

BIOCHEMICAL CHARACTERIZATION OF ARGONAUTE PROTEINS XAUSPARDA AND EAESPARDA CONTAINING NUCLEASE EFFECTOR DOMAIN

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Prokaryotic Argonautes (pAgos) are divided into long and short Agos. The majority (60%) are short pAgos containing only MID and catalytically inactive PIWI domains. Short pAgos have a guide-mediated target recognition function, while various effector domains (Sir2, TIR, DREN, fig. 1) of their associated APAZ proteins execute enzymatic reactions to kill the host cell, thus, preventing spread of the invader [1-4]. It has been demonstrated that short prokaryotic Argonaute associated with DNase/RNase effector nuclease (DREN-APAZ) (SPARDA) proteins using RNA guides (gRNA) recognize their DNA targets (tDNA), leading to nonspecific collateral cleavage of DNA and RNA [4-5]. However, the detailed SPARDA activation mechanism remains unknown. Here we present the in vitro study of previously uncharacterized SPARDA systems. Firstly, we determined the specificity of XauSPARDA and EaeSPARDA for gRNA and tDNA with a dsDNA substrate. Then we analysed collateral cleavage activity of XauSPARDA and EaeSPARDA with various substrates. Next, we purified 4 different active site mutants for XauSPARDA and measured their DNase activity. Finally, by varying different bases in guide RNA and target DNA sequences we determined key base pair positions responsible for activating each protein's enzymatic activity.

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