bled first, before the complex can leave the endoplasmic reticulum. Apparently, the DAP domain of nicastrin is not involved in assembly of the γ-secretase complex but, as Shah et al. (2005) confirm, the transmembrane domain of nicastrin is needed for the interaction of nicastrin with the other partners of the complex (Capell et al., 2003; Morais et al., 2003). The new work defines two clear functional domains in nicastrin: the DAP domain for substrate recognition and the transmembrane domain for the assembly and correct trafficking of the γ-secretase complex to the cell surface.

Although Shah et al. (2005) have solved an interesting puzzle, many questions remain unanswered. For example, what is the role of the two other γ-secretase components aph-1 and pen-2? Several lines of research suggest that presenilin has additional substrate binding sites. Furthermore, two *presenilin* (and two *aph-1*) genes exist in the human genome and thus at least four different γ-secretase complexes can be generated. One wonders whether presenilin or aph-1, in combination with nicastrin, could confer some specificity on the recognition of substrates (De Strooper, 2003). Finally, when contemplating the γ-secretase machinery, one infers that there must be active conformational changes in the complex to push the transmembrane domain of the protein substrate from the hydrophobic cell membrane into the catalytic (probably water-containing) pore of presenilin. Evidence that GTP or another energy source is required for the cleavage activity of γ-secretase has not yet been provided.

The experimental approaches and tools described by Shah et al. (2005) reveal new ways to study γ-secretase biochemistry in greater detail. It is particularly striking how efficiently the active γ-secretase complex can be produced in cells infected with baculovirus. One hopes to see in the near future some real images of the complex and more detailed biophysical studies of its activity. The next major challenge, a crystal structure of the complex, will be much more difficult to achieve, given the (estimated) 18 transmembrane domains of the core γ-secretase complex. In any event, many exciting discoveries remain to be made along the road to this ultimate goal.

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Salmonella's Sensor for Host Defense Molecules

The bacterial pathogen *Salmonella typhimurium* resides within phagosomes in host cells and is able to deflect the host immune response. In this issue of *Cell*, Bader et al. (2005) decipher an elegant mechanism by which the PhoQ sensor kinase of *Salmonella* is switched on by host cationic antimicrobial peptides, leading to changes in gene expression that enable *Salmonella* to combat the host immune response.

When a bacterium enters a host, certain events are triggered that will determine its fate. Different fates that might befall the microbe include destruction, passage through the host as a harmless transient, incorporation into the natural flora, or establishment of an infection with the initiation of host damage. The initial innate immune response of the host involves the activation of signaling pathways that recognize structurally conserved pathogen molecules using a series of receptors, in particular those of the Toll-like receptor (TLR) family (Miller et al., 2005). For example, the bacterial surface molecule lipopolysaccharide (LPS) engages TLR4, leading to the increased expression of numerous host defense genes including those encoding proinflammatory cytokines and chemokines, with the consequent marshalling of effectors of innate immunity to destroy or contain the microbe. The successful pathogen, however, recognizes environmental cues in the host and will remodel its metabolism and physiology to permit it to survive and grow in the host. For example, the small concentration of free iron in many hosts triggers production of bacterial iron acquisition systems (Bullen et al., 2005).

The study by Bader et al. (2005) in this issue of *Cell* sheds light on a particularly effective bacterial defense mechanism in the pathogen *Salmonella typhimurium*. The two-component sensor, PhoPQ, of *Salmonella* comprises a membrane bound sensor kinase PhoQ (which has a binding domain and a kinase/phosphatase domain) and a response regulator PhoP in the bacterial cytoplasm (see Figure 1). When the *Salmonella* bacterium encounters certain conditions (limiting concentrations of divalent cations or host cationic antimicrobial

