tion by histidine kinases and other transmembrane receptors. Previously, we created a chimeric protein called Taz1 (Figure 1). Taz1 has the external receptor domain, the transmembrane domain, and the HAMP domain from Tar, a protein that is activated by aspartate, and the cytoplasmic histidine kinase domain of EnvZ, a protein that is activated by high osmolarity. As a consequence, adding aspartate to the medium of bacterial cells expressing Taz1 leads to activation of the chimeric protein and induces the expression of the *ompC* gene, a downstream target of EnvZ (Figure 1). Later, our group found that the HAMP domains are interchangeable between signal transducers (Zhu and Inouye, 2003). In Taz1, the α2 helix of the Tar HAMP domain is directly connected to the long N-terminal helix of an EnvZ helical hairpin, which forms a dimer (Figure 1; Tomomori et al., 1999). This structure is likely to act as a single helix (II*). On this helix, there is the active-site histidine residue, which is the autophosphorylation site that plays an important role in phosphorylation and dephosphorylation of OmpR, the substrate of the EnvZ kinase. These two opposing enzymatic activities must be regulated by the signal passing through the HAMP domain. Therefore, on the basis of the model proposed by Hulko et al. (2006), the relative configuration between the helix with the active-site histidine and the ATP binding domain (Tanaka et al., 1998) may be altered by rotating helix II* by 26°. It is certainly feasible to test this experimentally. It may also be important to note that the HAMP domain in the Tar-EnvZ chimeric dimer can be heterologous (with one HAMP subunit from Tar and the other from EnvZ) and that this heterologous HAMP domain retains the ability to transduce the signal to regulate the histidine kinase domain (Zhu and Inouye, 2004). This suggests that the formation of the stable HAMP dimer may not be essential for signal transduction. In any case, the work by Hulko et al. (2006) is a major breakthrough for our understanding of the molecular mechanism of transmembrane receptor-mediated signal transduction.

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Plant Pathogens Trick Guard Cells into Opening the Gates

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The stomata of plants regulate gas exchange and water transpiration in response to changing environmental conditions. New work reveals that stomata also have an important role in host defense. In this issue of *Cell*, Melotto et al. (2006) show that stomata close upon detection of potential microbial pathogens to prevent the infection of the leaf interior. Moreover, pathogenic bacteria have evolved strategies to suppress the closure of stomata.

All microbial pathogens must access host nutrients for their own reproduction and thus frequently colonize particular host organs or cell types. For example, in plants, microbes can specifically colonize leaves, roots, fruits, or particular cell types such as root epidermal or phloem cells of the vasculature. Although multiplica-

tion of microbial parasites is usually specific to particular organs and/or cell types, microbial entry often takes place at a distant site. Until recently, plant immunologists had not paid much attention to the complexity of microbial entry or to the specific sites of pathogen propagation. In a new study reported in this issue of *Cell*, Melotto et al. (2006) show that stomata (the openings on the surface of leaves that are used for gas exchange) close in response to microbes. In addition, they show that plant pathogens have developed the capacity to stimulate the reopening of stomata to facilitate their invasion of the leaf interior.

A typical habitat of phytopathogenic bacteria is the leaf surface. *Pseudomonas syringae* is a common pathogen of flowering plants that causes spots of leaf necrosis or stem cankers and serves as a useful model for studying the interactions of microbial pathogens with plants. This bacterium enters leaves through wound sites, hydathodes (openings at the leaf margin), and stomata. The bacterium then multiplies in the leaf interior by forming microcolonies in close physical association with the cell wall of mesophyll cells. Extracellular propagation is typical for most phytopathogenic bacteria and differs from typical bacterial infections in mammals, where bacteria enter and multiply inside host cells (see review by Pizarro-Cerda and Cossart, 2006). The multiplication of *P. syringae* in the leaf interior explains why, until recently, artificial laboratory inoculation techniques involved mechanical injection of bacterial solutions into the mesophyll. The new work of Melotto et al. was prompted by a puzzling observation that *P. syringae* mutants, defective in producing the small compound coronatine, failed to cause disease when inoculated on the leaf surface but remained virulent when injected into leaves using standard procedures (Mittal and Davis, 1995). This observation suggested that coronatine might enable *P. syringae* to switch from an epiphytic mode of growth on the leaf surface to colonize the leaf interior. Coronatine is produced by several pathogenic variants (pathovars) of *P. syringae* and is a structural octadecanoid mimic of the phytohormone jasmonic acid and its precursor 12-oxophytodienoic acid, which promote wound responses, fruit abscission, and senescence. Because coronatine mimics many but not all of the effects of octadecanoids, it seems likely that these molecules have common cellular targets in plants.

