otide from the HPB-ALL T cell line. The V_β family of oligonucleotides used were as described [K. Wucherpfennig *et al.*, *Science* **248**, 1016 (1990)].
30. The HLA-A,B–negative B cell line, C1R, was transfected with the pSR-α Neo expression vector (mock) and pSR-α Neo expression vector containing the CD1a, CD1b, CD1c, or CD1d cDNA by electroporation, as described (*28*). The transfectants were selected in geneticin sulfate (G418; 2.0 mg/ml; specific activity 450 μg/mg, Gibco) and CD1-expressing cells were identified by indirect immunofluorescence. The transfected C1R cells were cloned by limiting dilution and maintained in complete medium containing geneticin sulfate (2.0 mg/ml).
31. We thank S. Porcelli, M. Brenner, and P. Bleicher for several of the C1R transfectants and antibodies; A. Aruffo and B. Seed for the CD1a, CD1b, and CD1c cDNA; D. Hafler for oligonucleotides and advice; and C. Terhorst for useful discussions and support. Supported by National Cancer Institute grant CA-01310 (to S.P.B.), National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases grants DK42166 (to E.C.E.) and 5 KO8 DK01886 (to R.S.B.), a Harvard Digestive Center pilot project grant 2P30DK34854-06, and a grant from the Crohn's and Colitis Foundation of America.

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A Mechanosensitive Channel in Whole Cells and in Membrane Patches of the Fungus *Uromyces*

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Bean leaf stomata provide a topographical signal that induces germlings of the phytopathogen *Uromyces appendiculatus* to develop specialized infection structures. Protoplasts from germ tubes of this fungus, when examined with patch-clamp electrodes, displayed the activities of a 600-picosiemen mechanosensitive ion channel. This channel passes a variety of cations, including Ca^{2+}, and is blocked by Gd^{3+} at 50 micromolar. This channel could transduce the membrane stress induced by the leaf topography into an influx of ions, including Ca^{2+}, that may trigger differentiation.

MOST PHYTOPATHOGENIC FUNGI must locate and recognize an appropriate site for entry into their hosts in order to establish a successful parasitic interaction. The rust fungi are the causal agents of the plant diseases wheat rust, coffee rust, and bean rust. Urediospore germlings of the bean rust fungus *Uromyces appendiculatus* are guided by the topography of the leaf surface to stomata, where they cease polarized growth and differentiate appressoria from the germling apices. Such appressoria are the first in a series of specialized infection structures needed for the pathogen to enter the plant host (*1*). The topographical signal that triggers formation of the appressorium is provided by a feature of the stomatal architecture that can be simulated on chemically inert plastic replicas of the epidermis (*2*) and on polystyrene membranes bearing microfabricated ridges 0.45 to 0.7 μm in height (*3*) (Fig. 1A).

The various postulated mechanisms by which a fungal germling can sense minute surface features include an ionic or electric change mediated by mechanosensitive (MS) channels. MS channels that respond to physiological stretch forces in the plasma membrane have been implicated in the regulation of cell volume, morphogenesis, osmotic pressure, guard cell responses, and neuronal growth-cone functions (*4, 5*). This type of ion channel has been identified in animal and plant cells and microbes, including the budding yeast, a free-living fungus (*5*). Contact of the bean rust germling with an inductive topography could produce localized stretching of the plasma membrane and cause MS channels to open. The resulting changes in membrane potential or cytoplasmic concentrations of ions, such as Ca^{2+}, may induce appressorium formation. We therefore examined the plasma membrane of *U. appendiculatus* germ tube protoplasts for MS channels with the patch-clamp method.

Collected urediospores were germinated and converted into protoplasts 4 to 7 μm in diameter (*6*). Standard patch-clamp electrodes were used to form gigaohm seals with the plasma membrane of selected protoplasts (Fig. 1B) (*7*). We report here the activities of a large conductance MS channel. Other smaller conductance channels were also detected that were voltage-sensitive but not MS. We detected the MS channel activities in every one of more than 50 protoplasts examined in the whole-cell mode or in excised inside-out or outside-out patches. In contrast to *Lymnaea* neurons (*8*), *Uromyces* protoplasts readily displayed whole-cell MS currents. Small pressures applied through the patch pipette tended to open these channels, yielding discrete, stepwise changes in the conductance of the membrane channels (Fig. 2). Opening appears not to be cooperative among these channels (Fig. 2B).

