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Commentary

The road less traveled: A new phosphorothioate antiviral defense mechanism discovered in Archaea

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The story of phosphorothioate modifications of DNA begins over 50 years ago and has unfolded like Frost's walk in the woods [1]. In 1970, Fritz Eckstein synthesized oligonucleotides in which a non-bridging oxygen in the phosphodiester backbone was replaced by sulfur – the first phosphorothioate-modified nucleic acid [2]. Twenty years passed until Zixin Deng and colleagues discovered a DNA degradation phenotype in bacteria possessing a 5-gene *dnd* cluster, with electrophoretic degradation caused by incorporation of sulfur into bacterial genomes [3]. These roads of chemistry and biology met again another 20 years later with the discovery by Wang et al. that Dnd proteins insert sulfur as a phosphorothioate in the genomes of diverse bacteria [4]. This walk in the woods now picked up speed.

As an epigenetic mark, the earliest functional role for phosphorothioates was identified in restriction-modification "immune surveillance", with modification regulated by Dnd proteins ABCDE and DndFGH performing the restriction cleavage of unmarked foreign DNA [5]. But the *dnd* cluster did not behave like a typical methylation-based restriction-modification system, with modification of all consensus sites in a genome. Advancements in next generation sequencing facilitated genomic mapping of phosphorothioates, which revealed that only ~10–15% of short consensus sequences (e.g., $C_{PS}CA$, $G_{PS}TTC/G_{PS}AAC$) were modified, with frequent partial modification at bistranded sites on the genome [6]. Beyond restriction-modification, new functions have emerged for phosphorothioates in regulating gene expression, cellular metabolism, and oxidative stress response [7,8].

With the walk moving at a rapid pace, the story of phosphorothioate DNA modifications recently took a sharp turn in the discovery by Xiong et al. of a completely new phosphorothioate-based anti-viral defense system in Archaea [9]. Archaeal microbiology is not as well studied as that of bacteria, which led Xiong et al. to explore phosphorothioate modifications on a road less traveled. And this road made all the difference. Using a combination of genomics and mass spectrometry, Xiong et al. initially found phosphorothioate modifications and functional *dnd*CDEA gene homologs in four archaea. It was not surprising that they also found homologs of the archaeal *dnd*C and *dnd*D genes in 2600 bacteria and 42 other archaeal genomes, and that short consensus sequences were only partially modified with phosphorothioates. What was surprising, however, was the discovery of a restriction system completely different than the previously recognized *dnd*F-I genes. The new "phosphorothioate-blocked DNA exclusion" gene cluster, *pbe*ABCD, did indeed restrict infection by bacteriophage lacking phosphorothioate modifications, but, remarkably, the *pbe*ABCD system did not result in the expected DNA cleavage associated with typical restriction-modification systems and caused by *dnd*F-I (Fig. 1) [10]. Instead, PbeABCD functioned to prevent viral DNA replication. While the biochemical mechanism of PbeABCD is a mystery, the fact that *pbe*ABCD genes were found to be widespread among both bacteria and archaea and sometimes combined with *dnd*F-I restriction genes points to an unappreciated complexity of bacteriophage defense mechanisms. By taking the road less traveled, Xiong et al. [9] have changed how we think about bacterial epigenetics. It is exciting to wonder where this new road will lead.

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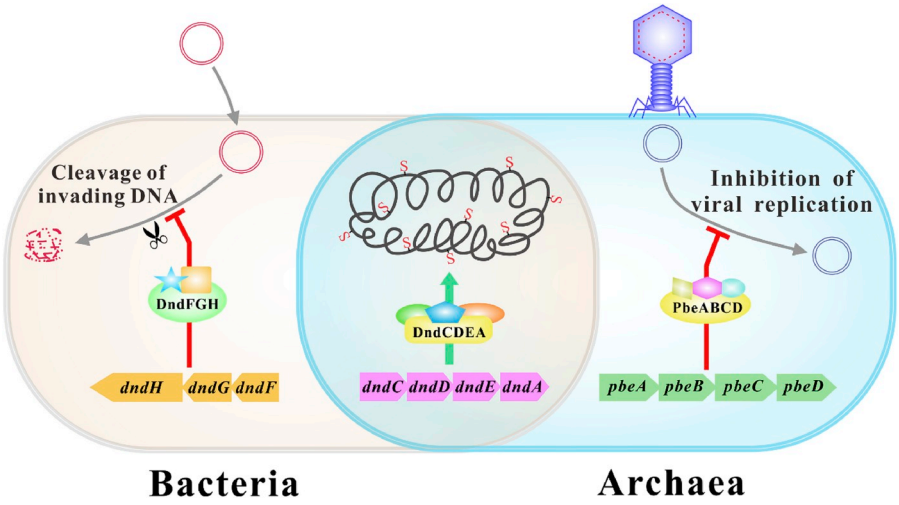

Fig. 1. PT modification and related defense systems in Bacteria and Archaea.

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