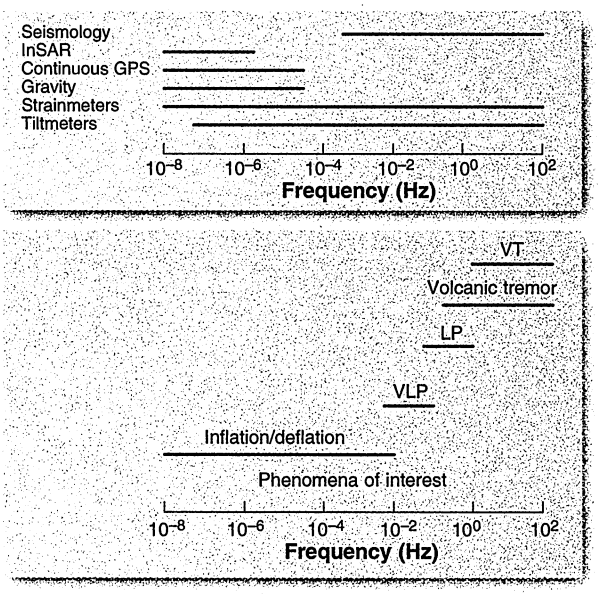

Toward volcanic prediction. Various ground-motion measurement techniques and the corresponding phenomena that can be studied with a variety of instrumentation. VT, volcano-tectonic; VLP, very-long-period; and LP, long period events.

noes characterized by frequent eruptions and a low explosivity index. The data on geophysical precursors are thus still too scarce for a statistically reliable detection methodology on a global basis. Successes are obtained empirically on the basis of eruptive history, physical-chemical properties of magma, and geophysical and geochemical monitoring.

Seismic monitoring is a powerful tool because it allows real-time data analysis. However, the seismic signals on volcanoes are complex and variable. So-called volcano-tectonic (VT) earthquakes are not directly linked to magma movement. Moreover, most data acquisition systems are tuned to the high-frequency band allowing detection of source parameters of the volcano-tectonic events. Most precursors are linked to long-period or very-long-period events, which occur in the frequency band of 1 to 100 s (*7*) and are closely linked to magma fluid dynamics. These movements may occur hours to weeks before the eruptions, constituting more reliable precursors than high-frequency volcano-tectonic earthquakes.

Unfortunately, the sensitivity of seismological instruments is in most cases insufficient for detecting magma and hydrothermal fluids movements. Quantitative methods for analyzing and simulating the behavior of multiphase systems also need further development.

Recent research indicates that broadband seismic radiation is a powerful tool for evaluating not only the source geometry but also the magmatic transport budget (*8–10*). The correct interpretation of these signals allowed the timely evacuation of the 30,000 inhabitants at Rabaul in 1994. This is very important because of the similarities with other calderas, such Campi Flegrei, near Naples, in Italy, or Miyake Island, Japan (*2*).

Another promising technique is the study of phenomena occurring in the frequency band below 10^{-2} Hz, the limit of many traditional seismometers. Data from borehole strainmeters, which can detect strains down to 10^{-10} (three order of magnitudes below GPS) and frequencies down to 10^{-7} Hz, provided important insights into two basaltic eruptions on Hekla, Iceland, in 1991 and 2000 (*11*), and one on Izu-Oshima, Japan, in 1986–87 (*12*), despite the fact that the networks were not designed for volcano monitoring.

The Hekla strainmeters provided the first direct measurements of magma movement, and in 2000, an early warning was officially issued 1 hour before the eruption and was used to alert the nearby airport. On Izu-Oshima, this kind of instrument provided a movie of the vent opening during an eruptive phase in November 1987. Additional instruments of this type have been installed or planned for installation in Mauna Loa, Long Valley, Komagatake, and Vesuvius. This is one of the most promising techniques for monitoring, despite the high costs for planning and borehole installation, which severely restrict their worldwide application.

Several kinds of strainmeters have been developed. In earthquake seismology, these instruments have led to the discovery of slow earthquakes (*13*) and their scaling laws (*14*). Because of their high sensitivity, systematic use of these instruments should allow modeling of the magma and gas transport systems. This will lead to a more quantitative approach in physical volcanology. High-risk volcanoes certainly need more sensitive instruments to model activity, as proposed by Kumagai *et al.* (*2*), and to detect early signals preceding the eruptions.

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PERSPECTIVES: STRUCTURAL BIOLOGY

Actin' Up

Enrique M. De La Cruz and Thomas D. Pollard

The protein actin together with its partner myosin forms the sliding filaments that enable muscle cells to contract. Even though animals with muscles arose relatively recently, actin has been helping eukaryotic organisms to move around for more than a billion years. Protozoa and nonmuscle cells such as macrophages still use actin to move about and to ingest bacteria and other prey. During embryonic development, the actin-based movements of nerve growth cones lay down a spectacular 1 million miles of nerve cell connections in our brains. Myosin proteins motor along actin filament tracks, producing forces that cleave dividing cells into two, transport intracellular vesicles, and power muscle contractions.