The probability that channels will open in response to a given applied pressure (open probability) fits a Boltzmann distribution in which the mechanical energy partitions the channel molecule between its open and closed conformations (Fig. 3). In three cells analyzed systematically, this curve inflects at pressures between 10 and 20 mmHg, which is similar to the sensitivities of MS channels of other species (*4, 5*). Because we have approached saturation of the open probability without breaking the protoplasts, we can estimate the number of MS channels in a protoplast. In one example (Fig. 3), there were 111 such MS channels in the whole membrane of this protoplast. The average density was about two channels per square micrometer in the ten protoplasts analyzed. The open probability of this channel under a small pressure increases strongly upon depolarization and slightly upon hyperpolarization below −50 mV (Fig. 3C). However, we could not activate the channel with voltage alone.

The conductance of the channel was 601 ± 10 pS (mean ± SD, $n = 9$) in symmetric 290 mM KCl solutions (Fig. 4A). This conductance is large for ion channels among eukaryotic species. We investigated the ion selectivity in the whole-cell mode by examining the changes in the current-voltage (*I-V*) plots of the unitary current after changing the ionic species and concentrations of the bath. Diluting the bath KCl concentration from 290 to 58 mM caused a shift of the *I-V* curve such that the current reversed at −35 mV, close to the calculated equilibrium potential of K^+ (Fig. 4A). Similar results were obtained in three other five-

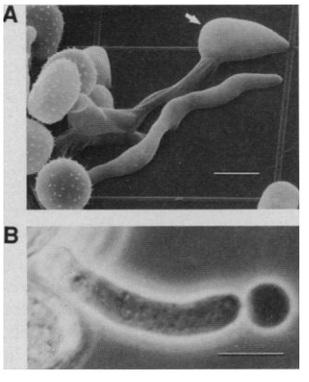

Fig. 1. Germlings of *U. appendiculatus*. (**A**) Scanning electron micrograph of two germlings grown on a polystyrene substrate bearing ridges 0.5 μm high and 2.0 μm wide, prepared as in (*3*). One of the germlings encountered an inductive ridge and developed an appressorium (arrow). (**B**) Protoplasts derived from young germlings (*6*). Bars represent 10 μm.

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or tenfold dilution experiments. Thus, the channel passes K^+ and excludes Cl^- in KCl solutions. Equimolar substitution of K^+ with Na^+, Cs^+, or Rb^+ did not greatly affect the *I-V* plot (Fig. 4B). This indicates poor selectivity among these monovalent cations, although a slight preference in the order of $K^+ \geq Cs^+ \geq Rb^+ > Na^+$ can be discerned.

To test the permeability of divalent cations, we replaced the bath K^+ with Ca^{2+} or Mg^{2+}. At positive voltages, K^+ carried the outward currents from the pipette as expected. At voltages more negative than −20 mV, inward currents were observed that could only be carried by the bath divalent cations (Fig. 4C). The unitary conductance for Ca^{2+} was found to be 565 ± 16 pS (n = 3) (Fig. 4D). From the shift of the reversal potential in these bi-ionic experiments, the permeability ratio was calculated by the expanded constant field equation (*9*) to be P_K:P_{Ca} = 1.1:1.0.

Neither tetraethylammonium ion nor Ba^{2+} blocked the activities of this channel. However, Gd^{3+}, which is known to block MS channel activities of several species, including budding yeast (*4, 5*), did reversibly block the MS channel of the bean rust fungus, with a half-maximal effective concentration of 50 μM (Fig. 4E). That MS channels may be involved in growth and differentiation of this fungus is supported by the observation that germ tube growth and differentiation are inhibited by 10 μM Gd^{3+} (*10*). The presence of 5 mM Ca^{2+} in the media causes a slight increase in differentiation and at pH 5.5 alleviates the inhibitory effects of up to 25 μM Gd^{3+}

In germlings of *U. appendiculatus*, contact with a topography inductive for appressorium development, such as a stomatal lip on the host leaf or a ridge on artificial substrata (*3*), probably results in ion fluxes. Although MS channels may be operative and essential for signal reception, they are probably not sufficient for completion of the appressorium. A different K^+-specific channel is apparently involved, because appressorium formation and, to a lesser extent, germ tube growth are inhibited by tetraethylammonium (6 mM, pH 7 or 5.5), a K^+ channel inhibitor, or elevated concentrations of external K^+ (*10*). These findings indicate that there might be a K^+-specific channel among the channels on the protoplast.

