of water skis, these show independence from Joe-related variables under all conditions, and drop out of consideration. Second, the Bayesian network approach allows the observer to start with a hypothesis about the structure of a network, and to perform targeted (as opposed to blundering) experiments to test particular network structures. Third, the particular approach used by Sachs *et al*. fails to identify cases in which downstream events feed back into upstream events (for example, when Joe's bank balance hits zero, he cannot take the bus). However, coupling measurements of variables at different time intervals with "dynamic Bayesian networks" may allow identification of feedback relationships. Finally, existing methods cannot identify causal connections between variables the Instrumentality does not know exist. In this example, the probability that Joe gets out of bed is influenced by whether he has filled a prescription for an antidepressant drug at the nearby drugstore the month before. Thus, Joe's antidepressant purchases seem to be relevant upstream "causal" input for the number of bus days. But if the Instrumentality has not yet learned about antidepressants and drugstores, it will not be able to discover the additional causal link.

When we return from the Instrumentality to our own world, we find that biologists are very good at making targeted perturbations. In genetically tractable organisms, performing these perturbations often depends on making the right mutant. In cell lines and in less tractable organisms, perturbations might be better effected by RNA interference, "protein genetic," or (for people) pharmacological approaches. We also see that for Bayesian network methods to realize their promise, researchers will need to get much better at measuring relevant variables. For intracellular events, variables include but are not limited to, numbers of regulatory molecules, modified molecules, and specific molecular complexes, and the percent occupation of regulatory sites upstream of genes. To be useful, measurement methods will need to operate on individual cells, or, at the very least, to allow large enough numbers of trials to yield causal assertions reliable enough to merit further experimental testing.

The Sachs *et al*. work is important because it suggests how researchers might develop a package of capabilities to enable systematic fishing expeditions. Such a package would generate testable ideas based on (relatively) error-prone high-throughput measurements made after (relatively) uninformed experimental treatments and could help experimenters refine those ideas after quick tests. Such capabilities seem well suited to one of the grand challenges of 21st- century biology: the grouping, ordering into pathways, and description of function for the numerous weakly acting and incompletely penetrant genes that quantitatively modify important phenotypes in humans and other organisms.

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PLANT SCIENCES

Recognition at a Distance

Paul Schulze-Lefert and Stéphane Bieri

A key step in the evolution of eukaryotic immune systems was the ability to discriminate between self and nonself. Evidence suggests that animals and plants independently evolved dedicated and highly variable receptor families for recognition of nonself structures. The outcome of interactions between plants and the pathogenic microbes that invade them largely relies on a repertoire of receptors that serve as a radar system for detecting pathogen-derived nonself molecules. The function and specificity of these receptors were originally defined by genetic studies. Such studies revealed that for plants to recognize their intruders and to mount an effective resistance response, there needed to be a match between a strain-specific pathogen effector and its corresponding plant host resistance (*R*) gene product (*1*). Detection of a pathogen effector by a plant R receptor frequently leads to rapid death of plant host cells at sites of attempted invasion as part of the immune response. Most known *R* genes encode intracellular receptors containing a nucleotide binding site and leucine-rich repeats (LRRs) or membrane-bound surface receptors containing extracellular LRRs (*2*). Two new studies—by Coaker *et al*. (*3*) on page 548 of this issue and by Rooney *et al*. (*4*) in this week's *Science Express*—describe encounters between pathogen-secreted effector molecules and their host targets in *Arabidopsis* and the tomato (*Lycopersicon*), respectively. Although this interorganismal molecular liaison has entirely different consequences for the effector target proteins, in both cases, their manipulation holds the key to a better understanding of how plant immune receptors recognize nonself.

Many *Arabidopsis* ecotypes contain the plasma membrane–associated intracellular R protein RPS2 (see the figure). This protein specifically detects and mounts an immune response to strains of the bacterial pathogen *Pseudomonas syringae*, which produce the AvrRpt2 effector protein. AvrRpt2 is delivered into the plant cytosol by a specialized bacterial secretion system and is cleaved near its amino terminus. The carboxyl-terminal cleavage product is sufficient to trigger the RPS2-dependent immune response and is predicted to adopt a secondary structure typical of a cysteine protease (*5*). Although attempts to detect direct interactions between RPS2 and AvrRpt2 have been unsuccessful, both proteins physically associate with the *Arabidopsis* protein RIN4. A complex between RPS2 and RIN4 is constitutively present in healthy (unchallenged) plants, but RIN4 disappears when AvrRpt2 is delivered into plant cells. Importantly, mutations in any of three amino acid residues in the carboxyl terminus of AvrRpt2 (predicted to be essential for catalytic activity of the putative *Pseudomonas* protease) disrupts the processing of AvrRpt2, the RPS2-dependent immune response, as well as elimination of RIN4 (*5*–*7*). This finding prompted the proposal that RPS2 might recognize the result of AvrRpt2's proteolytic activity, that is, the removal of RIN4.

