2008; Slavov et al., 2011). The mode of gene regulation affects the variability in single-cell responses (Munsky et al., 2012), raising another exciting question: Can high-promoter occupancy also reduce variability among the input-output responses of single cells? These implications and questions provide a fertile ground for further work characterizing the design principles of signal transduction.

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When Death Was Young: An Ancestral Apoptotic Network in Bacteria

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In this issue of *Molecular Cell*, Dwyer et al. (2012) characterize a RecA-dependent and ClpXP-regulated pathway that controls the acquisition of several apoptotic markers upon bactericidal treatment of prokaryotes, placing the hypothetical origin of apoptosis further downstream in evolution.

In metazoans, the life span of individual cells is regulated by an integrated suicide system (programmed cell death, PCD) that can be activated when cells become superfluous, accumulate damage, or menace organismal fitness. Among the distinct subroutines constituting PCD, apoptosis represents the best-studied one. Apoptotic death is a structurally and functionally conserved process in thus far that it is also observed in unicellular eukaryotes, such as protozoan parasites or yeast (Carmona-Gutierrez et al., 2010; Madeo et al., 1997). Dwyer et al. (2012) provide phenotypic and mechanistic evidence that may expand the evolutionary conservation frame of apoptosis into the realm of prokaryotes.

The authors demonstrate that bacterial cell death induced by treatment with different bactericidal antibiotics is accompanied by several biochemical markers of apoptosis, including DNA fragmentation, chromosomal condensation, exposure of phosphatidylserine to the outer leaflet of the plasma membrane, and dissipation of membrane potential (Dwyer et al., 2012). These results add to previous work by the same group (Dwyer et al., 2007; Kohanski et al., 2007) showing that bactericidal antibiotics promote the generation of reactive oxygen species (ROS), which are crucial apoptotic regulators in multicellular as well as in unicellular eukaryotes (Herker et al., 2004; Simon et al., 2000). In bacteria, ROS seem to play a similar role, since suppressing their formation reduces drug-induced cell death (Dwyer et al., 2007) as well as DNA fragmentation (Dwyer et al., 2012).

Now, Dwyer et al. (2012) identify and characterize RecA, a multifunctional protein crucial for DNA maintenance and repair, as an additional player involved in the antibiotic-triggered apoptotic demise of bacteria. Consistent with this finding, RecA plays a critical role in the recently described apoptosis-like death (ALD) pathway of *E. coli* (Erental et al., 2012). Dwyer et al. (2012) extend these observations by showing that the cell stress-triggered conversion of RecA into its active form is a prerequisite for its contribution to cell-death induction (Dwyer et al., 2012). The lethal activity of active RecA is thereby negatively regulated by the ClpP protease complex ClpXP. These factors also dampen the LexA-regulated bacterial DNA-damage (or SOS) stress response, which is necessary for the efficient induction of apoptosis in response to cellular stress (Dwyer et al., 2012).

In this network of interacting regulators, RecA seems to function in a similar fashion as do caspases, the central executionary cysteine proteases in many scenarios of mammalian apoptosis. Indeed, RecA can bind and hydrolyze synthetic caspase substrates and appears to be the only bacterial enzyme to

do so, at least in *E. coli* (Dwyer et al., 2012). However, RecA is not only involved in lethal signaling; it also harbors multiple essential functions associated with DNA repair and as a regulator of the SOS response. Thus, RecA combines both lethal and vital roles, paralleling the ambiguous involvement of mammalian caspases in multiple signal transduction pathways that may either factor cell death or survival (Galluzzi et al., 2012). It remains elusive whether RecA's vital and lethal functions might be dissociated from each other. This could be explored, for instance by generating specific point mutants that would preferentially affect one or the other function.