It has been known for more than 50 years that actin binds to the energy-releasing molecule adenosine triphosphate (ATP) (*1*). During the polymerization of actin monomers into filaments, ATP is hydrolyzed to adenosine diphosphate (ADP) and inorganic phosphate (P_i). Although ATP and ADP are known to bind to and stabilize actin monomers, it has not been clear how ATP hydrolysis is involved in actin polymerization (*2*). A unique crystal structure of ADP-actin (which, unlike previous actin structures, is not associated with other proteins)—presented on page 708 of this issue by Otterbein and col-

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leagues (*3*)—provides insights into how ATP hydrolysis and release of P_i contribute to the dynamics of actin filament assembly.

The discovery of "treadmilling" provided the first clue to how ATP and ADP could be involved in actin polymerization. The treadmilling effect comes from addition of ATP-actin monomers to the "barbed" end of the actin filament and the dissociation of ADP-actin subunits from the opposite "pointed" end (see the figure) (*4*). Treadmilling occurs because the ratio of the rate constants for monomer association and dissociation (the so-called critical concentrations for polymerization) is lower at the fast-growing barbed end of the actin filament than it is at the slow-growing pointed end (*5*). How ATP hydrolysis drives the slow flux of actin monomers and filament disassembly remains unclear.

Nucleotide-bound actin monomers also associate with proteins that regulate actin polymerization, such as thymosin-β_4, profilin, gelsolin, and cofilin (*6*). Thymosin-β_4 and profilin bind more tightly to ATP-actin monomers than to ADP-actin. In contrast, cofilin binds more efficiently to ADP-actin subunits in filaments and promotes filament disassembly.

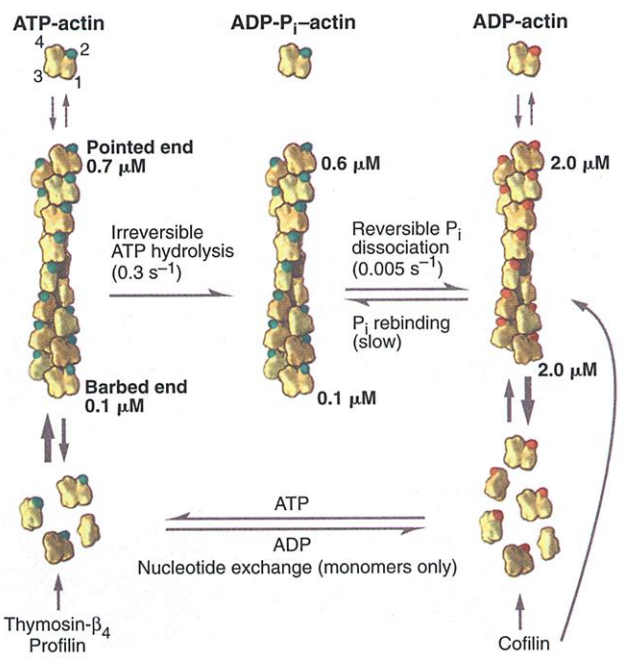
In what way do ATP- and ADP-bound actin subunits differ such that they dissociate from filaments at different rates and are recognized by separate sets of regulatory proteins? Crystal structures of ATP-actin and ADP-actin bound to DNAse I—an enzyme that fortuitously binds to actin monomers—are virtually identical (save for the absence of P_i) to each other and to structures of ATP-actin bound either to profilin or to gelsolin (*7*). If the structures of ATP-actin and ADP-actin are so similar, how can regulatory proteins distinguish between them? The Otterbein *et al.* crystal structure of ADP-actin provides an intriguing answer to this question (*3*).

Actin monomers labeled on cysteine-374 with the fluorescent dye tetramethylrhodamine maleimide are not able to polymerize into filaments. Capitalizing on this property of rhodamine-actin, Otterbein and colleagues determined a high-resolution crystal structure of ADP-rhodamine-actin. There is one crucial difference in subdomain 2 of their actin structure compared with previous actin structures: This segment is a short α helix instead of a β strand! The absence of P_i in the ADP-actin structure results in a series of linked conformational changes that are propagated from the nucleotide binding site over a distance of ~4 nm, favoring the helical conformation in subdomain 2. The orientations of the two halves of ADP-actin are also shifted relative to previous structures.

