A SECRET ALLIANCE BETWEEN SPLIT PROKARYOTIC ARGONAUTE PROTEINS AND TYPE I RESTRICTION - MODIFICATION SYSTEMS

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The ability to discriminate host DNA from foreign genetic material at the molecular level is fundamental to bacterial survival and evolution. The bacterial defense forces against genetic elements of exogenous origin are frequently clustered within genomic regions known as defense islands. This pattern suggests that genes of unknown function, which are consistently found near established defense systems, likely serve protective roles or may work synergistically with adjacent systems [1], [2]. Three split prokaryotic Argonaute (pAgo) operons have been identified in the genomes of thermophilic bacteria. Unpublished bioinformatic analyses from our laboratory indicate that these split pAgos, together with associated HEPN domain-containing nucleases, are embedded within operons encoding type I restriction-modification (RM) systems (Fig.1). This genomic co-localization suggests potential cooperation between these defense pathways.

The objective of this study is to investigate whether split pAgos and their associated type I RM systems function cooperatively to enhance bacterial defense against exogenous genetic elements *in vivo*. To model this alliance, genetically modified *Escherichia coli* strains have been constructed using Argonaute-mediated homologous recombination to integrate relevant defense modules into the genome [3]. The further experimental workflow includes the assessment of cytotoxic effects of individual components and their combinations, followed by functional assays to evaluate protection against plasmid acquisition and bacteriophage infection.

Understanding such alliances may provide insight into how bacteria deploy layered immune strategies to limit the constant influx of invading genetic elements.

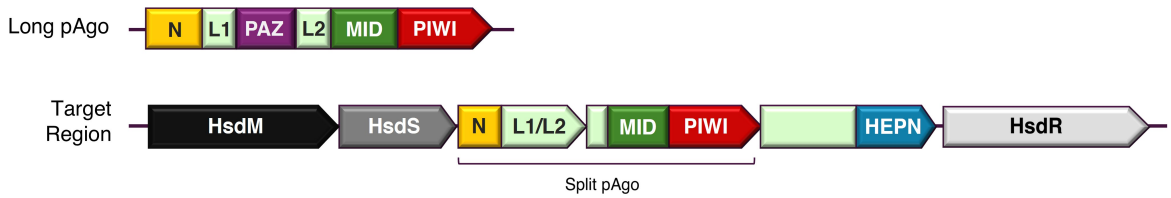

Fig. 1. Schematic representation of the genomic region encoding the target defense systems. For comparison, the domain architecture of a long pAgo is shown.

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