Catalytically active Argonaute proteins from mesophilic bacteria

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Argonaute proteins are an integral part of eukaryotic RNA interference machinery. They bind small noncoding RNAs and utilize them for guided cleavage of complementary RNA targets or indirect gene silencing by recruiting additional factors. Argonaute proteins are also encoded in many bacterial and archaeal genomes (pAgos). pAgos from thermophilic bacteria were initially studied to gain structural insight into eukaryotic RNA interference. They were later shown to cleave DNA substrates in a guided manner employing small RNAs or DNAs, which appear to be generated autonomously by pAgos. Thus, pAgos might be considered as means of prokaryotic defense against invasive genetic elements. Here we characterize pAgos from non-cultivable or pathogenic mesophilic bacteria. Candidate proteins were selected through bioinformatic screening of genomic databases. Corresponding pAgo genes were chemically synthesized and used for expression in a heterologous system. Upon expression in *E. coli* these proteins have been shown to associate with short (14-25 nt) 5'-phosphorylated DNA molecules. Such short DNA loading relies on the catalytic activity of pAgos and is abolished in catalytically dead protein variants, which bear amino acid substitutions in the DEDX catalytic tetrad. Further *in vitro* assays have shown that purified pAgos cleave various DNA substrates in a guide-dependent manner. They display high activity at temperatures ranging from 30 to 45 °C, with the efficiency of cleavage being greatly affected by ionic strength, supplied divalent cations and guide molecules. This suggests that all studied pAgos act as DNA-dependent DNA nucleases which may subsequently be used as means of targeted genome editing in eukaryotic organisms. This work was supported in part by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.