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Defense Responses in Plants and Animals—More of the Same

Pathogens are a ubiquitous fact of life, and the ability to resist their attentions constitutes one of the keys to biological success. However, as each defensive innovation is established in the host, new ways to circumvent it evolve in the pathogen. Over time, these coevolutionary struggles between would-be pathogens and their erstwhile hosts have generated some of the most complex and interesting interactions known to biology.

Because of the marked differences between their cellular structures and modes of life, it is not unreasonable to expect that very different strategies for attack, defense, and counterattack would have evolved in plants and animals and their respective pathogens. But have they? In fact, an emerging body of evidence indicates that several individual components of host–pathogen interactions are shared, either conceptually or mechanistically, in the two major branches of the eukaryotic lineage.

For example, in gene-for-gene nonself-surveillance systems in plants, disease resistance (*R*) genes mediate the recognition of specific pathogen-derived components (products of the *Avirulence* [*Avr*] genes) in much the same way that the animal adaptive immune system is capable of recognizing foreign molecules.

In addition, *R* gene–mediated and systemic pathogen resistance responses in Arabidopsis are triggered via a signal transduction pathway that includes NIM1/NPR1 (noninducible immunity/nonexpressor of pathogenesis related [PR] genes). This plant protein is related to IκB, which directs disease resistance responses in a range of animal species (Cao et al., 1997; Ryals et al., 1997). Moreover, the deduced amino acid sequence of *N*, an *R* gene from tobacco, includes a domain that is related to Toll, which is a critical regulator of disease resistance responses in Drosophila (for reviews, see Baker et al., 1997; Meister et al., 1997).

It is not only pathogen recognition and signal transduction pathways that appear to be conserved between plants and animals. Some of the defensive responses that are triggered when these pathways are activated are also similar in both lineages. For example, one of the earliest changes that can be detected following pathogen attack of plants and animals is a rapid increase in reactive oxygen species (see, e.g., Bauerle and Baltimore, 1996; Alvarez et al., 1998).

Mechanistic conservation can also be detected in the strategies adopted by plant and animal pathogens to invade their respective hosts. One well-known example includes the type III secretion systems encoded by the *hrp* (hypersensitive response and pathogenicity) genes of *Pseudomonas syringae* and other plant pathogenic bacteria and by the related *ysc* (Yop secretion) genes of animal pathogens in the genus *Yersinia* (Alfano and Collmer, 1996; Kubori et al., 1998).

A variety of distinct virulence determinants are delivered into host cells via these macromolecular complexes, suggesting that each determinant may trigger host defense responses in a different way (but see Hardt and Galán, 1997; Mills et al., 1997, for some possible exceptions). Nevertheless, the extensive sequence similarities among the Ysc and Hrp proteins (Bogdanova et al., 1996) and their common ability to produce secretion-associated appendages (Roine et al., 1997) suggests that the type III systems of plant and animal pathogens operate in fundamentally the same way (Alfano and Collmer, 1996).

Some of these parallels are, in fact, rather predictable. Is it really remarkable that plant and animal pathogens use similar secretion pathways to secrete virulence proteins? The immediate objective is the same, even though the overall interaction between pathogen and host is very different. Similarly, it is perhaps not all that surprising that homologous signal transduction components have been recruited to regulate distinct aspects of hosts' defense response pathways, particularly when the molecules that comprise those responses are similar in plants and animals.

But are there entire defensive systems that operate in both plants and animals? One possibility is the host iron-withdrawal mechanisms (and counteracting siderophore secretion by the pathogen) that have been uncovered in some plants (Expert et al., 1996) and animals (e.g., Wooldridge and Williams, 1993).

A second, and perhaps better documented, example involves the production of antimicrobial peptides (for reviews, see García-Olmedo et al., 1995; Broekaert et al., 1997; Meister et al., 1997; Galán, 1998; Medzhitov and Janeway, 1998). In this form of "innate immunity," peptides produced by the host directly affect the ability of the pathogen to survive and spread.

Genetic experiments have shown that pathogen sensitivity to host-produced antimicrobials is mediated by two general categories of bacterial proteins: those that affect the overall permeability of the pathogen's extracellular matrix, and those involved in peptide import. Mutations in the genes encoding the former class render the pathogen more susceptible to a range of general host defense mechanisms (see, e.g., Titarenko et al., 1997), whereas

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those in the latter class specifically affect the pathogen's ability to withstand antimicrobial peptides.