Uromyces protoplasts display whole-cell MS currents (Fig. 3), with distinctly quantized activity, cation selectivity, and saturation at higher pressure. We cannot be sure of the localization of the MS channels relative to the growing portion of the germ tube, where topographic sensing takes place. That topographic sensing occurs only at the apical region could simply reflect the local mechanical properties. Here the cell wall is still being polymerized and the subjacent membrane can more easily be stressed (*11*).

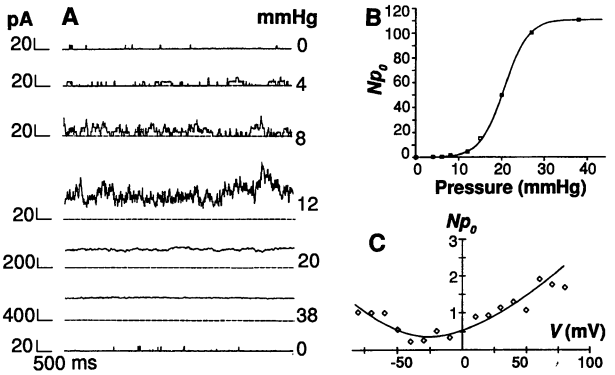

Fig. 3. Sensitivities of the MS channel to pressure. (**A**) Channel activities were recorded in whole-cell mode (+15 mV) at various applied pressures. Reference lines are aligned with the scale markers on left. Note scale changes and the near saturation of opening at 30 mmHg. These pressures did not damage the cell membrane or the seal, as the final recording (bottom trace) showed no additional leakage current. (**B**) Open probability, Np_0, of the MS channels plotted against the applied pressure. Np_0 was calculated from 20 s of recording, parts of which are shown in (A), as the integral of the total channel current during the analyzed period divided by the integral of a single-channel current through the same period. The points are fitted by linear regression to the Boltzmann curve. $Np_0/Np_0\text{max} = \exp[(P - P_{1/2})/S_p]/\{1 + \exp[(P - P_{1/2})/S_p]\}$, where Np_0 is the open probability of all channels in the patch, P is the positive pressure, $P_{1/2}$ is the pressure at which channels are open half of the time, and $1/S_p$ is the slope of the plot of $\ln[Np_0/(1 - Np_0)]$ versus pressure. (**C**) Open probability of the MS channels of another protoplast at various voltages in the whole-cell mode at 12 mmHg pressure.

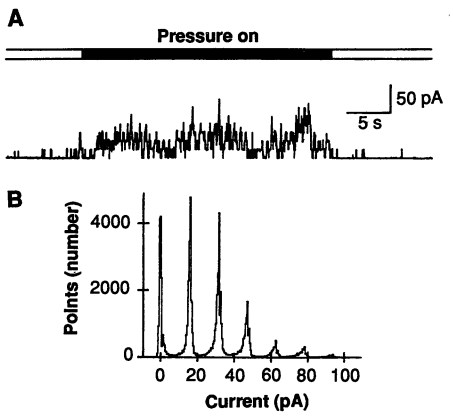

Fig. 2. MS channel activities in protoplasts of *U. appendiculatus*. (**A**) The application of pressure at 10 mmHg (1 mmHg = 133 Pa) to a protoplast through the pipette activated the channels, which were recorded in the whole-cell mode at membrane potential, V_m = +26 mV. (**B**) Current amplitude histogram of a 30-s record from the same protoplast showing seven discrete levels of current. This histogram can be adequately fitted by Poisson distribution (*14*). After gigaohm seal formation on a protoplast, the patch was disrupted by application of increased suction through the pipette to establish the whole-cell mode of recording at room temperature (19° to 22°C).