Coaker *et al*. started from the puzzling observation that processing of AvrRpt2 could be detected in all eukaryotic but not prokaryotic extracts tested, including those from *P. syringae*. This observation implies the existence of a eukaryotic cofactor required for AvrRpt2 processing. Using a combined biochemical and genetic approach, the authors identified this cofactor as a single-domain cyclophilin, a folding catalyst that facilitates cis/trans isomerization of prolyl bonds. Cyclophilin activity is required for proper AvrRpt2 self-cleavage, and this in turn may be a critical step for the correct subcellular localization of

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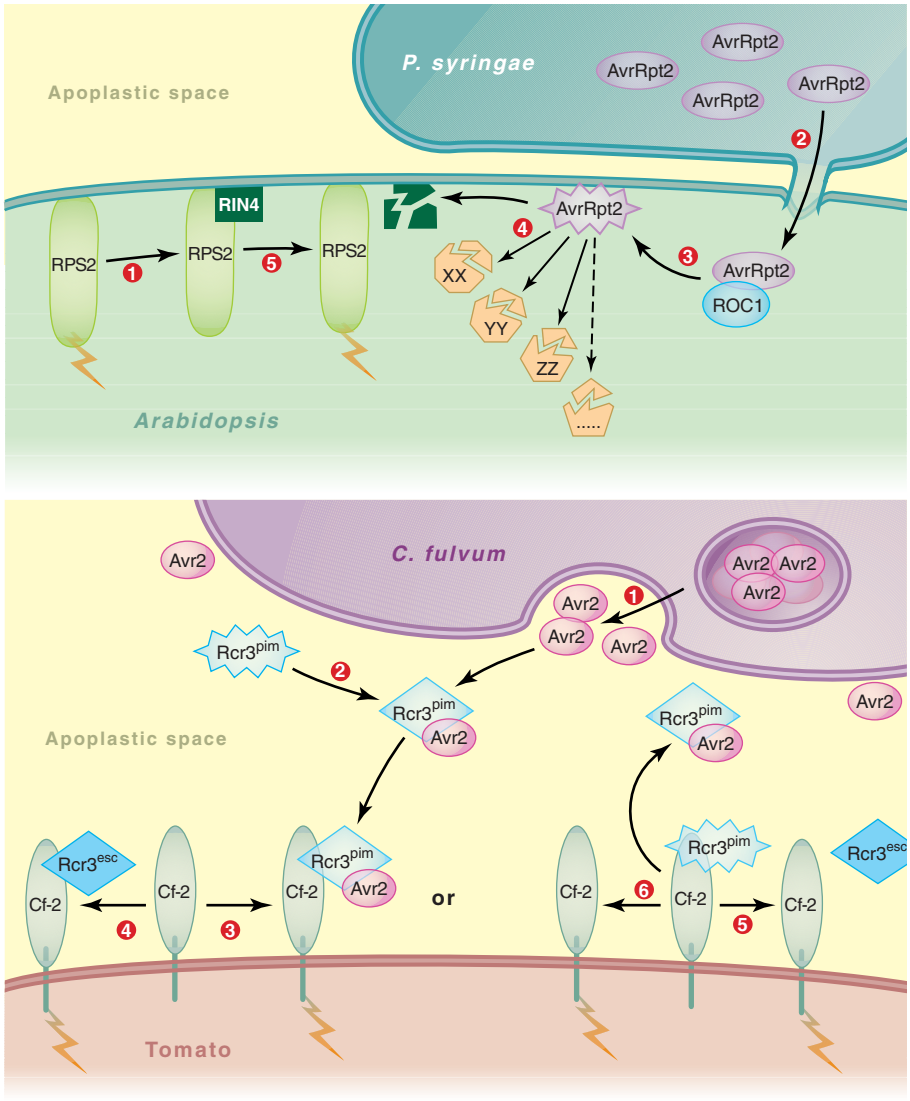
AvrRpt2 in plant host cells. Identifying the cyclophilin-dependent self-cleavage activity of AvrRpt2 provided the first indication that RIN4 might be a direct substrate of the bacterial protease. A similar stretch of amino acids surrounding AvrRpt2's self-processing site is also present in two regions within RIN4. Indeed, recombinant full-length AvrRpt2 and the single-domain cyclophilin were necessary and sufficient to specifically cleave RIN4 at these two sites in vitro.