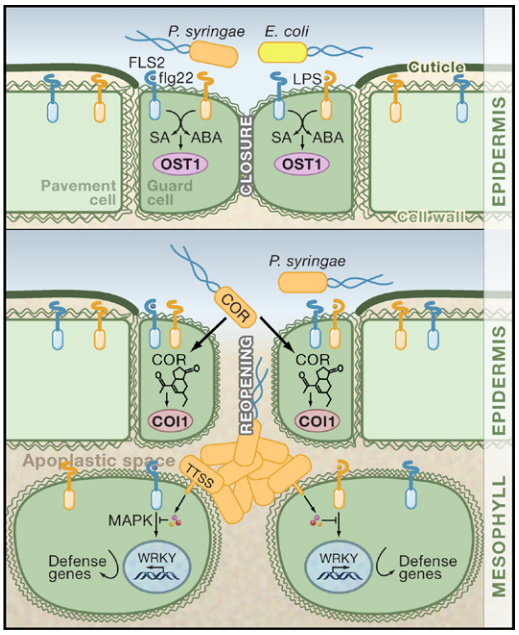

Figure 1. Stomata in Plant Defense

A cross-section of a leaf showing epidermal and mesophyll cell layers. (Top) The plant pathogen *P. syringae* and human pathogen *E. coli* grow epiphytically on leaf surfaces and are attracted toward open stomata of the epidermis. Stomata close upon detection of lipopolysaccharide (LPS) through the action of an unknown immune receptor and detection of flagellin (flg22) through the action of the flagellin receptor FLS2. The pathway for stomatal closure involves triggering of the salicylic acid (SA) and abscisic acid (ABA) signaling pathways. (Bottom) Coronatine (COR), a compound made by *P. syringae*, promotes stomatal reopening through the E3 ligase subunit COI1, a key component of jasmonic acid signaling. Opening of stomata allows entry of bacteria. Motile bacteria invade the apoplastic space of mesophyll cells, attach to cell walls, and inject effector molecules into the host cell cytoplasm via their type III secretion system (TTSS). These effectors suppress the expression of defense genes mediated by immune receptors, MAPK signaling cascades, and WRKY transcription factors. Finally, nonmotile bacteria form microcolonies, multiply, and cause disease.

A key observation by Melotto and colleagues is the ability of *P. syringae* to move toward open stomata when inoculated at the leaf surface. Stomata are formed by a pair of specialized cells known as guard cells, whose adjustable aperture regulates gas exchange and controls water transpiration. Although it remains to be determined how *P. syringae* can discriminate between open and closed stomata, this may be driven by a chemotactic process that could involve sensing of nutrients derived from the leaf interior and subsequent directed bacterial movement that is propelled by the flagellin-based motility apparatus. Directed motility by chemotaxis is required for virulence and competitive fitness of *Ralstonia solanacearum*, a soil-borne bacterial pathogen that invades host plants via their roots (Yao and Allen, 2006). *R. solanacearum* is attracted by diverse amino acids and organic acids present in exudates from roots of their host plants; mutants lacking core proteins that regulate chemotaxis exhibit reduced virulence despite retaining normal motility.

