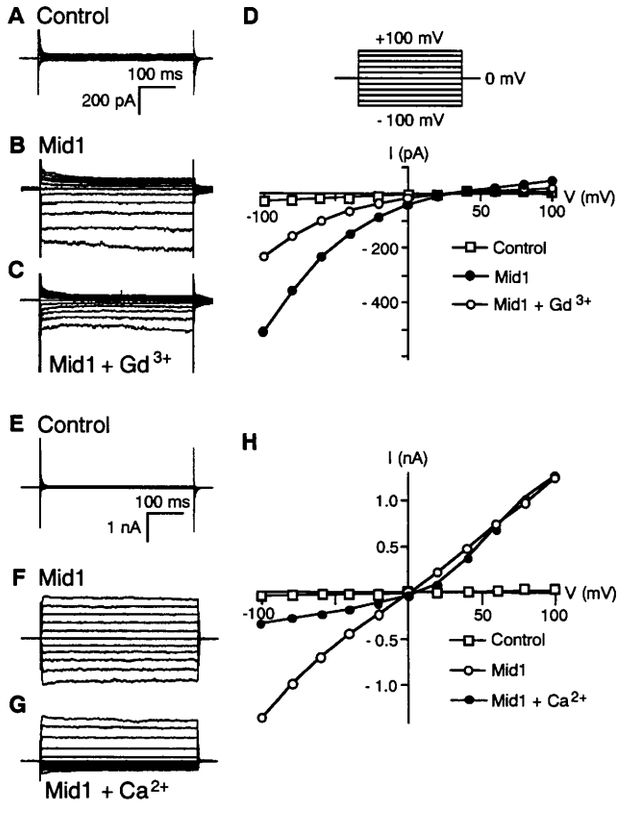


Fig. 2. Whole-cell current properties of Mid1-expressing CHO cells. [(A) to (D)] Inward Ca^{2+} currents in Mid1-expressing cells. (**A**) A control cell not treated with Zn; (**B**) A Mid1-expressing cell treated with $ZnCl_2$ for 24 hours; and (**C**) A Mid1-expressing cell treated for 24 hours with $ZnCl_2$ and then with 0.5 mM $GdCl_3$ in the bath solution. Note that the data in (B) and (C) were obtained from the same cell. (**D**) Steady-state *I-V* relationships for whole-cell Ca^{2+} currents calculated from (A) to (C), showing that Gd^{3+} blocks the inward Ca^{2+} current. Membrane potential was jumped from a holding potential of 0 mV in 20 mV steps between −100 mV and +100 mV for 400 ms at an interval of 1 s [upper panel in (D)]. [(E) to (H)] Cs^+ current in Mid1-expressing cells under symmetrical Cs^+-gluconate solutions. Pipette and bath solutions contained 150 mM Cs^+-gluconate, 1 mM EGTA-Cs, and 10 mM HEPES-Cs (pH 7.4). (**E**) A control cell not treated with Zn; (**F**) A Mid1-expressing cell; and (**G**) A Mid1-expressing cell in the presence of 2 mM $CaCl_2$ in the bath solution. Note that the data in (F) and (G) were obtained from the same cell. (**H**) The steady-state *I-V* relationships for whole-cell Cs^+ currents calculated from (E) to (G).

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rent (n = 5) (Fig. 2, A and D) and that cells treated with $ZnCl_2$ for 24 hours produced a Ca^{2+} current up to 492 ± 64 pA (n = 22) at −100 mV (Fig. 2, B and D). The inward Ca^{2+} current was increased in a time-dependent manner from 10 to 24 hours after addition of $ZnCl_2$ (*11*). The Ca^{2+} currents were inhibited to 198 ± 23 pA (n = 16) by Gd^{3+} (0.5 mM) in the bath solution (Fig. 2, C and D) and completely blocked by La^{3+} (0.1 mM).

