Antiviral death punch by ADP-ribosylating bacterial toxins

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Abstract

Toxin-antitoxin systems can defend bacteria against phages by shutting down infected cells, but the links between their molecular mechanisms and biological functions have remained underexplored. LeRoux *et al.* now show how the DNA-targeting ADP-ribosylation activity of DarTG impairs phage replication but is overcome by dedicated viral inhibitors and evolved tolerance.

Keywords

Toxin-antitoxin system; bacterial immunity; bacteriophage; antiviral defense; ADP-ribosylation

Main Text

Evolutionary ecologists often highlight the mystery of why there is such an exciting diversity of life on Earth and how it might be maintained [1]. Similar questions can be asked about the vast diversity of toxin-antitoxin (TA) systems: While they act by different molecular mechanisms, they are all variations of the same theme that a toxin is controlled by a cognate antitoxin until it is unleashed by some signal or event to shut down cellular physiology [2]. Nevertheless, bacteria like the model organism *Escherichia coli* K-12 encode dozens of different TA systems in their genomes, posing the question "Why so many, what for?" [3]. Diverse biological functions of TA systems have been explored to address this question including the long-known roles of some representatives in bacterial immunity against phages [2]. As an example, the RnlAB and LsoAB systems of *E. coli* inhibit replication of classical phage T4 if it lacks the Dmd "master key" antitoxin to shut them off [4].

LeRoux *et al.* started their project with a search for new TA systems with functions in bacterial immunity to establish a new model for their research on how the biology of TA systems is rooted in their molecular mechanisms [5]. They chose DarTG as a prime candidate because half of all representatives are encoded at genomic "defense islands" close to known immunity systems. Two subfamilies could be distinguished, DarTG1 and DarTG2, which have different antitoxins but share the same toxin, DarT. This protein had previously been shown to ADP-ribosylate DNA which inhibits bacterial growth by interfering with DNA replication [6].

Representative DarTG1 and DarTG2 systems from different *E. coli* indeed protected the *E. coli* K-12 laboratory strain against phage infection, albeit very differently: While DarTG1 targeted RB69, a relative of classic T4 among the "T-even phages", DarTG2 defended against other phages like T5 and SECφ18. As expected, protection depended on the catalytic activity of the

DarT toxin and resulted in detectable ADP-ribosylation of cellular DNA upon phage infection, coincident with a marked inhibition of viral DNA replication [5] (see the scheme in Figure 1).

However, LeRoux *et al.* found that this interference with phage replication did not rescue the infected cells but protected the bacterial population from viral spread. DarTG therefore differs from classic antiviral defenses such as restriction-modification systems that directly destroy foreign DNA and preserve cellular viability. Instead, this observation was reminiscent of abortive infection (Abi) systems that shut down cellular physiology when sensing phage infection [7]. However, unlike some other TA systems DarTG does not protect bacteria as a true Abi system because it targets viral genome replication and not a host process [4,7].

To delve deeper into the molecular details of antiviral defense by DarTG, LeRoux *et al.* studied phage mutants that displayed spontaneous resistance to this immunity system. For RB69, these mutants encoded single amino acid changes in Gp61.2, a small conserved protein of T-even phages, and mutant Gp61.2 specifically interfered with DarTG activity in different assays. These results suggested that Gp61.2 is a dedicated anti-DarTG factor analogous to the Dmd "master key antitoxin" and that the mutations of resistant RB69 clones generated an allele of *gp61.2* with specific activity against DarTG1 [4,5] (Figure 1).

Conversely, the DarTG2-resistant mutants of SECφ18 had mutations in the viral DNA polymerase gene *mga47*, suggesting that the wildtype allele might be a trigger of DarTG2 immunity. However, LeRoux *et al.* excluded this possibility because ectopic expression of *mga47* did not cause any toxicity in *E. coli* with DarTG2. Furthermore, the ADP-ribosylation of DNA in infected cells with DarTG2 was no different for wildtype SECφ18 and the immunity-resistant mutants, ruling out that Mga47 could be an inhibitor of DarTG like Gp61.2. Instead, the authors conclude that the DNA polymerase of DarTG2-resistant SECφ18 clones is altered in a way that retains its functionality despite the ADP-ribosylation of viral DNA (Figure 1).

The study by LeRoux *et al.* is a major breakthrough that shows how the molecular mechanisms of a TA system underly its biological function. However, it remains a clear gap in our understanding of DarTG immunity how infections of sensitive phages would unleash the DarT toxin from control by its cognate antitoxin. Previous work highlighted that the shutdown of host transcription by phage infection could trigger TA systems because toxins are commonly more stable than antitoxins and would thus get passively liberated [4]. However, transcriptional effects alone can inherently not release the toxins from tight protein-protein complexes with their cognate antitoxins [2]. LeRoux *et al.* therefore suggest that DarTG is directly activated by a yet unknown phage-derived trigger [5], e.g., analogous to the CapRel TA system where binding of the viral major capsid protein induces a conformational change that relieves antitoxin inhibition [8]. It will be interesting to see future work exploring this possibility for DarTG. Notably, the observation that DarTG1 and DarTG2 target different phages and are active under different conditions (DarTG1 during fast growth, DarTG2 during slow growth) could even be seen as evidence that they sense different triggers, e.g., via specific interactions with their non-homologous antitoxins (highlighted in Figure 1).