A second major observation by Melotto et al. is that, although inoculation of virulent *P. syringae* at the leaf surface stimulates the rapid closure of stomata, the stomata reopen within 3 hr (Figure 1). Importantly, mutant *P. syringae* lacking coronatine fail to colonize the leaf interior, and this correlates with a failure to reopen stomatal apertures. It may be that the initial rapid stomatal closure protects plant leaves from bacterial entry, which is counteracted by coronatine. The new study exploits the power of *Arabidopsis* genetics to test the plant defense and bacterial coun-

terdefense hypothesis. The authors capitalize on a previous observation that *Arabidopsis* mutants lacking the FLS2 immune receptor are more susceptible to *P. syringae* colonization, but only when inoculated at the leaf surface (Zipfel et al., 2004). FLS2 recognizes the flg22 peptide derived from the most conserved domain of bacterial flagellin. Melotto et al. show that flg22 is sufficient to prompt the closure of stomata in wild-type *Arabidopsis* leaves. This provides direct evidence that stomatal closure triggered by bacteria is subject to control by a plant immune receptor. Remarkably, inoculation of *Arabidopsis* leaves with the bacterium *Escherichia coli* (which occasionally causes infection of the human gastrointestinal and urinary tracts) mediated a sustained stomatal closure that coincided with the presence of flagellin but the absence of coronatine. Stomatal closure appears to be a common defense mechanism that integrates perception of multiple pathogen-associated molecular patterns (PAMPs) because application of lipopolysaccharide (LPS), the major structural component of the outer cell wall of Gram-negative bacteria, triggered closure similar to that triggered by flg22.

P. syringae injects a battery of effector proteins into host cells via its type III secretion system, and several of these effectors can suppress PAMP-mediated immune responses (Figure 1). Intracellular plant immune receptors constitute a second line of defense by recognizing cognate effectors that are specific to particular isolates of *P. syringae*. Upon effector recognition, these intracellular receptors trigger an immune response, which often leads to host cell suicide at sites of attempted colonization (reviewed in Chisholm et al., 2006). This second line of defense appears to play a minor role in restricting the entry of bacteria through stomata, suggesting that different classes of immune receptors restrict bacterial entry and colonization of the leaf interior.

The closure of stomata in response to a limiting supply of water is controlled by the hormone abscisic acid and the guard-cell-specific kinase OST1 (reviewed in Assmann, 2003). Stomata in *ost1* mutants of *Arabidopsis* fail to close upon treatment with flg22, and these mutants also support the multiplication of *P. syringae* regardless of whether it does or does not produce coronatine. Thus, the signaling pathway of stomatal closure regulated by abscisic acid and the immune pathway dependent on FLS2 must be interconnected. Although stomatal closure and resistance to *P. syringae* entry was found to require accumulation of the known plant defense-signaling molecule salicylic acid, it remains to be determined where exactly the abscisic acid- and salicylic acid-dependent pathways converge. The connection between seemingly unrelated signaling pathways is a subtext of the present study, and this is also relevant to the observation that stomatal reopening is dependent on coronatine. *Arabidopsis* plants lacking the E3 ubiquitin ligase subunit COI1 are insensitive to both coronatine and jasmonate treatment and are also defective in stomatal reopening upon inoculation with wild-type *P. syringae* that produces coronatine. When *P. syringae* lacking coronatine was injected into the leaf interior, the bacteria multiplied efficiently in both *coi1* mutant and wild-type plants, suggesting that jasmonate signaling is engaged by the pathogen to antagonize PAMP-triggered immune responses in stomatal cells. Although an antagonistic interplay between jasmonic acid and salicylic acid signaling pathways is well documented (reviewed in Dong, 2004), the molecular nature of this antagonism remains enigmatic. A further complication comes from the observation that jasmonic acid applied exogenously promotes stomatal closure instead of opening (Suhita et al., 2004). Thus, if the coronatine-dependent stomatal reopening by *P. syringae* occurs through antagonistic induction of the jasmonic acid signaling pathway, then as yet unknown differences in biochemical activity between the structural analogs coronatine and plant-derived octadecanoids might account for this discrepancy.

