Molecular Cell
Previews

Hydroxyurea Triggers Cellular Responses that Actively Cause Bacterial Cell Death

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DOI 10.1016/j.molcel.2009.11.027

In this issue of *Molecular Cell*, Davies et al. (2009) work out a sequence of active cellular events that lead to the death of *Escherichia coli* in the presence of the drug hydroxyurea.

Sometimes, we are met with unprecedented challenges and have to rely on our instincts for our decision of how to act. More often than not, this leads to improved results, but occasionally, we worsen our situation by our own attempts to improve it. For example, when sinking in quicksand, it would be advantageous to move very carefully; however, we tend to panic and struggle wildly instead, thus sinking further and actively worsening our situation. Bacteria also face life-threatening situations, for instance, when they are exposed to drugs that threaten their survival. Presumably, billions of years of evolution have "taught" organisms how to respond to such insults to ensure their own survival or at least that of their relatives. Hence, when exposed to attacks, single-celled organisms typically defy their fate by eliciting evolved protective responses. But do they—like humans—sometimes "overreact" and respond in a way that ultimately leads to their own death? In this issue, Graham Walker, Jim Collins, and their colleagues (Davies et al., 2009) show that the bacterium *Escherichia coli* responds to the drug hydroxyurea (HU) in ways that successfully ensure its survival for several hours but promote cell death at longer times.

It was recently shown that the formation of hydroxyl radicals, which can lead to oxidative damage of cell components, plays a key role in the mechanism by which antibiotics cause cell death (Kohanski et al., 2007). For the aminoglycosides—a class of antibiotics that target the bacterial ribosome and lead to mistranslation of proteins—hydroxyl radical formation was shown to be caused by a sequence of cellular events involving a membrane stress response mediated by the *cpxAR* two-component system (Kohanski et al., 2008). Besides hydroxyl radicals, toxin/antitoxin (TA) systems such as *mazEF* are also important for the killing mechanism of antibiotics (Kolodkin-Gal et al., 2008). These TA systems may have been selected for their role in stress response (Gerdes et al., 2005) or as a programmed cell death system, suggesting a scenario in which bacteria altruistically kill themselves under stress to benefit their kin in the surroundings (Kolodkin-Gal et al., 2007).

The drug HU causes a particularly interesting stress because it specifically targets ribonucleotide reductase (RNR), an enzyme with a key role in dNTP synthesis. HU thus depletes cells of the building blocks of DNA, causing DNA replication fork arrest. Like several antibiotics that target DNA replication, HU leads to cell death. The mechanism by which HU causes cell death, however, has been obscure.

Davies et al. (2009) combine genome-wide gene expression measurements in *E. coli* with a genetic approach and measurements of cell physiology to reveal a previously unknown sequence of cellular events that contribute to cell death in the presence of HU. Following HU exposure, cells first elicit a protective response, which includes initiation of the "SOS" DNA damage response (Figure 1). Genetically removing this response renders the cells more sensitive to HU. A further adaptive response is the upregulation of RNR, which presumably compensates for its inhibition, and upregulation of the primosome—a cellular system that may help restart DNA replication forks stalled by HU treatment. Consistent with the view that these early responses form a set of effective countermeasures, Davies et al. (2009) find that virtually all cells survive for the first 2 hr following HU exposure (Figure 1).

However, things quickly go downhill from there: cells start dying rapidly after about 2 hr in the presence of HU (Figure 1). Measuring and manipulating cellular hydroxyl radical levels using the dye 3′-(ρ-hydroxyphenyl)-fluorescein, the scavenger thiourea, and deletion of *ahpC* (which plays a role in reactive oxygen species removal) Davies et al. (2009) show that hydroxyl radical formation is the dominant cause of this increase in cell death and is specifically due to DNA replication fork stalling and not any other potential effect of HU. Moreover, the authors show that deletion of the major respiratory terminal cytochrome oxidase *cydB* prevents cell death, indicating that hydroxyl radical formation under HU results from improper reduction of oxygen in the electron transport chain. Interestingly, at these later times following HU treatment, cells also upregulate iron uptake genes. Because iron plays a role in RNR synthesis, increasing iron uptake may indicate another attempt that the cells make to counter the effects of HU. However, this strategy may be risky because iron can catalyze the formation of hydroxyl radicals via Fenton chemistry (Figure 1). Indeed, the authors show that deleting *tonB*, a key player in iron uptake, strongly increases survival in the presence of HU. Moreover, using an iron-responsive fluorescent reporter construct in a single-cell assay shows that strongly increased iron uptake correlates almost perfectly with cell death. These interesting results indicate that bacteria specifically respond to RNR inhibition in a way that ultimately facilitates their own death.

