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Bacterial Argonaute proteins as sensors of DNA damage and repair

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Prokaryotic Argonautes (pAgos) are programmable nucleases that participate in cell defense against invading genetic elements. They are evolutionary related to eukaryotic Argonautes (eAgos), which act as central components of RNA interference and are involved in silencing of foreign elements and gene regulation. Intriguingly, however, while all eAgos bind small RNA guides to recognize and silence RNA targets, all known pAgos preferentially target DNA. *In vitro,* pAgos can be programmed with small DNA or RNA guides to recognize and cleave complementary DNA targets. We show that nucleotide modifications and lesions in the DNA target can strongly affect the efficiency of its cleavage by pAgos, depending on the type of modification and its position relative to the site of cleavage. This potentially allows the use of pAgos as sensors of site-specific DNA damage. *In vivo*, pAgos preferentially target double-strand DNA ends and breaks and participate in their processing. We show that specific targeting of DNA breaks can be used for detection of DNA damage and repair at the whole-genome level. pAgos also target multicopy DNA elements including plasmids and phages, and can promote homologous recombination between plasmids and the chromosome, thus enabling genome editing in bacteria. Finally, we have discovered a new group of pAgos that use small DNA guides to recognize and cleave RNA targets. We show that these pAgos can sense modifications and lesions in RNA transcripts suggesting that they can find applications in RNA biotechnology.

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