The double-sided functional nature of RecA may account for the observation that its disruption leads to both (1) reduced acquisition of the apoptotic phenotype (Dwyer et al., 2012) and (2) an increased sensitivity toward bactericidal drugs (Kohanski et al., 2007), possibly as a result of the combined (1) loss of the main apoptotic executioner and (2) the fatal removal of the protein's vital functions. In the latter case, cell death might be accomplished through an alternative, nonapoptotic PCD subroutine like the *mazEF*-mediated death pathway, whose execution has been shown to be *rec*A independent (Erental et al., 2012). In fact, bacterial apoptosis and the *mazEF* pathway seem to be intertwined, the latter one possibly suppressing the first one by reducing *recA* mRNA levels (Erental et al., 2012). ClpXP is known to be involved in the synthesis of the quorum-sensing signaling factor EDF (extracellular death factor) (Kolodkin-Gal and Engelberg-Kulka, 2006), which is required for *mazEF*-mediated cell death and induces the endoribonucleolytic activities of MazF and ChpBK (Belitsky et al., 2011). Thus, it is conceivable that ClpXP, which suppresses RecA apoptotic activity (Dwyer et al., 2012), might partly do so by promoting MazF-mediated *recA* mRNA cleavage/splicing (Figure 1). Such indirect mechanisms might represent an additional regulatory process beyond the proposed direct proteolytic regulation and modification of RecA by ClpXP (Dwyer et al., 2012) that further refine the crosstalk between the *mazEF*- and apoptotic pathways.

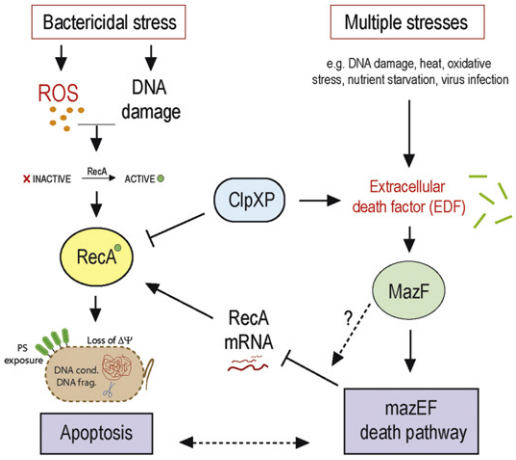

Figure 1. Bacterial Programmed Cell Death and Its Subroutines

Two types of prokaryotic PCD have been identified so far: *mazEF*-mediated cell death and apoptosis-like death. These two programs operate at different levels but also seem to be interwoven. The ClpXP protease complex, for instance, is involved in both pathways, namely (1) as a factor required for EDF production and consequently for *mazEF*-mediated cell death and (2) as a negative regulator of apoptosis. Moreover, the activity of RecA, the main apoptotic executor, is inhibited by the *mazEF*-mediated pathway via reduction of *recA* mRNA levels. ROS: reactive oxygen species, EDF: extracellular death factor.

The existence of mutually inhibitory PCD pathways—like in mammalian cells, in which some catabolic pathways reciprocally suppress each other—might function as a backup system to genetically ensure programmed demise even upon loss of one of the pathways and/or as a regulatory toolkit to modulate the cellular reaction to different types and intensities of stress.

The presence of a regulated suicide network in unicellular bacteria might—in analogy to PCD of eukaryotic microorganisms—increase the fitness of populations by facilitating the elimination of unwanted cells (Herker et al., 2004). To address the possible advantage of maintaining intact PCD programs in bacteria, it would be interesting to perform competition assays confronting wild-type, apoptosis-deficient, and/or *mazEF* mutant cells under conditions of antibiotic stress. Such experiments could be carried out not only in vitro but also in vivo, for instance in suitable mouse models of bacterial infection. It can be anticipated that the identification and detailed characterization of bacterial PCD pathways that respond to drug-induced cell killing will be instrumental to understand and counteract the surge of bacterial strains resistant to available antibiotic treatments. Of note, both the *mazEF* and apoptotic pathways permit survival of a small fraction of the bacterial population responding to external stress (Erental et al., 2012). Hence, elucidating the interweavement of distinct PCD pathways might pave the way for the development of new strategies of antibiosis.

