The report by Ahmed and colleagues opens up new possibilities for the treatment of chronic viral infections. Chronic viral infections are resistant to conventional medications and sometimes lead to tumorigenesis, such as liver cancer due to infections by HBV and hepatitis C virus. The invention of a safe and effective therapy is urgently needed. Interestingly, PD-1 deficiency leads to a relatively mild phenotype when compared to loss of another CD28 family member, CTLA-4, even though engagement of CTLA-4, like PD-1, results in the inhibition of T cell function. Also, the expression of PD-1 is restricted to activated lymphocytes. Thus, blocking PD-1 may have fewer side effects than other potential therapies. The next step may be to demonstrate in human patients that antigen-specific T cells survive during chronic infections, express PD-1, and are reanimated by blocking PD-1 signaling. Such findings could one day lead to the clinical use of PD-1 blockers for the treatment of chronic viral infections.

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# To Kill but Not Be Killed: A Delicate Balance

**Patrick Stragier**[1,*]
[1]Institut de Biologie Physico-Chimique, CNRS UPR9073, 75005 Paris, France
*Contact: stragier@ibpc.fr
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**Before launching a missile, it is necessary to design an efficient safety net for self-protection. In this issue of *Cell*, Ellermeier et al. (2006) describe the mechanism underlying a biological safety net for the soil bacterium *Bacillus subtilis*. This bacterium protects itself from a toxic protein it secretes by upregulating an immunity protein, which it does by sequestering a transcriptional repressor at the plasma membrane.**

When hungry, eat your sister... but beware that she does not eat you first! This is the motto of the soil bacterium *Bacillus subtilis*, according to results from Rich Losick's lab, first reported by González-Pastor et al. (2003), and now further analyzed by Ellermeier et al. (2006) in this issue of *Cell*. When *B. subtilis* is subjected to starvation, two independant gene clusters become transcribed, leading to the synthesis and release in the external medium of two toxic peptides, SkfA and SdpC. Both factors kill neighboring siblings, thereby releasing nutrients that sustain further growth of the bacteria producing the toxins. However, in order to survive, these bacteria have to protect themselves from the toxic peptides they produce. The SkfA killing factor is pumped out of the bacterial cells by the products of two genes belonging to the *skf* operon (González-Pastor et al., 2003). In this issue of *Cell*, Ellermeier et al. (2006) elucidate the mechanism that provides protection against the SdpC peptide and reveal a remarkable new signal transduction pathway (see Figure 1).

The core of the new signaling pathway is an operon encoding a soluble DNA binding protein, SdpR, and a transmembrane immunity protein, SdpI. When nutrients are plentiful, the *sdpRI* operon is not transcribed, due to the presence of the global repressor AbrB, which also represses the operon encoding the SdpC toxin (Fujita et al., 2005). As nutrients