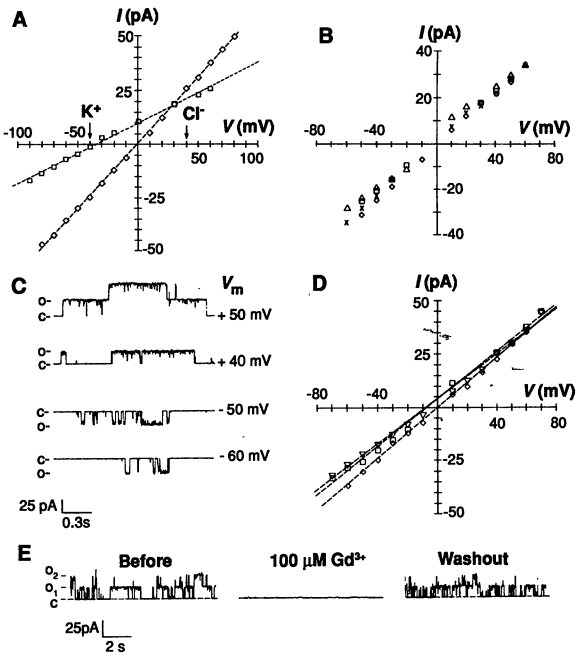

Fig. 4. Permeation or blockage of the MS channel by different ions. (**A**) *I-V* plots of unitary currents in KCl solutions. ◇, Recordings from a cell in solutions of 290 mM KCl, 10^{-2} mM $CaCl_2$, and 5 mM Hepes at pH 7.2, in both the pipette and in the bath; □, same cell after a change in the bath to 58 mM KCl, 5 mM Hepes, pH 7.2, and 464 mM sorbitol to maintain the osmotic balance. (**B**) *I-V* plots from another cell in baths of various alkaline metal ions (chloride salts), each at 290 mM. The bath solutions also contained 10^{-2} mM $CaCl_2$ and 5 mM Hepes at pH 7.2. x, CsCl; □, RbCl; △, NaCl; ◇, KCl. (**C**) Divalent ion permeation through the MS channel at negative pipette voltages (c, closed; o, open). Recorded from an excised inside-out patch in a bath solution of 200 mM $CaCl_2$ and 5 mM Hepes at pH 7.2 without any K^+. (**D**) *I-V* plots from this cell in baths of 200 mM Ca^{2+} (□) or Mg^{2+} (▽) or 290 mM K^+ (◇). (**E**) Recordings from a whole-cell mode under 10 mmHg pressure in 290 mM KCl, 1 mM $CaCl_2$, 5 mM Hepes, adjusted to pH 7.2 with KOH. Perfusion with the same bath solution containing 100 μM $GdCl_3$ completely blocked the channel activities, and the final reperfusion of the same bath solution without $GdCl_3$ restored activities.

Although membrane tension at the site of ridge contact can only be estimated, calculations (*12*) indicate that the germlings can experience tensions similar to those in patches, provided that signal transduction is limited to a small area such as a small portion of the cell apex. One might suspect that ridges <0.2 or >0.8 μm in height are noninductive for different reasons: too few MS channels may be affected, or contact may be made with an area in which the wall has already polymerized, respectively.

