and seed set. Thus, transgenic plants carrying both genes have the same yield potential as the wild type in the absence of infection.

What is the molecular basis of the PigmR-PigmS–mediated balance between immunity and yield? It is unclear how PigmR and PigmS interact in rice cells before and after infection. Also unclear is whether AvrPigmR is a conserved effector in *M. oryzae*, and how it interacts with PigmR or PigmS. Another question concerns the identities of the partners of PigmR and PigmS in the immune and yield signaling pathways. Furthermore, how plant hormones such as salicylic acid and jasmonic acid alter the *PigmR*-mediated yield penalty, and whether other R gene clusters have *PigmS*-like genes, should be examined. A key question is how epigenetic regulation affects *PigmS* expression to balance immunity and yield. Addressing these questions should enlighten the use of R genes for disease control without affecting crop yields.

For the foreseeable future, R genes will remain the best tool to combat blast disease in rice. The situation for wheat blast is currently dire because, so far, only one useful R gene has been identified in wheat (*12*). Therefore, it is important to know if there are orthologs of *PigmR* and *PigmS* in wheat and how they function. It should also be determined if *PigmR* and *PigmS* provide blast resistance when transferred into wheat. The work of Deng *et al.* and others indicate that pyramiding multiple R genes in a single cultivar may have fitness costs. China's progress in boosting rice yields through super high-yield hybrid rice is now seriously threatened by rice blast disease because of relative susceptibility of these hybrids to *M. oryzae* compared to conventional rice cultivars. This will also affect food security in South Asia and Africa, where hybrids derived from Chinese parental lines are being cultivated for higher yields. Therefore, better understanding the trade-off between immunity and yield will guide the development of new high-yielding cultivars that have strong, broad-spectrum, durable disease resistance. Such crops will be needed to feed the 9 billion people predicted to populate Earth by 2050. ■

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BIOPHYSICS

Membrane proteins scrambling through a folding landscape

Improved force microscopy reveals unfolding steps with microsecond time resolution

By **Daniel J. Müller**[1] *and* **Hermann E. Gaub**[2]

Single-molecule force spectroscopy (SMFS) (*1*) measures the extension of a molecule when subjected to force. The folding of a protein can be explored by pulling on one terminus to unfold it; upon relaxation, it may refold toward its native states (*2*, *3*). Transmembrane proteins typically unfold stepwise as structural segments (which can consist of parts of single or multiple secondary structures) are extracted from the membrane (see the figure, top panel) (*4*). Once extracted, the unfolded segment can insert back into the membrane and fold toward the native protein structure (*5*, *6*). Complex proteins extracted from the membrane may refold back into the membrane (*5*, *6*) but tend to misfold and require the assistance of chaperones, insertases, or both (*7*, *8*). Incremental folding steps of secondary structures are theorized to happen in the order of milliseconds (*9*) but have been undetectable because of the limited time resolution of SMFS-related techniques. On page 945 of this issue, Yu *et al.* (*10*) address this problem by applying a newly developed SMFS technique based on atomic force microscopy (AFM) that records the response of individual membrane proteins (bacteriorhodopsin) subjected to mechanical forces at microsecond resolution.

"The mechanical unfolding of bacteriorhodopsin presents just one of many potential applications to characterize the folding of membrane proteins..."

The heart of AFM-based SMFS is a microcantilever that determines the force sensitivity and time response of the measurements. As a rule of the thumb, shortening the length of a cantilever reduces viscous damping, which increases cantilever response time and reduces noise (*11*). Yu *et al.* have sculpted extremely short (20 to 30 μm) cantilevers with a focused ion beam and reduced their response time by a factor of ≈100 to approach microsecond time resolution. Removing reflective gold layers from the cantilevers reduced their thermal drift and further increased their force sensitivity by a factor of ≈10.

They used these cantilevers to image native purple membrane, a standard sample in the field, and extracted individual bacteriorhodopsins by pulling their C termini. At sufficiently high pulling forces, a structural segment consisting of transmembrane α-helices G and F and their connecting polypeptide loop unfolded first from the membrane protein (see the figure, bottom panel). Further extraction caused the stretching and unfolding of a structural segment consisting of transmembrane α-helices E and D. The stepwise unfolding of structural segments proceeded until the entire membrane protein was unfolded and extracted.

Superficially, this mechanical unfolding pathway of bacteriorhodopsin appeared as previously described (*4*). However, closer inspection of the force-spectroscopy data uncovered strikingly intricate details of smaller unfolding intermediates superimposed on the unfolding of each structural segment. Most of the smaller intermediates rapidly switched back and forth between unfolding and refolding with lifetimes of <10 μs.

These rapid structural transitions, which are considered a hallmark of equilibrium states, were separated by about two to three amino acids and represent the fast unfolding and refolding of α-helical turns. This multiplicity of closely spaced, transiently occupied intermediate states signifies small changes in local conformations of bacteriorhodopsin that had been predicted with molecular dynamics simulations (*12*). Thus, it is exciting to see experimentally that

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α-helical turns shape the smallest structural unit of larger structural folding segments; the folding pathway of individual proteins in native membranes can be scrutinized directly in unprecedented detail.

The innovations that Yu *et al.* introduce to AFM-based SMFS dramatically improve its potential to induce and detect fast inter- and intramolecular interactions and processes. The mechanical unfolding of bacteriorhodopsin presents just one of many potential applications to characterize the folding of membrane proteins, particularly the use of AFM to first image and then select individual molecules to be "force-probed" with superb time resolution. A wealth of new possibilities are opened up. For example, cell surfaces or protein arrays may be probed with increased throughput and improved precision.

