During the 20th century, only the 1943 Hendek earthquake with a magnitude of 6.4 affected the rupture zone of the 1999 earthquake. However, earlier earthquakes in 1719, 1754, 1878, and 1894 occurred in the Gulf of Izmit (*3*). The 1719 and 1754 earthquakes caused the death of 6000 and 2000 people, respectively, in the Gulf of Izmit, Istanbul, and Adapazari region (*3*). There is little information about the 1878 earthquake, which caused considerable damage and loss of life in the Sapanca and Adapazari regions. The 1894 earthquake caused damage and loss of life (1400 people) from Istanbul to Adapazari. The rupture zone for this earthquake is believed to be located either on the Yalova segment or the Cinarcik basin. Among these earlier earthquakes, the 1719 earthquake is perhaps most similar in magnitude and location to the 1999 earthquake, although it may have occurred closer to Istanbul. The remaining two earthquakes, in 1754 and 1878, probably occurred in the area between eastern part of the Gulf of Izmit and Adapazari.

Recent measurements with the Global Positioning System have indicated that the northern strand of the North Anatolian fault, which goes through the Gulf of Izmit and the northern Marmara Sea, has a slip rate of about 15 mm per year (*5*). This suggests that the recurrence interval for a 4 to 5 m displacement is about 300 years. Modeling of the 1939 to 1967 earthquake sequence (*6*) illustrates that during this period, stress has increased in the Gulf of Izmit region by a few bars. Given the estimated slip rate, information from historical earthquakes, and the modeling results, the location and severity of this earthquake should not have come as a surprise.

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PERSPECTIVES: BIOMEDICINE

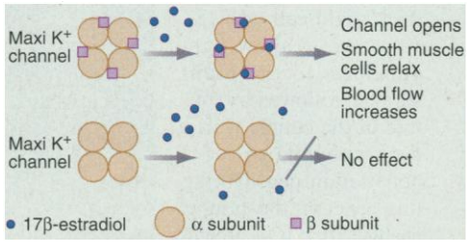
Beating the Odds with Big K

Shai D. Silberberg and Karl L. Magleby

When it comes to longevity, women have an advantage over men because they are less susceptible to cardiovascular disease, at least until the onset of menopause (*1*). This advantage is due largely to the beneficial effects of their estrogen hormones on blood vessels (*2*). Estrogen crosses the plasma membrane of vascular endothelial and smooth muscle cells and binds to specific intracellular receptors. The ligand-receptor complex then alters gene expression, which results in protection of blood vessels from injury and atherosclerosis. In addition to these long-term protective effects, estrogen induces rapid dilation of blood vessels without altering gene expression. However, it is not clear whether estrogen mediates this effect by binding to novel receptors in the plasma membrane, or by activating intracellular nongenomic pathways. In a study on page 1929 of this issue, Valverde *et al.* (*3*) seek to resolve this conundrum. They report that a potassium ion (K^+) channel known to participate in the rapid regulation of blood vessel tone is directly activated by 17β-estradiol, the major circulating estrogen in premenopausal women.

Contraction of vascular smooth muscle decreases the diameter of blood vessels, and thus controls blood flow and blood pressure. Graded changes in the voltage across the plasma membrane of smooth muscle cells in the blood vessel wall result in graded muscle contraction. When the intracellular potential becomes more positive (depolarization), voltage-dependent Ca^{2+} channels in the plasma membrane are activated. The entry of Ca^{2+} into smooth muscle cells through these channels then leads to muscle contraction. This process can be reversed by the opening of K^+-selective channels. The increased efflux of K^+ from muscle cells induces the membrane potential to become more negative, which closes the Ca^{2+} channels, resulting in muscle relaxation (*4*). The realization that modulating the activity of K^+ channels can be used to control blood pressure has sparked considerable interest in identifying K^+ channels in vascular smooth muscle cells and in developing drugs to modulate them (*5*).

