# Interactions of a bacterial Argonaute protein with DNA targets in vitro

P.18-108-Wed

**L. Lisitskaya**[I], I. Petushkov[I], D. Esyunina[I], A. Aravin [II], A. Kulbachinskiy[I]

*[I]Institute of Molecular Genetics, Moscow, Russia, [II]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, United States of America*

Argonaute proteins are central components of RNA interference in eukaryotes but the functions of homologous proteins in prokaryotes remain largely unknown. *Rhodobacter sphaeroides* Argonaute protein (RsAgo) was shown to preferentially recognize foreign genetic elements *in vivo* suggesting its role in RNA interference in bacterial cells. RsAgo was proposed to use guide RNAs to recognize complementary target DNA, leading to inhibition of transcription and also its nucleolytic cleavage by accessory nucleases. However, the mechanisms of specific DNA targeting by RsAgo and, in particular, the details of its interactions with double-stranded DNA molecules are unknown. In the present study, we analyzed the interactions of guide-loaded RsAgo with dsDNA targets *in vitro*. Using the gel shift assay, we showed that successful loading of RsAgo onto dsDNA requires prior DNA melting. The boundaries of the assembled ternary complex of RsAgo with guide RNA and dsDNA were revealed by footprinting methods. Possible interactions of RsAgo with RNA polymerases of *Escherichia coli* and *R. sphaeroides* were tested using the bacterial two-hybrid system, and the domains of the β- and β′-subunits of RNA polymerase that are likely involved in interactions with RsAgo were identified. The results suggest that recognition of dsDNA targets by RsAgo *in vivo* may be facilitated by DNA replication and/or transcription, a hypothesis that is now under investigation. This work was supported in part by the Grant of the Ministry of Education and Science of Russian Federation 14.W03.31.0007.