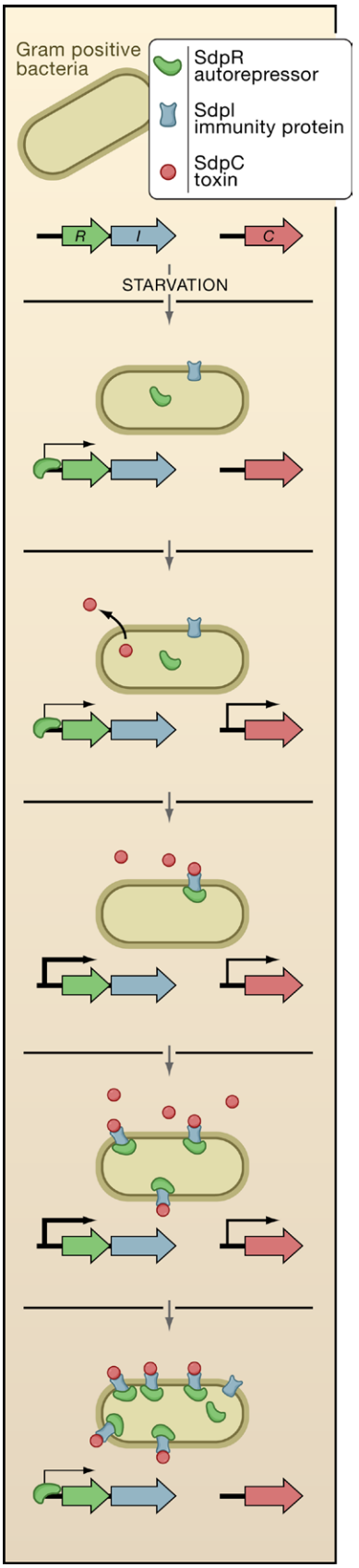


**Figure 1. Toxin-Producing *Bacillus subtilis* Makes Only as Much Immunity Protein as It Needs**

The *sdpRI* operon (green and blue straight arrows) encodes the cytoplasmic SdpR autorepressor (green) and the membrane-bound SdpI immunity protein (blue), whereas the toxin-encoding gene *sdpC* is part of a separate operon (red straight arrow). Transcription from *sdpRI* is induced by starvation and repressed by SdpR (thin arrow). Starved *B. subtilis* cells (rod shaped) also transcribe *sdpC* (medium arrow) and secrete the extracellular SdpC toxin (red). SdpC binds to SdpI, thereby triggering sequestration of SdpR at the membrane and activating *sdpRI* transcription (thick arrow). Increasing amounts of SdpI and SdpR molecules trap all SdpC molecules. Free SdpI cannot sequester SdpR, which remains in the cytoplasm, where it shuts off transcription of *sdpRI* (thin arrow). Transcription of *sdpC* is shut off once the global regulator Spo0A, which initiates this pathway in response to starvation, reaches a critical concentration.

become limiting, the global regulator, Spo0A, is activated and shuts off further synthesis of AbrB. Disappearance of AbrB leads to the synthesis of the three proteins, SdpR, SdpI, and SdpC. Results from the González-Pastor et al. (2003) and Ellermeier et al. (2006) studies suggest that the *sdpRI* operon is transcribed earlier than *sdpC*, presumably as a consequence of the respective affinities of AbrB for the two promoter regions. However, because SdpR binds to the *sdpRI* promoter, transcription of *sdpRI* remains limited and only a few SdpI molecules are synthesized and inserted into the cytoplasmic membrane. The SdpC toxin is secreted into the extracellular milieu and a striking switch occurs when it accumulates in the external medium. The SdpI immunity protein, presumably by binding to the SdpC toxin, triggers sequestration of the SdpR autorepressor at the bacterial membrane. The redistribution of the SdpR protein from DNA to the membrane was directly visualized by Ellermeier et al. (2006). Sequestration of SdpR promotes a high level of transcription of *sdpRI*, thereby increasing the amount of immunity protein for self-protection. Once active Spo0A reaches a critical concentration, it represses further transcription of the operon encoding SdpC (Fujita et al., 2005). The SdpC molecules become too scarce to bind to all SdpI immunity molecules. Then, free SdpR autorepressor accumulates in the cytoplasm, effectively shutting off new synthesis of SdpR and SdpI. Therefore, production of the immunity protein strictly correlates, both in time and in amount, to the presence of the toxin protein in the growth medium.

The whole pathway is under the control of the master regulator Spo0A, whose activity increases as nutrients become more and more limiting, triggering the successive activation or repression of several gene classes (Fujita et al., 2005). The complex mechanisms transducing metabolic imbalance into activation of Spo0A lead to heterogeneous populations of bacteria where individual cells have different levels of active Spo0A (Chung et al., 1994; Fujita and Losick, 2005). Ultimately, the cells embark on an irreversible developmental process culminating in the formation of a resting spore. The starved cells delay their commitment to sporulation by releasing SdpC (and the other killing factor SkfA) in the medium and, being fully armored to resist the toxicity of these two proteins, cannibalize their unlucky sister cells that are lagging behind in activating Spo0A. This is the last supper that precedes the metamorphosis of the toxin-producing bacteria into spores that may remain dormant for a very long time.