To evaluate the monovalent cation selectivity of the Mid1-induced conductance, we examined the membrane currents with 150 mM Cs^+-gluconate solutions in the pipette and the bath (*12*), and Cs^+ in the bath was subsequently replaced by K^+ (150 mM) or Na^+ (150 mM). Nearly equivalent inward and outward currents were recorded with Cs^+ in both solutions (Fig. 2F), and the overall *I-V* curve was nearly linear crossing 0 mV with little indication of voltage dependency (Fig. 2H). Replacement of extracellular Cs^+-gluconate with equimolar K^+- or Na^+-gluconate did not alter conductance or reversal potential. Little whole-cell current was recorded in cells not treated with Zn (Fig. 2E) or mock-transfected cells (*13*). Inward but not outward Cs^+ currents were inhibited by adding 2 mM Ca^{2+} to the bath solution (Fig. 2, G and H). These results indicate that the expression of Mid1 results in an increase in cation conductance with similar permeability among Cs^+, Na^+, and K^+, and that Ca^{2+} behaves as a blocker for monovalent cation currents as well as a permeant ion.

The Gd^{3+} effect (Fig. 2, C and D) indicates that Mid1 may be a SA Cat channel. Mid1 appears to be distinct from known ion channels, including voltage-dependent Ca^{2+} channels and ligand-gated Ca^{2+} channels: Mid1 has no overall amino acid sequence similarity to these ion channels, and its activity was not blocked by channel blockers we tested, including verapamil, nifedipine, diltiazem, ω-conotoxin, or heparin.

We made single-channel analyses with cell-attached patches that can be stretched by negative pressure in the pipette (*14*). The pipette solution contained 150 mM CsCl and the bath solution contained a high concentration of K^+ solution (137 mM) to depolarize the membrane potential to near 0 mV. Application of negative pressure in the pipette by suction increased channel activity, but the magnitude of the unitary current was not changed (Fig. 3A). This activation was reversed immediately after the cessation of suction in the pipette. The pressure dependence of the channel open probability (NPo) was sigmoidal over the range of tested pressures from 0 to 40 cm H_2O (Fig. 3B). Preliminary results indicated that addition of Gd^{3+} in the pipette solution reduced NPo with little changes in the conductance (*15*). Inward Cs^+ currents were recorded at various holding potentials in a Mid1-expressing cell under a negative pressure of 20 cm H_2O (Fig. 3C). The *I-V* curve displays a slope conductance of 32 ± 4.7 pS (n = 26) and a reversal potential of 0 mV. Similar results were obtained when NaCl or KCl was present in the pipette instead of CsCl, indicating similar permeability among monovalent cations, which is consistent with the property of whole-cell currents. We measured inward Ca^{2+} currents using a pipette solution containing 10 mM $CaCl_2$ under negative pressure (20 cm H_2O) in the pipette at various holding potentials (Fig. 3D). The *I-V* curve displays a slope conductance of 3.5 ± 0.46 pS (n = 21) with a positively shifted reversal potential, suggesting that Ca^{2+} is more permeable than monovalent cations (P_{Ca}/P_K = 7.13). These conductances did not change when chloride or aspartate was the anion in the pipette