The α-helical conformation probably exists in equilibrium with the β-strand conformation. The equilibrium constant for the transition is likely to depend on whether P_i is bound to the actin molecule—the strand conformation is probably favored by ATP-actin or ADP-P_i–actin, whereas the helical conformation is preferred by ADP-actin. To test this hypothesis, one could convert the helical conformation to the strand conformation by adding P_i to ADP-rhodamine-actin crystals, or by preparing crystals of ADP-P_i–rhodamine-actin or ATP-rhodamine-actin (which has not yet been achieved). Such an experiment would also establish whether it is the dissociation of P_i per se or the hydrolysis of ATP that actually triggers the conformational change. One would predict (although it is not yet proven) that the change from a strand to a helix occurs in newly polymerized actin filaments after ATP is hydrolyzed and P_i dissociates from the ADP-actin in the filament.

We assume that the Otterbein *et al.* crystal has the same conformation as ADP-actin in the filament, and that ATP-actin and ADP-P_i–actin in the filament have the β-strand conformation. If true, the two conformations of subdomain 2 may account for the differential association of ADP-actin and ATP-actin subunits with regulatory proteins in the filament. The strand conformation may help to stabilize the filament, whereas the helical conformation may induce ADP-actin to dissociate rapidly from the filament's barbed end. Similarly, the β-strand conformation may persuade thymosin-β_4 to bind to ATP-actin subunits (*8*), and the Arp2/3 complex to bind to the pointed ends of actin filaments. In contrast, cofilin has a much higher affinity for ADP-actin subunits in filaments than for ATP-actin, presumably because it favors interactions with the helical conformation. Profilin binds far from subdomain 2 (*6*) and actin-bound nucleotides have only a small effect on its binding (*5*, *7*). Given that the affinity of monomeric and filamentous actin for thymosin-β_4, profilin, cofilin, and the Arp2/3 complex depends on whether P_i is bound to actin, binding of these regulatory proteins must affect the affinity of actin for P_i. This reciprocal thermodynamic relationship is exemplified as follows: P_i bound to ADP-actin inhibits the binding of cofilin to filaments, and cofilin promotes dissociation of P_i from ADP-P_i–actin subunits (*9*).

The ADP-actin structure of Otterbein *et al.* reveals how a conformational change coupled to P_i release could result in filament instability and disassembly. The release of P_i has a small effect on the stability of actin filaments (*10*). However, the conformational change associated with P_i release has a dramatic effect on the binding of cofilin, which chops up ADP-actin filaments and promotes dissociation of ADP-actin subunits from filament ends. Profilin competes with cofilin for ADP-actin and, when bound to ADP-actin, promotes dissociation of ADP. ATP-actin monomers are then regenerated (by addition of ATP) and bind to thymosin-β_4 and profilin, forming a massive pool of unpolymerized actin ready to be added to new filaments as they form. P_i release also favors dissociation of actin filament branches induced by the Arp2/3 complex, another step in recycling actin



Fine-tuning filaments. The binding of regulatory proteins to ADP-actin and ATP-actin subunits in filaments. Equilibrium constants for assembly (critical concentrations) and binding reactions are shown for the actin-associated proteins: thymosin-β_4, profilin, and cofilin. An ATP-actin monomer concentration of greater than 0.7 μM is required for elongation of filaments at their pointed ends. In contrast, just 0.1 μM of ATP-actin is required for elongation at the filament's barbed end. Consequently, under steady-state conditions where the free ATP-actin concentration is usually slightly higher than 0.1 μM, there is a slow net addition of ATP-actin to barbed ends and a net loss of ADP-actin from pointed ends (treadmilling). [Adapted from (*11*)]

SOURCE: GRAHAM JOHNSON

from the polymer to the monomer form. The dramatic P_i-dependent conformational change in actin induced by binding of regulatory proteins enables P_i release to act as the timer that sets the tempo of filament turnover in cells. Some of the same signals that promote the formation of new actin filaments also suppress filament disassembly by inhibiting the ability of cofilin to promote P_i release. With the structure of ADP-actin in hand, the way is now clear to finally elucidate the intricate dynamics of actin filaments.

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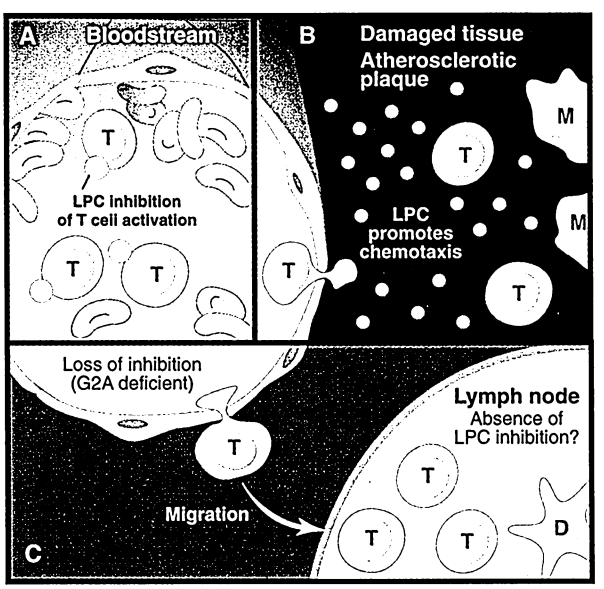
PERSPECTIVES: IMMUNOLOGY