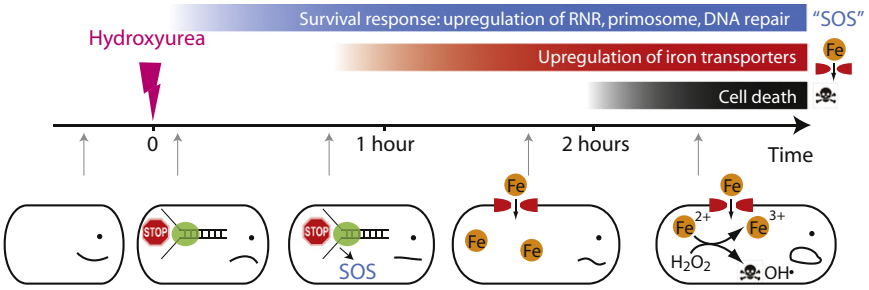

Figure 1. Sequence of Gene Regulation Responses and Cellular Events following Exposure to Hydroxyurea

A reminiscent programmed cell death function was suggested for TA systems such as *mazEF* and *relBE* (Aizenman et al., 1996). How could HU activate TA systems? The concentration of the "alarmone" guanosine tetraphosphate (ppGpp) is known to increase under the drug trimethoprim (Khan and Yamazaki, 1972), which has a mechanism of action similar to that of HU. Importantly, ppGpp represses *mazEF* expression (Aizenman et al., 1996), thus offering a molecular explanation for MazF-toxin activation under HU (Kolodkin-Gal et al., 2008). Davies et al. (2009) confirm that these TA systems play a role in drug-mediated cell killing and propose a novel mechanism that links them to hydroxyl radical formation. Specifically, they use a recently developed tmRNA-based system (Moore and Sauer, 2005) and find that HU leads to toxin-dependent formation of truncated proteins—a plausible scenario, given that toxins like *mazF* were shown to cleave mRNAs (Zhang et al., 2003). Such improperly translated proteins can trigger a membrane stress response mediated by the *cpxAR* two-component system that affects respiratory genes and leads to hydroxyl radical formation (Kohanski et al., 2008). Supporting the relevance of this scenario for HU-mediated cell death, Davies et al. (2009) find that *cpxA*-deletion leads to strongly increased survival.

The work by Davies et al. (2009) identifies specific active cellular behaviors that cause cell death following HU treatment. These results highlight the importance of gene regulation responses and the interplay of multiple cellular systems in the killing mechanism of drugs. A puzzling question raised by the work of Davies et al. (2009) is why these bacteria—which we often think of as "survival machines"—respond to drugs in ways that ultimately facilitate their own death. For TA systems, a scenario has been suggested in which cell death could be beneficial at the population level if some bacteria sacrifice themselves to ensure the survival of their kin (Kolodkin-Gal et al., 2007). It remains unclear whether a similar scenario may also apply to the novel cell death mechanism proposed for HU. A possible alternative explanation could be that the gene regulation responses to HU reflect a strategy that is successful in other conditions but simply misguided under prolonged exposure to HU—reminiscent of our unfortunate friend who is sinking in quicksand and led astray by instincts that were likely evolved for survival when drowning in water. Related work has recently led us to conclude that even tightly controlled key systems such as ribosome synthesis may not be well regulated in the presence of antibiotics (Bollenbach et al., 2009). Laboratory evolution experiments and the synthetic manipulation of bacterial response strategies to drugs will enable us to better understand such seemingly counterproductive microbial responses to drugs.

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