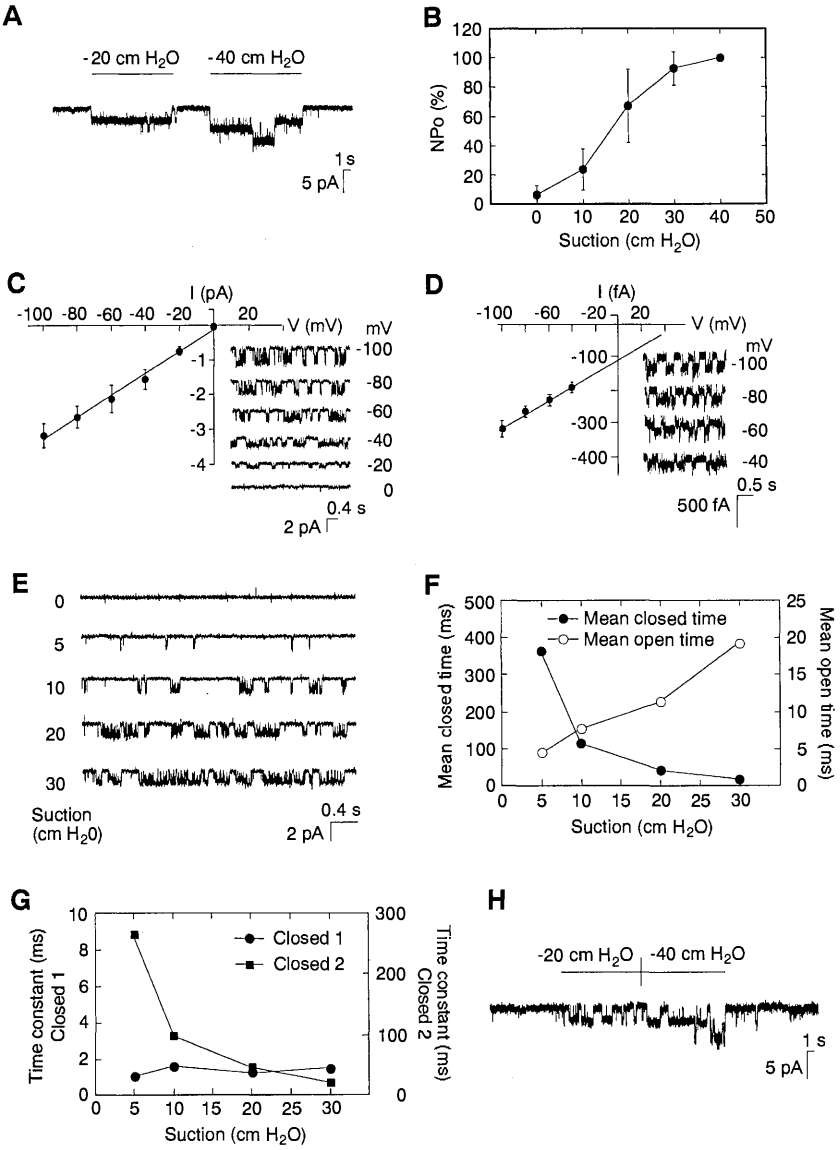

Fig. 3. Single-channel currents from Mid1-expressing CHO cell. (**A**) A typical record of single-channel currents from a cell-attached patch on a Mid1-expressing cell in response to suction (0 to 40 cm H_2O) in the pipette at −60 mV. Solutions were 150 mM Cs^+-gluconate, 1 mM EGTA-Cs, and 10 mM HEPES-Cs (pH 7.4) (pipette) and 137 mM KCl, 5 mM NaCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, and 10 mM HEPES-Na (pH 7.4) (bath). (**B**) Dependence of channel open probability (NPo) on pressure in the pipette. The NPo gradually increased with negative pressure (suction). NPo was normalized to that obtained at 40 cm H_2O suction. (**C**) The *I-V* relationship with 150 mM Cs^+ in the pipette. (**D**) The *I-V* relationship with 10 mM Ca^{2+} in the pipette. (**E**) Changes in channel activity with various pipette suctions at −60 mV; (**F**) Pressure dependency of mean open and mean closed times calculated from (E); (**G**) Pressure dependency of short (closed 1) and long (closed 2) closed times calculated from (E). (**H**) A typical channel current recorded from an excised inside-out patch derived from a Mid1-expressing cell. Membrane potential was held at −60 mV, and negative pressure (0 to 40 cm H_2O) was applied under the conditions described in (*18*). Values are the mean ± SD for three to four experiments.

solution, confirming that the currents were carried by an influx of cations, rather than by outward flux of anions, which would display negative reversal potentials in these experimental conditions. The inward cation current activated by suction was not detected in mock-transfected cells. These properties of single-channel currents of the Mid1 channel appear to be similar in most respects to a mechanosensitive ion channel observed in the *S. cerevisiae* plasma membrane, except for its conductance and permeability for Ca^{2+} (*16*).