Some of the most informative investigations of antimicrobial peptides and the associated pathogen efforts to render them ineffective have come from studies of interactions between the facultative intracellular pathogen *Salmonella typhimurium* and a number of its animal hosts. For example, experiments with *S. typhimurium* mutants that show decreased virulence in mice led to the identification of an operon that mediates pathogen resistance to host-produced antimicrobial peptides (Groisman et al., 1992).

Subsequent cloning and sequencing of the five *sap* (sensitivity to antimicrobial peptides) genes that comprise this operon demonstrated that they encode a multimolecular structure related to the ATP binding cassette (ABC) transporters that have been identified in a large number of organisms (Parra-Lopez et al., 1993). This work also confirmed the critical role of the *sap* operon as a potent *S. typhimurium* virulence determinant.

ABC transporters are a large class of proteins responsible for transporting organic molecules across membranes in both prokaryotes and eukaryotes (Higgins, 1995). Unlike other membrane transport systems, ABC transporters use energy directly from ATP hydrolysis (as opposed to a pH gradient established by H^+ pumps), and they generally possess distinct membrane-spanning and extracellular ligand binding components.

Although the organization of the ABC transporter encoded by the *sap* genes remains to be determined precisely, it is thought that several components of the complex reside in the bacterial inner membrane where they function to transport antimicrobial peptides into the cytoplasm for degradation. One component (possibly that encoded by *sapA*) may bind the antimicrobial peptide and bring it to the membrane-spanning component of the transporter. This modular organization facilitates versatility in terms of peptide binding specificity (e.g., there could be several different ligand binding components, each capable of recognizing a distinct peptide), while allowing the core components to remain unchanged.

Several hints that a similar antimicrobial peptide–based system may be operating to direct host-range specificity in interactions between plants and their bacterial and fungal pathogens have emerged over the past few years (Broekaert, et al., 1997). For example, the spatial and temporal expression patterns of such peptides correlate with increased pathogen resistance in plants, and the overexpression of some peptides can enhance plant tolerance to pathogens (e.g., Molina and García-Olmedo, 1997). Nevertheless, demonstrating directly that these presumed antimicrobial peptides really do function to defend the plants in which they are found endogenously has been difficult, primarily because the corresponding plant mutants (i.e., those that do not produce the antimicrobial peptides) are not available.

Now, in a definitive piece of work reported **on pages 917–924 of this issue, López-Solanilla et al.** have obviated this problem by shifting their focus from the plant host to the bacterial pathogen. In this way, they provide comprehensive genetic and molecular evidence that bacterial pathogens of plants possess systems that are closely related to those used by animal pathogens to detect and respond to their hosts' antimicrobial peptide–based defenses. These findings imply strongly that peptide-based antimicrobials are an important factor in plant defense responses and that the ability of pathogens to evade plant-produced antimicrobials may be a major determinant of their host-range specificity.

The authors chose to base their studies on *Erwinia chrysanthemi*, a soilborne facultative plant pathogen that causes serious soft rots in a number of major crops. *E. chrysanthemi* has a broad host range and perhaps because of this versatility, it has proven difficult to control in the field. Nevertheless, antimicrobial peptides that appear to contribute to disease resistance have been identified in some *E. chrysanthemi* hosts, including potato.

For these reasons, and because *E. chrysanthemi* is closely related to *S. typhimurium*, López-Solanilla et al. followed a hunch that similar genes may be involved in directing the ability of both pathogens to evade the defense mechanisms of their respective plant and animal hosts. As they had anticipated, they were able to identify an *E. chrysanthemi* operon that contains close relatives of the *S. typhimurium sap* genes. Furthermore, these genes are organized similarly in *E. chrysanthemi* and in *S. typhimurium* and their deduced amino acid sequences are over 70% identical.

To demonstrate that the *E. chrysanthemi sap* genes are required for virulence, the authors have generated a strain in which the *sap* operon has been interrupted and inactivated by an insertional mutagen. In a series of experiments with this strain, which they call BT105, López-Solanilla et al. show that the *sap* operon is indeed an important virulence determinant for *E. chrysanthemi* and that one of its functions is to protect the bacterium from host-produced antimicrobial peptides.