The existence of morphological and biochemical signs of apoptosis in prokaryotes has broad evolutionary implications. Given the core role of mitochondria in the eukaryotic apoptotic machinery, it is possible that eukaryotic apoptosis evolved during endosymbiosis, when the prokaryotic protomitochondrion was introduced into the primitive protoeukaryotic cell. One intriguing scenario predicts that eukaryotes obliged to develop a control system that would avoid lysis of the endosymbiont activating its endogenous PCD machinery. The development of pathways to control these imported lethal mechanisms might have also promoted the eukaryotic ability to make use of them when required. Hence, the phylogenetic origin of PCD in mitochondrial precursors might explain the architecture of eukaryotic cell-death control (which involves mitochondrial permeabilization as a central gateway to death) as well as its complex regulation.

However paradoxical it appears, the emergence of life might have promoted the advent of regulated death to maintain evolutionary progress and ecological balance, thus ultimately assuring the long-time establishment of life itself. If the lethal programs that we harbor in our own cells constitute a modern, adapted version of ancestral pathways that we can still recapitulate in prokaryotes, a more detailed comprehension of when death was young will have a profound scientific, medical, and even philosophical impact.

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Stemming Danger with Golgified BAX

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In this issue of *Molecular Cell*, Dumitru et al. (2012) report that hES cells localize a conformationally activated form of proapoptotic BAX to the trans Golgi network, a previously unanticipated launch pad for mitochondrial assault in response to DNA damage.

BCL-2 family proteins are charged with protecting the organism from unwanted cellular excess or demise. To achieve the proper balance, antiapoptotic BCL-2 family proteins such as BCL-X_L have the capacity to protect mitochondria, the power plants of the cell, from permeabilization by proapoptotic members such as BAX, thereby preserving cell survival. Conversely, when persistence of damaged cells threatens the organism, activated proapoptotic proteins with the ability to form destructive mitochondrial pores become the saviors, eliminating renegade cells for the benefit of the whole. To render the appropriate life-death decision in response to a litany of cellular stressors across a broad diversity of tissues, BCL-2 family proteins are subject to exquisite regulation. In particular, pore-forming proapoptotic members, such as BAX, must be carefully controlled to avoid wanton activation and cellular destruction, yet stand ready for rapid deployment in the face of threatening external and internal stimuli. Dumitru et al. (2012) report the provocative finding that a conformationally activated form of BAX specifically localizes to the trans Golgi network (TGN) in human embryonic stem (hES) cells, enabling rapid apoptosis of DNA-damaged stem cells to potentially avoid the developmental consequences of menacing genetic defects.

To date, a variety of mechanisms have been implicated in BAX regulation. Chief among them is the autoinhibitory structure of BAX itself, which buries the hydrophobic pore-forming surfaces at the core of the protein (Suzuki et al., 2000). Only when triggered by a change in physiologic conditions, such as pH (Khaled et al., 1999) or heat (Pagliari et al., 2005), or directly activated through protein interaction (Gavathiotis et al., 2010), does a major conformational change ensue, moving BAX from cytosol to mitochondria to exert its proapoptotic effect (Figure 1, top). Thus, inherent in the BAX activation mechanism is regulation by subcellular localization (Wolter et al., 1997). An autoactivated isoform of BAX, BAX-β, eliminates this cytosolic step, existing in a tonically mobilized form that constitutively targets the mitochondria (Fu et al., 2009). Once at the mitochondria, activated BAX can be restrained, at least temporarily, through sequestration of its critical death domain by a specialized groove on the surface of antiapoptotic BCL-2 family proteins (Sattler et al., 1997). Antiapoptotic protein shuttling of BAX from mitochondria back to the cytosol or "retrotranslocation" has recently emerged as another BAX-suppressive mechanism (Edlich et al., 2011). In the case of BAX-β, proteosomal degradation is a key mode of negative regulation (Fu et al., 2009). Dumitru et al. (2012) find that, in the uniquely privileged context of embryonic stem cells, BAX has a previously unrecognized subcellular localization and mode of action.

Motivated by elucidating the mechanistic basis for the especially rapid apoptotic response of hES cells to DNA damage, the authors employed shRNA analyses to implicate BAX, rather than BAK, as the driving executioner protein. Surprisingly, BAX was already at least