A programmable pAgo nuclease with universal guide and target specificity from the mesophilic bacterium *Kurthia massiliensis*

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Prokaryotic Argonaute (pAgo) proteins were proven to have the ability to specifically target and cleave DNA and RNA. Although the majority of previous studies focused on thermophilic bacteria pAgos, mesophilic-derived pAgos are active under moderate temperatures, thus a good target for pAgo-based applications. In this context, this study aims to characterize a mesophilic pAgo from *Kurthia massiliensis* (KmAgo) with relaxed specificity towards nucleic acids.

The main results of the study show that KmAgo, synthesized and purified from heterologous expression in *E. coli*, is a guide-directed endonuclease; it preferentially binds 16-20 nt long, 5'-phosphorylated nucleic acids guide with no strict sequence specificity and is active in a wide range of temperatures. Following DNAse and RNAse treatment of extracted KmAgo-associated nucleic acids, the authors claim that it is associated with small DNA (smDNA) guides, *in vivo.* However, using a set of synthetic guide and target oligonucleotides, *in vitro* experiments showed that it acts with all nucleic acids combinations, displaying highest activity for DNA-guided DNA cleavage. Moreover, plasmid cleavage activity with empty KmAgo or loaded with (one or two) DNA guides, showed that the presence of two guides enables it to introduce double-stranded breaks only at high temperatures. Noteworthy, they demonstrated that KmAgo RNA processing activity depends on the RNA structure as it only cleaves single-stranded RNA.

In conclusion, this study showed that KmAgo is the first argonaute with broad specificity to guide and target DNA/RNA with variable efficiency rates modulated by temperature. These findings open the possibility to use KmAgo as a flexible tool for biotechnology applications, such as genome editing.

In this study it is not clear what was the evidence to look for this particular pAgo. The results support the proposed claims to a certain extent but not fully convincingly. Although the *in vitro* results are promising, the ultimate goal of such a study should be the validation of the *in vivo* application which is missing. Also, the toxic effect on *E. coli* could compromise its *in vivo* application. Further studies are necessary to reveal the full potential of the KmAgo application in RNA-centric studies.

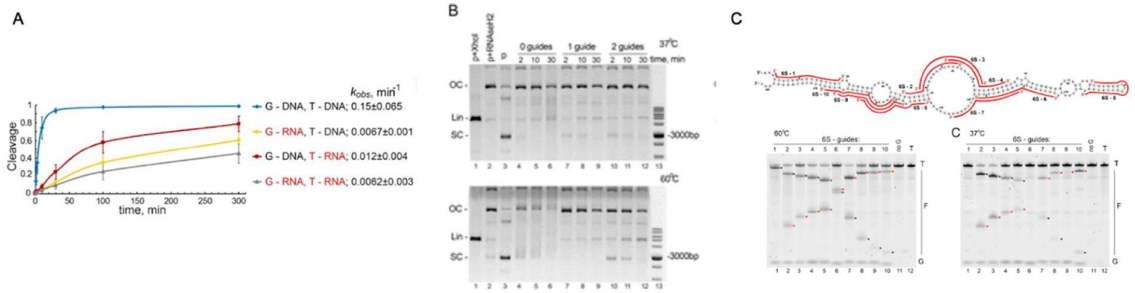

Figure. A. Guide and target specificity of KmAgo *in vitro*. Kinetics of nucleic acid cleavage by KmAgo measured with radiolabeled target DNA or RNA. **B. Plasmid DNA cleavage by KmAgo.** The target plasmid was incubated with empty KmAgo (lanes 4-6) or KmAgo loaded with one or two guide DNAs (gS0 and gAS0; lanes 7-12) at 37 °C (top) or 60 °C (bottom) for indicated time intervals. Control samples containing linear plasmid obtained by treatment with XhoI (lane 1), relaxed plasmid obtained by treatment with RNase H2 (lane 2), and supercoiled plasmid (lane 3) were incubated in the absence of KmAgo. **C. RNA probing with KmAgo.** (up) Secondary structure of 6S RNA used in the experiments. Positions of small guide DNA loaded into KmAgo are shown with red lines. Positions of the expected cleavage sites for each guide smDNA are indicated with arrowheads. (bottom) Analysis of the cleavage products obtained after incubation of KmAgo with 6S RNA for 30 min at 60 °C and 37 °C, respectively. Positions of the 5'-terminal and 3'-terminal cleavage fragments are indicated with red and black asterisks, respectively.