Pathogen Surveillance—
the Flies Have It
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The ancient origins of the battles between infectious microbes and their hosts are illustrated by the similarities in frontline defense adopted by insects and mammals. In mammals, the innate immune system defines a rapidly induced first response to infection that directly activates host defenses and also stimulates the adaptive immune system. Insects share features of the mammalian innate immune response. In both groups, pathogens are recognized through interactions of stereotypical microbrial structures with host proteins called pattern recognition receptors (1). Mammalian pattern recognition receptors include the Toll-like receptors (TLRs), so-called because they resemble the Toll receptor of Drosophila. The Toll receptor and the TLRs activate immune responses to infection that are regulated by the transcription factor NF-κB. However, unlike the TLRs, Toll does not interact directly with microbial compounds but rather acts as a receptor for small molecules that can activate the immune system.

References and Notes
2. Named by the nomenclature committee of the Meteoritical Society, NWA stands for Northwest Africa.
4. For Earth, the composition of midocean ridge basalts is plotted. Basalt compositions from the Moon, Mars, and Venus are taken from an Apollo 12 basalt, the Shergotty meteorite, and eucrite meteorites, respectively. Venus data are from the Russian Venera 13 and 14 missions. All data are normalized to average solar system abundances represented by C-chondrites. It is therefore assumed that all planets have the same bulk composition.

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mammalian immune responses, revealed evolutionary links between *Drosophila* and mammalian innate immunity, facilitating the identification of TollRs in mammals.

The *Drosophila* immune deficiency (Imd) pathway is a second independent signaling cascade in fat-body cells that fights Gram-negative bacteria (see the figure) (4, 5). In contrast to the Toll pathway, the Imd pathway is not involved in development. This finding has facilitated the identification of additional components of this pathway through genetic screens. The Imd pathway induces antimicrobial gene expression via a third *Drosophila* NF-kB-like factor, Relish. It shares some similarities with the mammalian tumor necrosis factor receptor pathway, pointing to evolutionary conservation between these two signaling cascades.

In vertebrates, multiple TollReceptors enable detection of different microbial compounds. TLR4, for example, activates immune responses to the lipopolysaccharides (LPS) that are found in Gram-negative bacterial cell walls. In contrast, TLR2 mediates responses to peptidoglycan, a major component of the cell walls of Gram-positive bacteria (6). The presence of pathogen-specific pattern recognition receptors in *Drosophila* is clearly indicated by the preferential activation of the Toll pathway by fungal and Gram-positive bacterial infections and the activation of the Imd pathway by Gram-negative bacteria. In addition, the *Drosophila* genome encodes eight Toll homologs, suggesting that fruit flies may use different Toll receptors to differentiate among pathogens. Nevertheless, studies of these proteins in cultured cells have not demonstrated their involvement in microbial recognition. Thus, our understanding of pathogen detection in insects has lagged behind that in mammals.

The facility of genetic screens for isolating immunocompromised *Drosophila* mutants is closing this gap. Michel et al. (3) started to search for fly mutations that block antimicrobial gene expression induced by Gram-positive bacteria. They found that Gram-positive bacterial infections activate PGRP-SA, a protein circulating in the fly hemolymph that then activates the Toll pathway. PGRPs were initially isolated from the moths *Bombyx mori* and *Trichoplusia ni* because of their high affinity for bacterial peptidoglycan (7, 8). Mutations in PGRP-SA block the activation of the Toll pathway in response to Gram-positive bacteria and significantly decrease resistance to this type of infection, in perfect agreement with the prediction that this protein detects the peptidoglycan in Gram-positive bacterial cell walls. The steps that link PGRP-SA and the Toll ligand Spatzele are not known, but they probably involve serine proteases because a serine protease inhibitor, Necrotic, represses Toll activation. Mutations in PGRP-SA do not affect Toll activation induced by fungal infection, indicating that additional fungal-specific pattern recognition receptors may act upstream of Toll. Interestingly, the *Drosophila* genome contains 12 additional PGRP genes that are predicted to encode either short extracellular proteins (like PGRP-SA) or longer intracellular and membrane-spanning proteins (9). PGRP-SA’s involvement in Gram-positive bacterial recognition, together with the large number of PGRP proteins in flies, suggests that this family mediates the recognition of multiple microbial species.