The Push-Me Pull-You of T Cell Activation

Monica J. Carson and David Lo

Explanations for an autoimmune response directed against a single tissue seem simple. Immune cells activated in response to a pathogen protein could aberrantly attack a healthy tissue that expresses a structurally similar protein (molecular mimicry) (*1*). Alternatively, dysregulation of immunoregulatory molecules could lead to recruitment and activation of self-reactive T cells within the healthy tissue (*2*). Yet it is difficult to understand how either of these processes could explain chronic intermittent multiorgan autoimmunity, typified by the disease systemic lupus erythematosus (SLE). The puzzle about the molecular events initiating multiorgan autoimmunity may be partly solved by Kabarowski and colleagues (*3*). In their report on page 702 of this issue, they identify lysophospholipids as ligands for the lymphocyte G protein–coupled orphan receptor G2A. By linking lysophospholipids to G2A, these authors bring together two independent lines of research that cumulatively suggest what neither could alone. Lysophospholipids may be potent super-regulators of T cell activation inflammation at sites of tissue damage and in the early stages of atherosclerosis. Defects in the lysophospholipid-G2A pathway may lead to chronic intermittent multiorgan inflammation by lowering the threshold for T cell activation.

Realizing the similarity of G2A to OGR1—the high-affinity receptor for the lysophospholipid sphingosylphosphorylcholine (SPC)—Kabarowski *et al.* tested SPC and the structurally similar lysophosphatidylcholine (LPC) to see whether they would bind to G2A. LPC, and to a lesser extent SPC, turn out to be high-affinity ligands for G2A capable of inducing G2A-dependent calcium fluxes, chemotaxis of immune cells, ERK protein kinase activation, and the internalization of G2A receptors. The fact that G2A overexpression blocks oncogene-induced expansion of pre-B cells and transformation of fibroblasts in vitro hints that the interaction of G2A with its ligand inhibits the proliferation of immune cells (*4*).



Don't leave it to the professionals. The effects of LPC on T cell activation and migration. (**A**) Physiological concentrations of LPC may inhibit T cell activation in the bloodstream. (**B**) Production of LPC in oxidized LDLs promotes chemotaxis of T cells into damaged tissues and into atherosclerotic plaques in blood vessels. Chronic accumulation and activation of macrophages (M) results in release of large quantities of inflammatory mediators that overcome the inhibitory effects of LPC within atherosclerotic plaques. (**C**) Lymphoid tissues may be exposed to less LPC and so their T cells may be inhibited less strongly, permitting potent T cell activation by dendritic cells (D). In G2A-deficient mice, loss of LPC inhibition in the bloodstream allows a lower stimulation threshold, predisposing the animals to tissue infiltration by lymphocytes, lymphoproliferative disease, and an SLE-like syndrome.

The extent to which G2A, and perhaps LPC, regulate T cell activation and the progression of inflammation is dramatically revealed by the phenotype of mice lacking this receptor (*5*). G2A-deficient mice develop a late-onset multiorgan inflammation that is strikingly similar to SLE. But unlike other murine SLE models, young G2A-deficient mice appear healthy: lymphoid tissues seem normal, and T and B cell lineages display the usual patterns of differentiation. As they age, however, G2A-deficient animals develop progressive enlargement of lymph nodes due to polyclonal (nonmalignant) expansion of lymphocytes. After 1 year, mice develop a wasting disease similar to SLE that is characterized by lymphocytic infiltration of multiple organs, including lung and liver, coupled with immunoglobulin deposition in kidney glomeruli. In vitro analysis reveals that in the absence of G2A, T cells are hyperresponsive to activating stimuli even when they are isolated from young, apparently normal G2A-deficient animals.

When considered together, these data suggest a tantalizingly simple way to integrate two opposing requirements: The need to generate sensitive and rapid antigen-specific responses to pathogens, and the need to avoid unwanted activation of large-scale immune responses caused by transient exposure of lymphocytes to self antigens, molecular mimics, or weak antigenic stimuli (see the figure). In the presence of G2A, physiological concentrations of serum LPC (100 μM) would be expected to increase the threshold for antigen-driven activation of circulating T cells. Conversely, in G2A-deficient mice, the activation threshold would be lower, and thus antigen presentation to T cells would be more effective

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