Application of negative pressure in the pipette increased the channel activity (Fig. 3E), where the mean open time increased and the mean closed time decreased with increased suction (Fig. 3F). Analysis of the dwell time histograms for open and closed times suggested that the Mid1 channel has one open state and two closed states, because the open-time histogram was well fitted by a single exponential function and the closed time by a double exponential function using the maximum likelihood method (*17*). Suction in the pipette produced minor changes in the flickery closed state (short closed time), but significant decreases of the interburst closed state (long closed time) (Fig. 3G).

We also analyzed the pressure dependence of Mid1-channel activities with the inside-out patch configuration (*18*) and found that they were essentially the same as those obtained from the cell-attached patch-clamp configuration (Fig. 3H), suggesting that the single-channel currents observed are activated directly by the membrane stretch, not by an intracellular second messenger.

To examine the stretch-activated, whole-cell activity of the Mid1 channel, Mid1-expressing CHO cells were cultured on a fibronectin-coated, thin silicone membrane as in (*19*) and subjected to uniaxial stretch (120%) for 2 s, and the average $[Ca^{2+}]_c$ from about 60 cells was measured under a fluorescence microscope (*20*). Although the $[Ca^{2+}]_c$ during stretch could not be measured because of the out-of-focus cell image with this method, we could approximate the degree of $[Ca^{2+}]_c$ increases from the tail response. Control cells not treated with Zn showed essentially no change in $[Ca^{2+}]_c$ (Fig. 4A). By contrast, cells treated with Zn for 12 hours did show an increase in $[Ca^{2+}]_c$ (Fig. 4B), which was abolished by removal of extracellular Ca^{2+} (Fig. 4C) or by externally applied $GdCl_3$ (20 μM) (Fig. 4D). The Gd^{3+} effect was dose-dependent, and 50 μM Gd^{3+} completely blocked the response. Essentially the same results were derived from cells loaded with another Ca^{2+} indicator, fluo3, in conjunction with confocal microscopy. These observations are essentially the same as those obtained with cultured endothelial cells (*19*) and confirm that the Mid1 protein expressed in CHO cells acts as a Ca^{2+}-permeable SA Cat channel. We do not have data that can directly explain the difference in Gd^{3+} sensitivity of the Mid1 channel between the whole-cell currents (Fig. 2, C and D) and the $[Ca^{2+}]_c$ increase (Fig. 4D). It is possible that the whole-cell currents were measured under a putative constant stretch generated by turgor force, as suggested above in the hypertonic inhibition of steady Ca^{2+} influx. On the other hand, the $[Ca^{2+}]_c$ response by stretch pulse (Fig. 4D) might be mediated by the SA Cat channels that had not been activated by the putative background turgor force. Presumably, the pharmacological property of the Mid1 channel in an adapted state by turgor force may be different from that in a non-adapted one, but it remains to be solved.

Our results indicate that Mid1 can function as an SA Cat channel in CHO cells, although it remains to be determined whether Mid1 is an SA Cat channel itself or a subunit that up-regulates the activity of endogenous SA Cat channel in CHO cells. We have obtained electrophysiological data from cell lines of different species including mouse Balb/c 3T3 cells and green monkey COS-7 cells, similar to those obtained with CHO cells (*21*), which suggests that the latter possibility is unlikely. In either case, further functional and mutational studies on Mid1 should provide important new clues for molecular characterization of eukaryotic SA Cat channels.