Results from a second genetic screen provide support for this hypothesis. Choe et al. (2) demonstrate that fly mutations—originally isolated in a search for genes that induce antimicrobial gene expression after Gram-negative bacterial infection—affect the PGRP-LC gene. These results have been confirmed by two other groups using different approaches (10, 11). PGRP-LC is predicted to encode a transmembrane protein with an extracellular PGRP domain. This protein activates the nuclear translocation of Relish and antimicrobial gene expression via the Imd pathway after both Gram-negative and Gram-positive bacterial infection. Although the localization of PGRP-LC in fat-body cell membranes and its direct interaction with microbial compounds has yet to be demonstrated, these genetic results suggest that PGRP-LC is a pattern recognition receptor for the Imd pathway in flies.

The demonstration that PGRPs operate in both the Toll and Imd pathways clarifies several aspects of innate immunity. First, it is now clear that *Drosophila* has two different systems for sensing microbes: (i) circulating pattern recognition receptors such as PGRP-SA that are present in the hemolymph (the Toll pathway), and (ii) transmembrane recognition receptors such as PGRP-LC (the Imd pathway) (see the figure). One obvious advantage of circulating recognition molecules is that they are able to sense microbes throughout the body cavity and to amplify signals via proteolytic signaling cascades. Extracellular signaling proteins, however, require enclosed compartments and cannot work in tissues exposed to the external environment. This may explain why the Imd pathway is the predominant cas-

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**Invaders pay the Toll.** The Toll and Imd pathways regulate the *Drosophila* antimicrobial innate immune response. Antimicrobial peptide genes are regulated by the NF-kB-like proteins Dif, Dorsal, and Relish, which are the end targets of two distinct signaling cascades: The Toll pathway, which is principally activated by fungi and Gram-positive bacteria, and the Imd pathway, which is largely activated by Gram-negative bacteria. PGRP-SA is a circulating protein required for Toll activation in response to Gram-positive bacteria (2). PGRP-LC is a putative pattern recognition receptor required for the activation of the Imd pathway (2).
MADS-Box Genes Reach Maturity

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Since Adam’s first visit to the fruitless Garden of Eden, humankind has been totally dependent upon the angiosperm flower and the fruit it bears. Much of our food and clothing is derived from flowers and their fruits. Unraveling the pathways that regulate how flowers, fruits, and seeds develop has significant implications for agriculture. Ripening is a vital aspect of fruit production. For many fruits to be palatable they must be fully ripe, and ripening is essential for propagation of the species. In terms of shipping, storage, and shelf life, we need to know how to control the ripening of edible fruits. On page 343 of this issue, Vrebalov et al. (1) reveal that a tomato plant whose fruit cannot ripen, called ripening-inhibitor (rin), carries a mutation in a gene encoding a MADS-box transcription factor. This work not only establishes the involvement of MADS-box factors in fruit ripening, but also has important agricultural implications. In today’s world of global distribution, the control of fruit ripening is of strategic importance. Unfortunately, our understanding of the genetic regulation of ripening is limited, reducing our ability to manipulate this process. In the tomato and many other fruits, early ripening events involve an increase in biosynthesis of the plant hormone ethylene accompanied by a burst of respiration. The fruit then undergoes a complex and coordinated transformation (2). Cell wall structure is modified, improving texture and inducing softening (see the figure). The production of compounds conferring flavor and aroma increases. Starch is converted into sugars, adding sweetness, and red pigments (such as carotene and lycopene) begin to replace the green chlorophyll. Ethylene signaling is one of the best known plant hormone regulatory pathways (3) and is a key factor in the control of ripening. However, developmental pathways also influence ripening, and many types of fruit do not require increased ethylene biosynthesis to ripen. So far, an overall developmental regulatory pathway, tightly regulated, genetic analyses of immune systems would be hampered by functional redundancies among protein components of immune pathways. Mutations in Drosophila and mouse proteins, however, reveal that disrupting genes that belong to large families, such as those encoding TLRs and PGRPs, can generate specific immune defects. Such results validate this approach in the ongoing dissection of the battle between pathogens and their hosts.

Perspectives: Plant Biology

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