Functional activities of DNA-guided and RNA-guided bacterial Argonaute proteins

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Specific targeting of nucleic acids by Argonaute (Ago) proteins lies at the heart of RNA interference. Eukaryotic Ago's bind small RNAs and use them as guides for target RNA recognition and cleavage. Argonaute proteins are also found in bacterial and archaeal genomes where their roles remain unclear. Structural and biochemical studies of a few prokaryotic Ago proteins showed that they can function as endonucleases *in vitro* and may provide cell defense against foreign genetic elements *in vivo*. However, most prokaryotic Ago's are predicted to lack endonuclease activity; they also often have unusual domain architectures and are associated in the same operons with putative nucleases or helicases. In this study, we focused on prokaryotic Ago's from several cultivable bacterial species. We showed that although eukaryotic Ago's work only with RNA, prokaryotic proteins included in our analysis can use either RNA or DNA guides to recognize DNA targets. To define the specificity of bacterial Ago's we expressed them in *Escherichia coli* and analyzed associated short nucleic acids. Furthermore, we tested nucleolytic activity of three proteins *in vitro* using different guide and target molecules, and also measured the affinity of Ago's to guides and targets. The slicer activity of Ago's is known to depend on four conserved amino acid residues in the catalytic center. We showed that, in agreement with bioinformatic predictions, two of the three proteins possess the endonuclease activity. Our results indicate that bacterial Ago proteins can cleave target nucleic acids with high specificity *in vitro* and can function in the heterologous *E. coli* system *in vivo*. These properties likely underlie the process of DNA/RNA interference in bacterial cells and may promote horizontal transfer of the Ago genes. This work was supported by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.