In *S. cerevisiae*, Mid1 is activated in cells exposed to mating pheromone after a lag period of 30 min or more (*4*), and during this time, the remodeling of the cell wall is induced to form a polarized mating projection (*22*). This remodeling possibly causes increased stretch in the plasma membrane because of turgor and activates the Mid1 channel. Although eukaryotic SA Cat channels are known to respond to stretch produced by extracellular forces, they may also respond to stretch generated by the activity of the cell itself directing cell polarity during cell division, cell morphogenesis, or cell migration.

A potential Mid1 homolog, Yam8 (SWISS-PROT number Q10063), from the fission yeast *Schizosaccharomyces pombe* was cloned, and it complemented the mating pheromone–induced death phenotype of the *mid1* mutant (*23*). Because the divergence of homologous genes between *S. cerevisiae* and *S. pombe* is similar to that between yeasts and mammals (*24*), it is possible that Mid1 homologs are present in other eukaryotes.

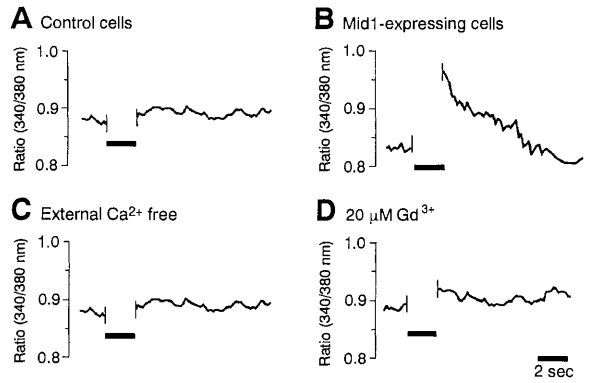

Fig. 4. Stretch-activated Ca^{2+} response in CHO cells. Cells cultured on elastic silicone membranes were incubated with 1 μM fura-2/AM and subjected to an uniaxial stretch pulse (120% of length for 2 s at room temperature). (**A**) Control cells not treated with $ZnCl_2$; (**B**) Mid1-expressing cells treated with $ZnCl_2$; (**C**) Mid1-expressing cells in the absence of extracellular Ca^{2+}; (**D**) Mid1-expressing cells in the presence of 20 μM external Gd^{3+}. A thick solid bar in each chart indicates the period of stretch during which fluorescence signals could not be recorded because of loss of focus and visual field of the sample. Data represent typical examples from at least five experiments for each condition.

