Catalytically active Argonaute nuclease from Synechococcus elongatus

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Argonaute proteins, which are found in almost all eukaryotes and in many prokaryotes, use small nucleic acid guides for the recognition and cleavage of complementary nucleic acids. While the role of eukaryotic Argonautes in RNA interference is well understood, the functions of prokaryotic Argonautes remain largely unknown. It was proposed that they may provide defense against invading nucleic acids, preferably acting on DNA targets. In this work, we studied the SynAgo protein from the cyanobacterium *Synechococcus elongatus*. We expressed affinity-tagged SynAgo in *S. elongatus*, purified the protein, and sequenced and mapped associated nucleic acids. We showed that SynAgo is bound with ~18 nt small DNAs coming from all genomic regions with no obvious gene specificity. Mass-spectrometry of co-purified proteins from *S. elongatus* also revealed several possible protein partners of SynAgo. Biochemical analysis demonstrated that SynAgo is an active nuclease that can cleave both target DNA and RNA with varying efficiency, depending on the reaction conditions and the presence of mismatches between the guide and target strands. Finally, we introduced the SynAgo gene in the *E. coli* genome and tested its effects on plasmid maintenance and phage infections. This work was supported by the grant 18-29-07086 of the Russian Foundation for Basic Research.