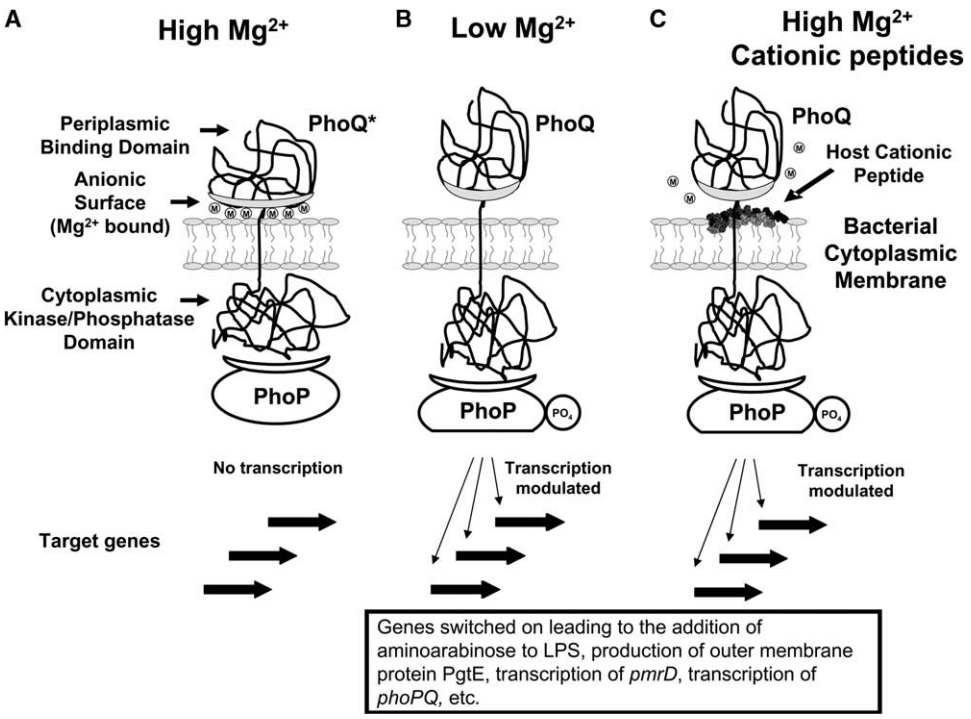

Figure 1. Activation of *Salmonella*'s PhoPQ Defense System

(A) In the presence of a high concentration of Mg^{2+} (M) or other divalent cations (and the absence of host cationic antimicrobial peptides), the divalent cations form a bridge between the negatively charged lipids in the outer leaflet of *Salmonella*'s cytoplasmic membrane and the anionic surface of the PhoQ binding domain. The phosphatase activity of PhoQ is activated (PhoQ*), leading to dephosphorylation (inactivation) of the transcriptional regulator PhoP in the bacterial cytoplasm. In this unphosphorylated state, PhoP is incapable of modulating transcription.

(B) If the concentration of divalent cations is low, the PhoQ protein undergoes a conformational change, resulting in increased kinase activity of its cytoplasmic domain. This causes phosphorylation of the PhoP protein, which is then able to bind to target DNA sequences, leading to modulation of transcription (that is, an increase in transcription of those genes for which PhoP is an activator and a decrease for those genes where PhoP serves as a repressor).

(C) Host cationic antimicrobial peptides enter the bacterial outer membrane and then bind to the anionic patch of the PhoQ binding domain (displacing Mg^{2+} ions, if the Mg^{2+} concentration is high), leading to a conformational change in PhoQ (as observed under limiting Mg^{2+} conditions) and activation of its kinase activity. As in (B), this results in phosphorylation (activation) of the transcriptional regulator PhoP, which modulates the expression of a number of different bacterial genes that help to combat the host immune response.

peptides), the PhoQ protein dimerizes and becomes autophosphorylated at a conserved histidine residue. The dimeric PhoQ protein then transfers the phosphate to an aspartate residue within PhoP, leading to increased affinity of PhoP for a conserved DNA binding motif and modulation of expression of target genes containing this conserved site (Castelli et al., 2000). PhoPQ is found in a number of Gram-negative bacteria and is essential for the virulence of Gram-negative bacterial pathogens in humans and mice (Gunn, 2001). *Salmonella* PhoPQ is activated when the bacteria are taken up into the phagosomes of host macrophages. In vitro experiments have shown that the PhoPQ system is activated by low concentrations of Ca^{2+}, Mg^{2+}, or Mn^{2+} ions (Castelli et al., 2000). However, it is not intuitively obvious that PhoPQ would be activated inside host cells as most host tissues contain repressing (1–2 mM) concentrations of free Ca^{2+} and Mg^{2+}, and the concentration of Ca^{2+} within phagosomes (0.4 to 0.6 mM) also is repressing (Christenson et al., 2002). These observations suggest that, in vivo, *Salmonella* may be responding to a signal other than limiting divalent cation concentrations.