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6. Urediospores (0.5 g) were germinated in water at 16° to 19°C until the germ tubes were 40 to 70 μm long (~2 hours) and before all of the cytoplasm had migrated out of the spore. To produce protoplasts, germlings were digested for 25 to 40 min with Novozyme (Novo BioLabs, Bagsvaerd, Denmark), 5 mg/ml, in 0.5-M sorbitol buffered with 17 mM MES, pH 6.0 [D. Huang, R. C. Staples, W. R. Bushnell, D. L. Maclean, *Phytopathology* **80**, 81 (1990)]. All protoplasts were formed from the germling apices. After filtration through a 20-μm mesh Nytex cloth (Tetko, Inc., Elmsford, NY), the protoplasts were pelleted at 2000*g* for 4 min and resuspended in sorbitol without enzyme. The protoplasts were washed twice through centrifugation in the recording bath solution (*7*). Protoplasts selected for patch-clamping appeared uniformly dark, smooth, and empty when viewed with phase contrast optics.
7. The protoplast membrane of *U. appendiculatus* (*6*) formed gigaohm seals more easily than that of *Saccharomyces cerevisiae*. After seal formation, we were often confronted with cell-attached patches, in which the MS channels could be activated with large suctions, albeit the activities were not as clear as in other modes. For *U. appendiculatus* protoplasts, a 10- to 30-mmHg suction sustained over several minutes, instead of pulses of very large suctions, was most effective in converting the cell-attached mode to whole-cell mode. Whole-cell current through the MS channels was verified with a small positive pressure. Once established, the whole-cell preparation usually lasted for hours. Pressure application, pressure measurement, and recording, digitizing, and analyzing of data have been reported (*5*). Unless stated otherwise, the bath solution was 220-mM KCl, 50-mM $MgCl_2$, 5-mM Hepes, pH 7.2, and the pipette solution was 290-mM KCl, 10^{-2}- mM $CaCl_2$, 5-mM Hepes, pH 7.2 (pH adjusted with KOH).
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12. The membrane tension (T) is related to applied pressure (P) according to Laplace's law: $T = Pd/4$, where d is the cell diameter. Low but significant channel opening was observed at a pressure of 10 mmHg in protoplasts with an estimated diameter of 4 to 7 μm. The resulting values for T are 1.3×10^{-3} to 2.3×10^{-3} N/m. In germ tubes, the tension experienced on contact with a ridge can be calculated as follows: $T = K_A \Delta A/A$, where K_A is the area elasticity and ΔA is the change in the area (A) experiencing the tension (*13*). If tension from the observed membrane deformation [Y. H. Kwon, thesis, Cornell University (1991)] is experienced over the whole germ tube membrane, then $\Delta A/A$ is negligible. With a K_A of 100×10^{-3} N/m, representative of most biological lipids (*13*), the tension in germ tubes is far less than that which opened channels in patched protoplasts. However, the region of deformation on contact is limited; therefore, a tension of approximately 2.1×10^{-3} N/m is produced. The K_A would be larger for membranes with underlying cytoskeleton, thus the value calculated for T in the germlings could be underestimated.
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14. Channel activity was quantified as $Np_0 = \Sigma_{n=1}^{N} np_n$, where p_0 is the open probability of the single channel, p_n is the probability that n channels are open simultaneously, and the N is the apparent number of channels. The value of p_n was derived as in Fig. 2B. The observed occupancies of conductance levels zero through six were 0.156, 0.287, 0.303, 0.199, 0.037, 0.009, and 0.008. This distribution can be fitted to a Poisson distribution of $p_k = (\lambda^K e^{-\lambda})/K!$ (K = 0,1, . . .,6), where $Np_0 = \lambda$. The first seven terms of this Poisson distribution are 0.177, 0.306, 0.265, 0.153, 0.066, 0.029, and 0.008. The observed distribution does not differ significantly from that calculated in a χ^2 test.
15. We thank B. Martinac, P. Minorsky, R. C. Staples, W. Sigurdson, and F. Sachs for valuable discussions. Programs used to analyze single-channel records were developed by Y. Saimi. Supported by the Lucille P. Markey Trust (C.K.) and the NSF (H.C.H.).

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Depletion of CD4$^+$ T Cells in Major Histocompatibility Complex Class II–Deficient Mice

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The maturation of T cells in the thymus is dependent on the expression of major histocompatibility complex (MHC) molecules. By disruption of the MHC class II A^b_β gene in embryonic stem cells, mice were generated that lack cell surface expression of class II molecules. These MHC class II–deficient mice were depleted of mature CD4$^+$ T cells and were deficient in cell-mediated immune responses. These results provide genetic evidence that class II molecules are required for the maturation and function of mature CD4$^+$ T cells.

THE NORMAL DEVELOPMENT OF MATURE CD4$^+$ T lymphocytes requires their interaction with MHC-encoded class II molecules in the thymus (*1–6*). These polymorphic molecules are present on thymic cortical epithelial cells, and their engagement with the αβ T cell receptor on immature thymocytes is thought to result in the positive selection of CD4$^+$ T cells and their ultimate export to the periphery (*4–7*). Gene targeting (*8*) is a method by which specific genes can be altered in embryonic stem (ES) cells regardless of their expression (*9*) and subsequently passed through the germ line (*10–17*). We have used this technique to generate mice that are devoid of cell surface expression of MHC class II molecules by introducing a loss of function mutation into the A^b_β gene (*18*) in ES-D3 cells. ES-D3 cells are derived from mice of the H-2^b haplotype (*19*) and thus harbor a deletion in their E^b_α gene that prevents the expression of I-E molecules on the surface of class II expressing cells (*20*). Disruption of the A^b_β gene in ES-D3 cells would similarly prevent the cell surface expression of I-A molecules on class II expressing cells. Therefore, mice of this genotype should be deficient in the cell surface expression of both I-E and I-A class II MHC molecules.

The targeting vector (Fig. 1A) incorporates the neomycin resistance (*neo*^{r}) gene into the second exon of the A^b_β gene, contains 5.4 kb of homologous flanking sequence, and contains the herpes simplex virus (HSV-1) thymidine kinase (*tk*) gene, allowing positive-negative selection of transfectants (*21*). Of the 2×10^7 ES-D3 cells transfected with this construct, 720 colonies were G418^r (calculated from control plates), whereas 143 were resistant to both G418 and gancyclovir. Of these 143 colonies, 86 were screened by Southern (DNA) blot analysis and four clones contained a disrupt-

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