Whether a mechanically perturbed membrane protein unfolds and refolds structural segments at once, or via much smaller steps, is pertinent to larger fundamental questions in biology. Understanding these processes helps influence our thinking about how membrane proteins fold, how they are stabilized, and how they misfold. These processes, in turn, underlie cellular processes related to diseases, including neurodegeneration and cancer. ■

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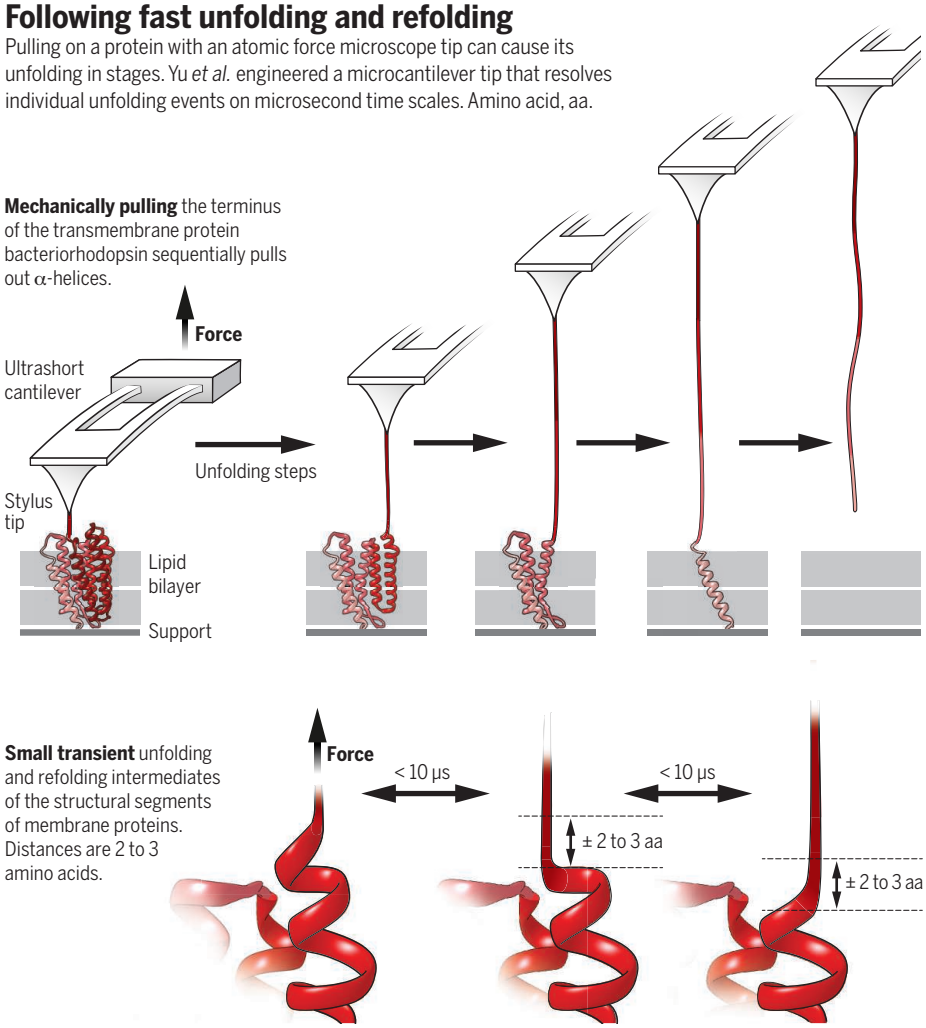
Following fast unfolding and refolding

Pulling on a protein with an atomic force microscope tip can cause its unfolding in stages. Yu *et al.* engineered a microcantilever tip that resolves individual unfolding events on microsecond time scales. Amino acid, aa.



GRAPHIC: K. SUTLIFF/*SCIENCE*

GEOPHYSICS

A measure of mantle melting

Laboratory measurements suggest that the mantle is 60 K hotter than previously thought

By **Paul D. Asimow**

Earth's interior is hot, as is evident from geothermal heat flow, the existence of volcanoes, and the mobility of tectonic plates. But just how hot is it? The temperature increases rapidly with depth through the rigid lithosphere in order to conduct geothermal heat flow, but this cannot continue downward indefinitely without reaching the melting point of rocks. Yet there is no global molten layer below the lithosphere. The propagation of shear waves through the upper mantle shows that it is solid. Rather, the conductive heat flow gives way at some depth to the transport of heat by solid-state convection in the ductile asthenosphere. Convecting systems evolve to an adiabatic temperature profile that can be characterized by a single reference, the "potential temperature." Determining the average and range of variability of the potential temperature of the mantle below the lithosphere allows geoscientists to link observations of phenomena such as postglacial rebound and seismic wave speeds, through laboratory data on viscous and elastic properties, to the composition of Earth. On page 942 of this issue, Sarafian *et al.* (*1*) report new experimental observations of the melting of mantle rocks with the appropriate amount of water and infer a higher value of the potential temperature than previous estimates.

The main avenue to determining the potential temperature is the study of volcanic lavas, particularly at mid-ocean ridges. A mid-ocean ridge forms where two plates of oceanic lithosphere are pulled apart, hot asthenospheric mantle is drawn upward, and it partially melts. Partial, rather than complete, melting is the result both of the

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