One fundamental explanation how the diversity of life is maintained are divergent niche adaptations that enable the coexistence of even very similar organisms without direct competition [1]. TA systems obviously do not face competitive exclusion like living organisms, but a wide variety of complementary biological functions could very well explain their abundance and diversity. LeRoux *et al.* indeed argue that the vast diversity of toxin functions and possibly also triggering mechanisms might enable different TA systems to target phage infections at different stages and in different ways [5]. TA systems would thus be entangled in a coevolutionary arms race with viral counterstrategies like *gp61.2*, *dmd*, and others that have recently been discovered [9], not unlike the arms race of restriction-modification systems and their specific viral counterstrategies. We anticipate that future studies will unravel the detailed

molecular biology of additional TA systems with functions in phage defense and dedicated viral strategies that subvert their activity. These results will reveal the extent to which a phage-host arms race can reasonably explain the diversity of TA systems in bacterial genomes compared to the implicit null hypothesis that many of them are merely selfish loci without benefit for the bacterium [2,10].

Figure legend

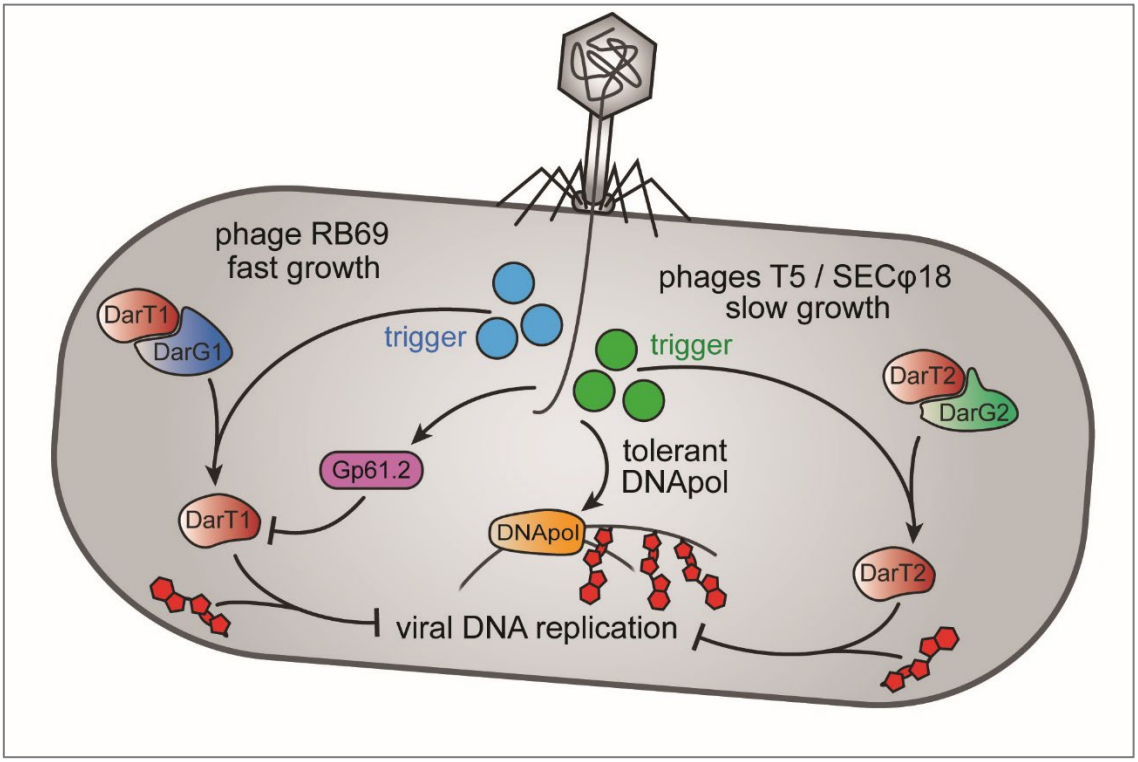

Figure 1: Bacteriophage defense by the DarTG TA system and viral counterstrategies

The schematic shows how DarTG1 (left) and DarTG2 (right) defend bacterial cells against phage infection by ADP-ribosylation (red) of viral DNA under different conditions and how they can be overcome by distinct viral counterstrategies. While DarTG1 is inhibited by Gp61.2, a dedicated anti-immunity factor of T-even phages, the activity of DarTG2 can be bypassed by mutant phages expressing a DNA polymerase that is tolerant to DNA ADP-ribosylation. We highlight the speculated activation of DarTG1 and DarTG2 by different direct viral triggers (blue and green) possibly acting on the non-homologous antitoxins DarG1 and DarG2.

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