Stomata-based immunity might also have relevance for other classes of pathogens, such as parasitic fungi. Epiphytic hyphae of the basidiomycete rust fungus move directionally toward stomata to enter the leaf interior. Also, chitosan, a β-1,4-linked glucosamine derived from fungal cell walls, induces stomatal closure (Lee et al., 1999). Unlike bacteria, however, many parasitic fungi including rusts must penetrate the plant cell wall to accommodate specialized feeding structures in plant cells for nutrient uptake. Recent evidence suggests that fungal entry into plant cells is restricted by two secretory pathways that likely deliver defensive compounds into the extracellular space upon PAMP recognition (reviewed by Ellis, 2006). Ascomycete powdery mildew fungi appear to engage a plasma membrane protein of the host to manipulate these secretory pathways for entry into plant cells.

The Melotto et al. (2006) study provides new opportunities to tackle poorly understood relationships between the outbreak of epidemic disease and adverse environmental conditions. For example, can PAMP-triggered stomatal closure override abscisic acid-dependent stomatal opening under conditions of high humidity? Conversely, are plants more resistant to bacteria when stomata are forced to close because of drought? In addition, the findings might provide insights into the phenomenon of pathogen-specific "colonization niches" in eukaryotic organisms. It remains to be seen whether the interplay between chemotactic motility, multiple cell-type-specific immune responses, and the capacity of a parasite to subvert the gates of the innate immune response combine to determine organ- or cell-type-specific patterns of colonization.

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Mind the GAP: Wnt Steps onto the mTORC1 Train

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The TSC1/2 tumor-suppressor complex controls protein synthesis through the regulation of mTOR. In this issue of *Cell*, Inoki et al. (2006) report that the kinases GSK3 and AMPK cooperate in the activation of TSC2 to inhibit mTOR activity. Surprisingly, the phosphorylation of TSC2 by GSK3 is markedly suppressed by Wnt signaling. This suggests that components of the mTOR pathway may be therapeutic targets for diseases linked to hyperactive Wnt signaling.

In order to maintain homeostasis, cells interpret and coordinate responses to diverse environmental cues such as growth factors, energy status, and the availability of glucose and other nutrients. Mutations in the pathways that coordinate these responses can contribute to metabolic or inflammatory disorders and often promote tumorigenesis, as in tuberous sclerosis complex (TSC). TSC is an autosomal-dominant disorder that is characterized by the development of benign tumors, called hamartomas, in many vital organs including the brain, kidneys, heart, and lungs (reviewed in Kwiatkowski, 2003). It has a prevalence of roughly 1 in every 10,000 births and results from mutations in either *TSC1* or *TSC2* (which encode proteins also called Hamartin and Tuberin, respectively). Recent studies in both flies and mammals have placed the TSC1/2 proteins in the middle of an evolutionarily conserved signaling pathway that controls mTOR, a serine/threonine kinase that stimulates ribosome biogenesis and protein synthesis (reviewed in Shaw and Cantley, 2006). mTOR integrates distinct signals reflecting nutrient availability, presence of growth factors, and bioenergetic status into the regulation of cell growth and proliferation. Work by Guan and colleagues (Inoki et al., 2006) now reveals a pathway by which bioenergetic status and the Wnt pathway are integrated to control the activity of mTOR. They show that the sequential phosphorylation of TSC2 by AMP-activated protein kinase (AMPK), which is activated by low cellular energy, and glycogen synthase kinase 3 (GSK3), which is inhibited by Wnt signaling, stimulates the activity of TSC2, leading to the inhibition of mTOR.

TSC2 is an ~180 kDa protein that is phosphorylated on multiple sites (Figure 1) by various kinases. Depending on the site that is phosphorylated, the GAP (GTPase-activating protein) activity of TSC2 toward the small GTPase Rheb (a Ras homolog enriched in brain) is inhibited or activated. Rheb is a positive regulator of the mTORC1 complex (which consists of mTOR, Raptor, and mLST8) and is sensitive to rapamycin (Shaw and Cantley, 2006). Although the precise mechanisms remain unclear, mitogen signaling inhibits the ability of TSC2 to negatively regulate Rheb, resulting in augmented mTORC1 signaling. For