One class of K^+ channel that participates in the relaxation of smooth muscle is the large-conductance, calcium-activated K^+ channel (*4*), also affectionately referred to as the Maxi K^+ or Big K channel because of its unusually large conductance. Maxi K^+ channels differ from most other K^+ channels in that their activation is under dual control—switched on by either depolarization or by an increase in intracellular Ca^{2+} (*6*). This dual (often synergistic) activation is possible because of the Maxi K^+ channel's structure. Each of the four α subunits that assemble to form a functional Maxi K^+ channel (*7*) can be divided into two parts: a core (which is similar to that in other voltage-activated K^+ channels) complete with a voltage sensor, and an extended tail that houses an intracellular Ca^{2+} binding domain (*8*). In addition to the pore-forming α subunits common to all Maxi K^+ channels, those in vascular smooth muscle have an auxiliary β subunit that combines with α subunits in a one-to-one stoichiometry (see the figure) (*9*). The β subunit has profound effects on Maxi K^+ channel activity, decreasing by 5- to 10-fold the concentration of intracellular Ca^{2+} required to keep the channel open 50% of the time compared with the β subunit-deficient Maxi K^+ channels in skeletal muscle (*9*, *10*). That the activation of Maxi K^+ channels in vascular smooth muscle leads to rapid dilation of blood vessels raises the question of whether estrogen binding to the β subunit is involved in this process.



Maximizing the benefits of K^+ channels. The Maxi K^+ channel of vascular smooth muscle cells is composed of both α and β subunits (**top**), whereas that of skeletal muscle cells is composed of α subunits alone (**bottom**). 17β-Estradiol binds to and increases the activity of Maxi K^+ channels with β subunits. The resulting efflux of K^+ from the vascular smooth muscle cells results in closure of Ca^{2+} channels and relaxation of the muscle in the blood vessel wall.

Valverde *et al.* (*3*) address this question by expressing human Maxi K^+ channels in *Xenopus* oocytes and examining the effects of estrogen on channel activity. They found that 17β-estradiol increased the activity of Maxi K^+ channels composed of both α and β subunits, but had no effect on channels composed of α subunits alone. When estrogen was coupled to bovine serum albumin (which prevents the hormone from crossing the plasma membrane), the Maxi K^+ α/β channel

S. D. Silberberg is in the Department of Life Sciences and the Zlotowski Center for Neuroscience, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel. E-mail: silber@bgumail.bgu.ac.il. K. L. Magleby is in the Department of Physiology and Biophysics, University of Miami School of Medicine, Post Office Box 016430, Miami, FL 33101, USA. E-mail: kmagleby@miami.edu

was still activated, demonstrating that estrogen acts extracellularly. Estradiol can still activate Maxi K^+ channels reconstituted into artificial membranes (provided both α and β subunits are present), indicating that no intracellular signaling is required and that the Maxi K^+ channel is a receptor for estrogen. To corroborate this deduction, Valverde and colleagues performed binding studies. Oocytes expressing Maxi K^+ channels composed of α and β subunits, but not of α subunits alone, bound greater amounts of ^{3}H-estradiol. Human embryonic kidney cells expressing the α/β channels show greater fluorescence after exposure to estradiol tagged with a fluorescent label compared with cells expressing channels containing only the α subunit. These experiments indicate that the direct binding of estradiol to an external site on Maxi K^+ channels—which is available only when the β subunit is present—increases channel activity.

New methods for treating cardiovascular disease are continually being sought. There is accumulating evidence that postmenopausal estrogen-replacement therapy decreases the risk of major coronary heart disease (*1*). However, the benefit of estrogen treatment decreases with long-term hormone use because of the increased risk of breast cancer (*11*). Consequently, tissue-specific, estrogen-like drugs that preserve the beneficial effects of estrogen on the cardiovascular system without having deleterious effects on other organs are needed. The finding by Valverde *et al.* that estrogen directly activates vascular smooth muscle cell Maxi K^+ channels may pave the way for the rational design of new drugs for the prevention of cardiovascular disease.