For example, the authors' data indicate that the BT105 strain exhibits increased sensitivity in vitro to physiologically relevant concentrations of the potato antimicrobial peptide snakin-1. Further evidence that the *E. chrysanthemi sap* genes confer tolerance to specific antimicrobial peptides comes from experiments showing that protamine, an unrelated peptide from salmon, has no differential effect on the in vitro growth rate of the BT105 mutant. Moreover, because the BT105 mutants do not exhibit nonspecific changes in outer membrane permeability, in vitro growth rate, or colony size, the authors

conclude that these phenotypes are directly related to the increased sensitivity of the BT105 mutants to potato antimicrobial peptides.

López-Solanilla et al. also show that necrotic lesions forming on potatoes inoculated with the BT105 mutant are significantly smaller than are those that form on potatoes infected with wild-type *E. chrysanthemi.* This finding correlates with the 100-fold reduction in the BT105 population (relative to that of wild type) that the authors detect in inoculated potato tubers and indicates that the *sap* operon is required for full virulence in vivo.

This work offers definitive evidence that plants and animals share an innate defense mechanism, one that may be at least as important for determining host range as the well-known gene-for-gene resistance systems in plants. However, it also raises several intriguing questions about the evolutionary origins of host-produced antimicrobial peptides and the counteracting Sap system of their bacterial pathogens, as well as about the molecular mechanisms that mediate Sap specificity in different bacterial species.

Because both plants and animals appear to possess the ability to produce functional antimicrobial peptides, López-Solanilla et al. suggest that these defense responses may have been around prior to the evolutionary separation of eukaryotes into their two main lineages. This would imply that the corresponding bacterial evasion mechanisms, such as the Sap system, also made early appearances on the evolutionary stage. However, it is also possible that the *sap* operon has been distributed via horizontal gene transfer from one bacterial species to another. Indeed, the acquisition of "pathogenicity islands" encoding discrete virulence functions appears to be an important factor in the evolution of bacterial pathogenicity (Groisman and Ochman, 1996).

One way to address questions concerning *sap* evolution is to extend the analysis of the distribution of the *sap* operon initiated by the authors. Can related genes be found in other bacterial pathogens of plants or animals and do they function similarly in these species, or are antimicrobial peptide defense mechanisms restricted to a subset of pathogens? Both *S. typhimurium* and *E. chrysanthemi* exhibit a very broad host range and are capable of surviving and propagating in a number of different environments and host cell types. Perhaps these rather unusual pathogenic features correlate with the presence of a functional *sap* operon, a feature that may not be required for more fastidious pathogens to evade their hosts' defenses.

More detailed analyses of the *sap* sequences themselves will also help to determine how peptide recognition specificity is determined. Is this specificity a function of a single component of the Sap system (e.g., SapA) that may contain hypervariable domains analogous to those capable of mediating S RNase specificity (Matton et al., 1997), or do several different Sap proteins contribute to the specificity of the system in each species? Refined mutational analyses can be used to address these questions in the *E. chrysanthemi*–potato interaction, whereas cloning, sequencing, and functional investigations of *sap* operons from other bacterial species will be required to provide more fundamental insight into the role of individual Sap components in determining peptide specificity.

Questions regarding Sap specificity are not only of academic interest. Indeed, resistance to antimicrobial peptides may turn out to be a key determinant of host range specificity in *S. typhimurium*, *E. chrysanthemi*, and a number of other pathogens. Extrapolating from this contention, López-Solanilla et al. suggest that attempts to restrict pathogen host range may be bolstered by manipulating the expression of antimicrobial peptides in crop plants.

In conclusion, López-Solanilla et al. provide an elegant demonstration of common mechanisms of innate resistance in plants and animals. Their work adds to the accumulating body of evidence suggesting that similarities in the attack and defense mechanisms adopted by plants and animals and their respective pathogens are the norm rather than the exception. What is particularly notable about the work reported by these authors is that their combined use of an appropriate mutant, purified antimicrobial peptides, and virulence assays cleanly links concept with a comprehensive and effective mechanism that pathogens use to evade their hosts' defense responses.

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