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the *MID1* mRNA by Northern blotting as in (*26*). The transfected cells were seeded in 100-mm dishes for immunoblotting, or in 35-mm dishes containing cover slips or in 4-cm^2 silicon chambers coated with fibronectin (50 μg/ml) for $[Ca^{2+}]_c$ measurements. To transiently induce expression of the *MID1* gene, 80 μM $ZnCl_2$ was added to the medium.
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7. The $[Ca^{2+}]_c$ of cells grown on cover slips was monitored by use of fura-2 as in (*25*). Briefly, cells cultured on a cover slip were incubated with 2 μM fura-2/acetoxymethyl ester (AM) (Dojin, Kumamoto, Japan) for 20 min at room temperature, and then each cover slip was examined by fluorescence microscopy, with the fluorescence measured using the ARGUS calcium imaging system (Hamamatsu Photonics, Hamamatsu, Japan).
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9. M. Kanzaki *et al.*, unpublished data.
10. We used the single-channel variation on the cell-attached patch-clamp technique for analysis of Mid1 molecule unitary channel events and measured changes in membrane currents with the whole-cell mode of the patch-clamp technique, as in (*26*). The resistance of the patch pipettes was 4 to 6 megohms for whole-cell analysis and 7 to 7.5 megohms for single-channel analysis, and the indifferent electrode was an Ag-AgCl plug connected to the bath via a KCl agar bridge. All experiments were done at 22° to 26°C. For whole-cell patch-clamp analysis, the pipette solution contained 120 mM *N*-methyl-D-glutamate (NMDG)–aspartate, 10 mM HEPES (pH 7.0), and 1 mM EGTA. The bath solution contained 120 mM NMDG-aspartate, 10 mM $Ca(OH)_2$, and 10 mM HEPES (pH 7.4 adjusted with aspartate). The osmolarity of these solutions was adjusted to 320 mosM with sucrose. Cells were held at 0 mV, and the *I-V* relations were recorded using jump pulses of 20 mV from −100 mV to +100 mV for 400 ms at an interval of 1 s.
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12. To evaluate permeability of monovalent cations, the pipette solution contained 150 mM Cs^+-gluconate, 1 mM EGTA, 10 mM HEPES (pH 7.2). The bath solution contained 150 mM Cs^+-, K^+- or Na^+-gluconate and 10 mM HEPES (pH 7.4). The *I-V* relations were obtained by applying voltage pulses as mentioned above. The osmolarity of these solutions was adjusted to 320 mosM with sucrose.
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14. For the single-channel analysis, seals of ~20 gigohm were achieved by applying slightly negative pressure (~5 cm H_2O) in the patch pipette, and after formation of a stable seal, the suction in the pipette was released, establishing a reference point for zero applied pressure. To investigate mechanosensitivity of Mid1, negative hydrostatic pressures of up to 40 cm H_2O were applied. The total number of functional channels (N) in the patch was estimated by observing the number of peaks detected on the amplitude histogram. As an index of channel activity, NPo (the number of channels times the open probability) was calculated as in [M. Kanzaki, M. A. Lindorfer, J. C. Garrison, I. Kojima, *J. Biol. Chem.* **272**, 14733 (1997)]. Kinetics of open and closed events were analyzed for patches containing only one active channel (determined by an all-points amplitude histogram).
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28. Supplementary materials are available at www.sciencemag.org/feature/data/1038393.shl
29. We thank S. Ozawa (Gunma University) for helpful discussion. Supported in part by Grants-in-Aid for Scientific Research on Priority Areas to H.I. and I.K., Grant-in-Aid for Scientific Research (C) to H.I., and Grant-in-Aid for Scientific Research (B) to M.S. from the Ministry of Education, Science, Sports and Culture of Japan, and by a grant from the Yamada Science Foundation to H.I., from ETL to C.S., from Japan Space Forum to I.K. and M.S., and from CREST to M.S.

12 January 1999; accepted 28 June 1999

Requirement of Rsk-2 for Epidermal Growth Factor–Activated Phosphorylation of Histone H3

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During the immediate-early response of mammalian cells to mitogens, histone H3 is rapidly and transiently phosphorylated by one or more unidentified kinases. Rsk-2, a member of the pp90^{rsk} family of kinases implicated in growth control, was required for epidermal growth factor (EGF)–stimulated phosphorylation of H3. *RSK-2* mutations in humans are linked to Coffin-Lowry syndrome (CLS). Fibroblasts derived from a CLS patient failed to exhibit EGF-stimulated phosphorylation of H3, although H3 was phosphorylated during mitosis. Introduction of the wild-type *RSK-2* gene restored EGF-stimulated phosphorylation of H3 in CLS cells. In addition, disruption of the *RSK-2* gene by homologous recombination in murine embryonic stem cells abolished EGF-stimulated phosphorylation of H3. H3 appears to be a direct or indirect target of Rsk-2, suggesting that chromatin remodeling might contribute to mitogen-activated protein kinase–regulated gene expression.

In mammalian cells, various environmental stimuli induce a Ras-dependent MAP (mitogen-activated protein) kinase cascade that results in the transcriptional activation of immediate-early–responsive genes (*1*, *2*). These transcriptional responses are thought to depend on modulation of the nuclear localization, DNA binding, and activation properties of transcription factors, but the roles of MAPK phosphorylation in this process remain poorly defined (*1*).

Remodeling of chromatin structure appears to have a primary role in transcriptional regulation (*3*), and posttranslational modifications of histones are thought to contribute to this remodeling. Widespread phosphorylation of histones, particularly histones H1 and H3, correlates with mitosis in many cells

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