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PERSPECTIVES: PLANT BIOPHYSICS

Forcible Entry

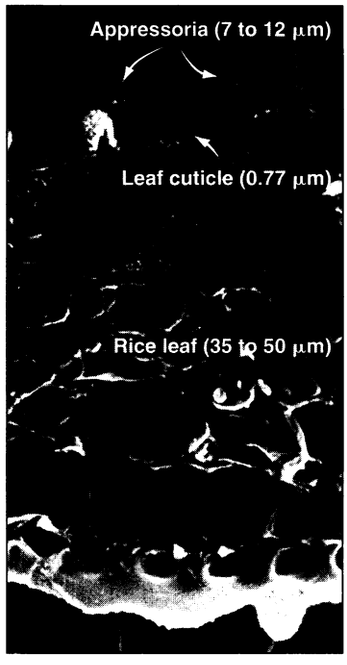
Nicholas J. Talbot

When a fungal pathogen lands on a plant leaf, the most obvious obstacle it faces is how to gain entry to the underlying tissue. Unlike bacteria, which have to circumvent the problem by locating stomata (pores in the plant epidermis), wounds, or other natural openings, many fungal species can rupture the cuticle (the tough outer layer of a plant) directly (*1*). How they do so remains controversial (*2*). In the case of some fungi, enzymatic action is clearly visible at the point of infection, suggesting that the plant cuticle is dissolved ahead of the infecting pathogen (*1*). In other species, specialized infection structures called appressoria are formed that can generate high pressures, indicating a mechanical infection process (*3*). On page 1896 of this issue, Bechinger *et al.* (*4*) report that enormous invasive forces are applied by appressoria of a fungal pathogen, directly demonstrating for the first time that appressoria can exert sufficient pressure to enable mechanical infection of plants by fungi. By allowing appressoria to form on an optical waveguide, the forces exerted by the fungal penetration pegs could be visualized and quantified.

Many of the most severe and economically important plant diseases are caused by fungi, and the initial infection processes have been studied extensively to develop effective disease control strategies (*1*). Fungi have evolved many methods for entering plants, including mechanisms for locating stomata and the ability to rapidly colonize wound sites (*3*, *5*). But many fungal species simply penetrate plant cuticles directly, either as threadlike fungal cells called hyphae or, more frequently, by growing specialized appressoria (see the figure) (*3*). Appressoria are swollen, dome-shaped, or cylindrical cells that differentiate from the end of fungal germ tubes and during maturation can further differentiate to produce a thick, rigid cell wall (*3*). Appressoria allow tight adhesion to the plant surface, followed by rupture of the cuticle with a narrow hypha called a penetration peg. During this process, the fungus changes its axis of growth and reestablishes polarized growth as the penetration peg extends into the plant.

The infection process by appressoria can involve enzymatic action, and the external matrix around appressoria often contains cutinase, cellulases, and other nonspecific esterases to help soften the cuticle, thereby aiding adhesion and penetration (*1*, *3*). However, experimental proof of an absolute requirement for enzymatic activity has remained elusive (*2*), and it has been apparent ever since the pioneering work of Miyoshi (*6*) and others that some fungi can physically break their way through plant cuticles.

Fungi such as *Colletotrichum* and *Magnaporthe* species produce appressoria with tough melanin-pigmented cell walls (*3*, *7*). Howard *et al.* previously showed that appressoria of *Magnaporthe grisea*, the causal agent of rice blast disease, generate very high internal pressure (turgor) (*8*). A cell collapse assay was used to predict the appressorial turgor of *M. grisea* by calculating the concentration of polyethylene glycol required to collapse an appressorium. Howard *et al.* showed that *M. grisea* appressoria generate pressures of between 6 and 8 megapascals—the equivalent of 30 to 40 times the pressure of an average car tire—an astounding pressure for a cell to generate. Appressoria were also shown to be able to puncture artificial plastic membranes. In the same series of experiments, ap-



Breaking in. A spore from the rice blast fungus *Magnaporthe grisea* has germinated on the surface of a rice leaf and formed a dome-shaped appressorium. The appressorium has to breach the thin but tough rice leaf cuticle to invade the leaf and cause disease.

The author is in the School of Biological Sciences, University of Exeter, Washington Singer Laboratories, Exeter EX4 4QG, UK. E-mail: n.j.talbot@exeter.ac.uk. Until October 1999, he is on sabbatical at Paradigm Genetics, Research Triangle Park, NC 27709, USA. E-mail: ntalbot@paragen.com