These findings are biologically relevant because substitutions of RIN4 residues at its carboxyl-terminal cleavage site abolish both RIN4 elimination and RPS2 activation in plants (*8*). The authors of this study also determined whether RIN4 cleavage results in a loss of physical association with RPS2, or merely derepresses RPS2 activity while maintaining physical contacts with RIN4 cleavage products. They used in vivo coexpression of DNA constructs encoding RIN4 cleavage products and RPS2. Activity of RPS2 in the presence of RIN4 cleavage products indicated that in vivo release of RPS2 from its RIN4 partner is essential for triggering the immune response. This probably explains previous findings showing autoactivation of RPS2 in *rin4* mutant plants in the absence of the pathogen. Collectively, these data strongly favor a model in which RIN4 negatively regulates RPS2 activity. This mode of regulation permits indirect activation of the AvrRpt2-dependent R receptor through proteolytic elimination of RIN4.

This indirect intracellular perception of a *Pseudomonas* effector protein may be analogous to extracellular recognition of the Avr2 effector protein of the fungus *Cladosporium fulvum* by the *Cf-2 R* gene product of tomato (see the figure), the subject of a complementary paper by Rooney *et al*. (*4*). Cf-2 is a transmembrane receptor–like protein with extracellular LRRs. Previous genetic analysis of Cf-2-mediated resistance revealed that a papain-like protease of the tomato plant, Rcr3, in the extracellular leaf space is required for Cf-2 activity (*9*). Rooney *et al*. showed that this protease is not a signaling component of the Cf-2–triggered immune response but rather is crucial for Cf-2–dependent recognition of the Avr2 fungal effector protein. To monitor Rcr3 protease activity during coimmunoprecipitation experiments, the authors used a biotinylated "suicide" substrate that irreversibly and covalently binds and inhibits the active site of the protease. They demonstrated that Avr2 specifically associates with and inhibits Rcr3 protease activity in the tomato plant in vivo and also after heterologous expression in yeast. Heterologously synthesized Rcr3 or Avr2 or the suicide substrate–locked form of Rcr3 all failed to trigger a Cf-2–dependent immune response when injected into *rcr3* mutant tomato plants containing wild-type *Cf-2*. In contrast, Cf-2 was specifically activated when Rcr3-Avr2 complexes were injected into tomato leaves containing mutant *rcr3* and wild-type *Cf-2*. This finding and the existence of an autoactive *Rcr3* allele (*9*), which activates Cf-2 in the absence of Avr2, suggests that a conformational change in Rcr3 imposed by binding of Avr2 or mimicked by the autoactive Rcr3 allele is the trigger for Cf-2 activation. Whether Cf-2 activity is negatively regulated by binding to Rcr3 in healthy plants—analogous to the negative regulation of RPS2 by RIN4 in



A subtle approach to resisting invasion. Indirect recognition of pathogen effector proteins by plant immune receptors in *Arabidopsis* (**top**) and the tomato (**bottom**). (Top) (1) RIN4 binds to and suppresses the activity of the R receptor RPS2 in healthy plants. (2) The bacterial effector protein AvrRpt2 is secreted by *P. syringae* into host plant cells by a specialized secretion system. (3) *Arabidopsis* cyclophilins (for example, ROC1) interact with AvrRpt2 and activate this bacterial effector protein. The AvrRpt2 protease becomes localized to the plasma membrane after autoproteolytic cleavage of its secretion signal peptide. (4) Activated AvrRpt2 then cleaves RIN4 and at least three other *Arabidopsis* proteins (*16*). (5) RIN4 cleavage products can no longer suppress RPS2 activity. (Bottom) (1) The fungal effector protein Avr2 of *C. fulvum* is secreted into the apoplastic space surrounding host tomato plant cells. Avr2 associates with the secreted plant protein $Rcr3^{pim}$ (an allele originating from wild tomato *Lycopersicon pimpinellifolium*), and inhibits its protease activity. (2) There is a binding-dependent conformational change in Rcr3. (3) Altered Rcr3 then binds to the extracellular R receptor Cf-2, which is activated by indirect recognition of Avr2. (4) An autoactive $Rcr3^{esc}$ variant (originating from *L. esculentum*) may be a conformational mimic of the $Rcr3^{pim}$ state enforced by Avr2 binding. (5) Alternatively, $Rcr3^{pim}$ (but not $Rcr3^{esc}$) may inhibit Cf-2–triggered immune responses. (6) In this case, conformational changes in $Rcr3^{pim}$ enforced by Avr2 binding sequester $Rcr3^{pim}$ away from Cf-2, thereby derepressing the R receptor.

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Arabidopsis—is not known because validated *rcr3*-null mutant plants are not available (*9*). Thus, we can infer two possible modes of Cf-2 activation (see the figure). Secretion of Avr2 during pathogenesis may sequester Rcr3 away from constitutive Cf-2–Rcr3 complexes, thereby derepressing Cf-2 activity. Alternatively, formation of Avr2–Rcr3 complexes may trigger a conformational change in Rcr3, enabling it to bind to and activate Cf-2. In either case, Cf-2–dependent recognition of Avr2 is likely to be indirect, taking place without physical interaction between the fungal effector protein and the plant host R protein.