Intriguingly, cationic antimicrobial peptides, which are key components of host innate immunity (Zasloff, 2002; Bowdish et al., 2005), induce upregulation of the *Salmonella phoPQ* operon (Bader et al., 2003) as well as the *Pseudomonas aeruginosa pmrAB* operon, which encodes another type of bacterial two-component sensor (McPhee et al., 2003). Bader et al. (2005) now reveal that these host cationic antimicrobial peptides bind directly to the binding domain of *Salmonella*'s PhoQ membrane bound sensor via an acidic patch that seems to overlap with the PhoQ binding domain for divalent cations (see Figure 1). Binding of these peptides activates the kinase activity of PhoQ, resulting in phosphorylation and activation of PhoP, which then alters the transcription of specific bacterial target genes. Using a sophisticated combination of genetic, structural, and biophysical approaches, Bader and colleagues construct a model in which the PhoQ binding domain of *Salmonella* interacts with negatively charged

lipids in the outer leaflet of the bacterial cytoplasmic membrane via a divalent Mg^{2+} bridge (see Figure 1). Removal of Mg^{2+} ions or their displacement by cationic antimicrobial peptides induces a structural transition in PhoQ that is translated across the cytoplasmic membrane, promoting phosphorylation of PhoP. Cationic antimicrobial peptides, despite great diversity of structure, tend to fold into amphipathic structures that lie on the surface of the bacterial cytoplasmic membrane with their positive charges facing outwards; hence, they are ideally suited to interacting with the membrane-facing anionic patch of PhoQ.

Cationic antimicrobial peptides are a ubiquitous component of the innate immune systems of complex eukaryotes: they are found in plants, insects, amphibians, crustaceans, and mammals, including humans. These antimicrobial peptides not only have direct antimicrobial activity but also are involved in the modulation of other innate immune pathways (Zasloff, 2002; Bowdish et al., 2005) because they can act as chemokines. In this capacity they stimulate host gene expression, selectively suppress endotoxemia/sepsis, and stimulate wound healing and angiogenesis. In humans, they are prominent components of dedicated antiinfective (phagocytic) cells and are also found in other cell types (myeloid precursor cells, epithelial cells, mast cells, keratinocytes, and lymphocytes), in other tissues (the mucosa, intestine, skin, oral cavity, cervix, lungs), and in bodily fluids (gastric juices, saliva, semen, sweat, plasma, airway surface liquid, and breast milk). The concentrations of these antimicrobial peptides vary from very high in sites where their action is likely to be direct—in the granules of phagocytes or the crypts of the intestine—to modest in locations such as mucosal surfaces where their immunomodulatory properties may be more important.

Salmonella bacteria enter host cells by binding to the gastric mucosa and are taken up by a variety of host cell types including phagocytes. Once inside host cells, this pathogen resides in vesicles of host origin called *Salmonella*-containing vesicles and resists destruction. It is striking that *Salmonella* has evolved a mechanism that harnesses host antimicrobial peptides to switch on its own defense system, PhoPQ, which enables the pathogen to become resistant to the antimicrobial peptides as well as to other cationic defense molecules such as the antibiotic polymyxin. When PhoPQ is activated, the lipid A portion of LPS—which anchors LPS in the outer membrane of Gram-negative bacteria—becomes modified by aminoarabinose and fatty acids, which decreases the negative charge and fluidity of the bacterial outer membrane. This results in decreased binding and uptake of cationic antimicrobial peptides across the bacterial outer membrane, leading to resistance of *Salmonella* to these peptides. Modification of the lipid A portion of LPS by PhoPQ also reduces by up to 100-fold the ability of *Salmonella* LPS to induce host innate immunity via TLR4 (Kawasaki et al., 2004), resulting in muting of the host immune response. Furthermore, the regulation by PhoPQ of more than 200 bacterial genes involved in chemotaxis/motility, drug resistance, transport, and heme biosynthesis indicates that a substantial shift in bacterial physiology is triggered by induction of this clever defense system (Minagawa et al., 2003).

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