The SdpC toxin is synthesized as a larger cytoplasmic protein whose maturation into its extracellular active form requires the products of two genes cotranscribed with *sdpC*. A similar strategy is observed with colicins, proteins that are synthesized in an inactive form by some *Escherichia coli* strains and are secreted in the medium where they kill nonimmune neighboring bacteria. Presumably, this two-step process has been selected in order to avoid self-destruction of the toxin-producing bacteria. What would be the phenotype of *B. subtilis* cells producing the mature form of SdpC in their cytoplasm? Assuming such an experiment is feasible, would mature SdpC be toxic, from the inside, to the cells engineered to produce it? The molecular basis of SdpC toxicity

remains obscure. It involves neither SdpI nor SdpR, although these two proteins were identified when searching for targets of SdpC (González-Pastor et al., 2003). If SdpC only acts on certain membrane proteins, it will only affect bacteria displaying these specific receptors, thereby limiting its toxicity to *B. subtilis* or very closely related bacteria. Conversely, if SdpC acts merely by inserting itself into the plasma membrane and promoting, for instance, membrane destabilization, then its spectrum of toxicity could be much wider. This would turn an unusual cannibalism process into a more traditional form of biological warfare. A similar wider function can be suggested for the other killing factor, SkfA, which is toxic to the rice pathogen *Xanthomonas oryzae* (Lin et al., 2001). Whatever the mechanisms of SdpC toxicity, it should be stressed that it is effective only at high concentrations. Therefore, it is safe to predict that it operates mostly in colonies (usually constituted of siblings) and on mixed communities of bacteria, such as those existing in natural biofilms.

From its sequence, the SdpI immunity protein is inferred to be an integral membrane protein. Located in the bacterial membrane—the interface between the external medium and the cytoplasm—SdpI behaves as a receptor (for the SdpC toxin) and as a signal transduction protein (through its ability to interact with the SdpR repressor). Ellermeier et al. (2006) isolated two classes of *sdpI* mutants that enabled separation of these two functions. Some mutants are still immune to SdpC, but they do not induce expression of *sdpRI* in the presence of the toxin. These mutants appear to have lost the ability to interact with SdpR but still recognize SdpC. Another class of mutations leads to the opposite phenotype, with derepression of *sdpRI* in the absence of the SdpC toxin. Evidently, these mutations lock the SdpI transducing protein into the conformation that sequesters SdpR. As these constitutive forms of SdpI confer immunity on the toxin (R. Losick, personal communication), they probably still interact normally with SdpC.

The mechanism of immunity is a matter of conjecture. Assuming that SdpI binds directly to SdpC (which remains to be demonstrated), the simplest model is that trapping of the toxin molecules by SdpI is sufficient to prevent SdpC from interacting with its yet-to-be-identified target. In that case, SdpI is an antitoxin which, as is commonly the case, induces its own synthesis in response to the presence of the toxin. However, its modus operandi is quite peculiar. Membrane sequestration is usually designed to prevent transcription activation factors from reaching their DNA targets, in prokaryotes as well as in eukaryotes (Alba and Gross, 2004; Brown et al., 2000). In the case of SdpI and two other bacterial proteins (Bohm and Boos, 2004), membrane sequestration is used to activate transcription by trapping a repressor away from the genes it controls.

SdpR is an autorepressor that blocks transcription of the *sdpRI* operon, but it is likely that this is not its sole function. Expression of several other operons is dependent on SdpR and their products may contribute to delaying commitment to sporulation (González-Pastor et al., 2003). SdpR was also independently identified in a screen for transposon insertions inhibiting expression of *sigW*, a gene encoding a regulatory protein involved in detoxification and antibiotic resistance (Turner and Helmann, 2000). Therefore, the exact scope of the SdpR regulon remains to be determined. But the results reported by Ellermeier et al. (2006) show unambiguously that SdpR (and the operons it may control) is not involved in sensitivity to the SdpC toxin. Likewise, SdpR sequestration at the bacterial membrane (and its putative transcriptional consequences other than induction of *sdpI*) is not required for immunity to SdpC.

Orthologs of the *sdpRI* operon exist in five other *Bacillus* species, with SdpR being strongly conserved and SdpI much less conserved. Given that these bacteria do not contain an ortholog of *sdpC*, it seems logical to propose that an interaction similar to that described in *B. subtilis* takes place between their SdpI and SdpR orthologs in response to some signal other than SdpC. This raises the intriguing possibility that extracellular signals other than SdpC might also trigger membrane sequestration of SdpR in *B. subtilis*, thereby activating another set of genes and a specific response that has yet to be identified. The presence in the immediate vicinity of *sdpRI* of two operons encoding ABC transporters might be a clue to the identity of these new responses as well as to additional extracellular signals other than SdpC that could affect localization of SdpR. We eagerly await the results of the next set of experiments characterizing these interesting signaling pathways.

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