Work on other plant resistance responses mediated by pairs of host resistance and pathogen effector proteins supports an indirect mode of nonself recognition (*10, 11*). Of particular note is the recognition of the *P. syringae* effector AvrRpm1 by the intracellular and plasma membrane–associated RPM1 receptor of *Arabidopsis*. Both proteins were found to physically associate with *Arabidopsis* RIN4 rather than interacting directly with each other. Thus, RIN4 appears to be a host target for multiple *Pseudomonas* effector proteins (*11*). However, RIN4 does not disappear upon delivery of AvrRpm1 into plant cells. The exact biochemical alteration in RIN4 mediated by AvrRpm1 is poorly understood, but a change in RIN4 phosphorylation seems likely to be involved in RPM1 activation (*11*). An indirect mode of recognition appears to be the common theme in these cases, and clearly plant immune receptors are capable of recognizing biochemically diverse alterations of effector targets, including phosphorylation status, proteolytic cleavage, and conformational changes.

Indirect recognition of nonself in plants is an elegant alternative solution to direct nonself recognition by the adaptive immune systems of vertebrates. Vertebrates evolved dedicated somatic recombination systems for the generation of receptor diversity and specialized immune cells to recognize any potentially harmful nonself molecular structures (*12*). Indirect pathogen detection in plants appears to be as effective as direct nonself recognition in vertebrates. However, fewer receptors are needed—for example, there are only ~120 nucleotide binding LRR-type receptors in the *Arabidopsis* genome (*13*)—and specialized immune cells are not required. R protein–mediated surveillance of only those host protein assemblies that are critical for successful invasion by parasites may have been an important step in helping plants, with their limited set of receptors, to survive. Indeed, it is conceivable that *Arabidopsis* RIN4 and tomato Rcr3 are virulence targets. However, the roles of these two host proteins in cellular reprogramming during pathogenesis remain mysterious. In addition, although conformational changes in R proteins are likely to be critical for their activation (*14*), we do not have detailed insights into this process owing to a lack of R protein crystal structures. Such structures might help to identify the immediate targets of activated R proteins, which are as yet unknown. Finally, it will be important for future studies to compare the current findings with the recognition mechanics of a second nonself perception system in plants, the so-called PAMP (pathogen-associated molecular pattern) receptors. These receptors detect conserved pathogen-derived molecular structures present in multiple microbial species, such as a peptide derived from the bacterial motor protein flagellin (*15*) .

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APPLIED PHYSICS

Toward a Universal Memory

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When it comes to computers, mp3 players, digital cameras, and other electronic gadgets, there is no such thing as too much memory. Whether it is more Flash memory for taking high-resolution digital pictures, a bigger hard drive for digital video files, or more random access memory (RAM) to view them on the computer, the appetite for ever more memory at ever-increasing densities appears insatiable. An emerging technology, magnetoresistive RAM, promises additional functionality and improved memory performance that will enable yet more applications and open up system designs that are not possible today.

Today's dominant solid-state memory technologies—static RAM, dynamic RAM, and Flash—have been around for a long time, with Flash the youngest at 21 years (*1*). Their longevity can be explained in part by mutually beneficial differentiation. Each technology does a single thing very well, but many systems need all three memory types to deliver overall good performance at reasonable cost. However, the gain from differentiation comes at the cost of increased system and fabrication complexity, particularly in so-called embedded applications, where an entire electronic system is implemented on a single chip with static RAM, dynamic RAM, and Flash often used side-by-side.

All three technologies have advantages and disadvantages. Static RAM has excellent read and write speeds, integrates readily into the process technology of embedded applications, and requires little power for data retention. However, its large cell size (a typical memory bit requires six transistors) makes it impractical for embedded applications that require a lot of memory. Embedded static RAM is used for cache memory in microprocessors, where high speed is more important than large amounts of memory.

Dynamic RAM uses a single transistor and a storage capacitor per cell and thus provides a denser architecture than static RAM, at the expense of increased embedded-process complexity. Because the stored charge tends to leak out of the capacitor, dynamic RAM requires constant power to refresh its bit state every few milliseconds. Because of its high power consumption, large amounts of dynamic RAM are impractical for portable electronics with limited battery life.

In contrast to static and dynamic RAM, Flash memory offers nonvolatile data storage; that is, its information is not lost when the power is turned off. Nonvolatility is highly desirable in portable electronics, because nonvolatile data retention does not consume any battery power. Flash also has high density and moderately fast read access time, but its write mode is too slow and its write endurance far too limited for many applications. In addition, embedded Flash needs its own high-voltage drivers, complicating the design and manufacturing process.

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