RESEARCH HIGHLIGHT

Filament assembly powers *Nba*SPARDA in bacterial defense

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The SPARDA system, a short Argonaute-based bacterial defense mechanism, employs guide RNA to recognize invading complementary DNA and degrade both invader and the host genome, leading to cell death or dormancy. A recent paper published in *Cell Research* elucidated the activation mechanism of *Novosphingopyxis baekryungensis Nba*SPARDA by capturing cryo-EM snapshots, demonstrating that guide RNA–target DNA heteroduplex formation triggers filament assembly and subsequent activation of collateral nuclease activity on environmental nucleic acids.

Argonaute proteins (Agos) are evolutionarily conserved across all domains of life and function by employing small oligonucleotides (typically 15–30 nucleotides) as guides to recognize complementary nucleic acid targets. Eukaryotic Agos (eAgos) mediate RNA-guided RNA cleavage, playing essential roles in RNA interference and gene expression regulation. Prokaryotic Agos (pAgos), which can be further classified into long pAgos and short pAgos based on domain architecture, utilize RNA or DNA guide to provide innate immunity against invading genetic elements. Long pAgos exhibit structural similarity to eAgos, containing N, PIWI (P-element Induced Wimpy Testis), MID (middle), and PAZ (PIWI-Argonaute-Zwille) domains. In contrast, short pAgos contain only the MID and PIWI domains required for guide recognition. Notably, their PIWI domains lack the DEDX catalytic tetrad, abolishing the nuclease activity. Instead, short pAgos typically associate with effector proteins that contain an APAZ (analog of PAZ) domain, along with diverse enzymatic domains, to mediate immune defense through abortive infection or suicide mechanisms. Based on their phylogeny, short pAgos can be further classified into four clades including S1A, S1B, S2A, and S2B.[1] In clades S1A and S1B, the APAZ domain is fused to a Sir2 (Silent information regulator 2, also known as Sirtuin) domain, known as SPARSA (Short Prokaryotic Argonaute SIR2-APAZ) systems. In clade S2A, the APAZ domain is fused to a TIR (toll/interleukin-1 receptor/resistance protein) domain, known as SPARTA (Short Prokaryotic Argonaute TIR-APAZ) systems. Both SPARTA and SPARSA systems employ short pAgos associated with NADase-containing effectors, which hydrolyze nicotinamide adenine dinucleotide (NAD^+) upon RNA-guided recognition of target DNA (tDNA), thereby leading to cell death to confer population-based immunity.[1–3] The S2B clade exhibits greater domain diversity, with the APAZ domain fused to a variety of effector domains (Schlafen/Alba, Mrr-like, DUF4365/DREN, RecG/DHS-like, or HNH-like), and can be further classified into 9 subclades, designated S2B-1 through S2B-9.[1,4]

DUF4365, recently designated as DREN (DNA and RNA effector nuclease), belongs to the PD-(D/E)XK superfamily of metal-dependent nucleases and is the most abundant effector domain in clade S2B.[5] Nuclease domain-containing short pAgo systems, including DREN, Mrr, and HNH, are referred to as SPARDA (Short Prokaryotic Argonaute, DNase and RNase associated), and mediate the degradation of both invader and the host genome, eventually inducing cell death.[4,6,7] Recent studies show that *Novosphingopyxis baekryungensis Nba*SPARDA, composed of *Nba*Ago and DREN-APAZ, preferentially binds 16–24-nt guide RNAs (gRNAs) carrying either 5′-phosphate or 5′-hydroxyl groups and featuring 5′-AU/5′-AC motifs.[4] *Nba*SPARDA is activated upon recognition of complementary target single-stranded DNA (ssDNA) and exhibits Mg^{2+}- or Mn^{2+}-dependent collateral nuclease activity against a wide range of substrates, including ssDNA, double-stranded DNA (dsDNA), plasmid DNA, single-stranded RNA (ssRNA) and RNA–DNA hybrids. Using fluorophore-quenched ssDNA or dsDNA reporters as substrates for collateral activity, *Nba*SPARDA enables nucleic acid detection with a sensitivity of 150 pM without sample pre-amplification, and as low as ~30 molecules when combined with pre-amplification and exonuclease digestion of the non-target strand.[4,7]

Wang et al.[8] characterized the biochemical properties of *Nba*SPARDA and elucidated its filament assembly and activation mechanism by determining a series of cryo-EM structures in five different functional states. *Nba*Ago comprises the MID and PIWI domains, while DREN-APAZ includes the DREN, a bridging loop, and APAZ domains. In the absence of gRNA, apo *Nba*SPARDA exists as a heterodimer formed by *Nba*Ago and DREN-APAZ (Fig. 1a). Upon loading with a 21-nt gRNA, *Nba*SPARDA undergoes dimerization through the formation of APAZ–APAZ and Ago–Ago interfaces (Fig. 1b). The bound gRNA is highly flexible, with only the first five nucleotides stably resolved in the structure. The MID domain forms extensive hydrophilic interactions with the gRNA, enabling specific recognition of the 5′-phosphate group and the 5′-AU motif. Additionally, the MID and PIWI domains form hydrogen bonds with the 2′-hydroxyl groups of nucleotides at positions 2–4, thereby accounting for RNA guides over DNA.

A gRNA–tDNA heteroduplex of sufficient length and perfect central complementarity is essential for filament assembly and nuclease activation. When a short 13-nt ssDNA is loaded, the dimeric *Nba*SPARDA complex dissociates into monomers, as the dimeric complex can only structurally accommodate heteroduplexes shorter than 8 bp (Fig. 1c). Upon loading of a 21-nt ssDNA, *Nba*SPARDA is activated and assembles into a filament composed of an outer backbone and an inner layer, indicating a monomer-to-filament transition driven by the propagation of the guide–target heteroduplex (Fig. 1d). *Nba*Ago forms the backbone,

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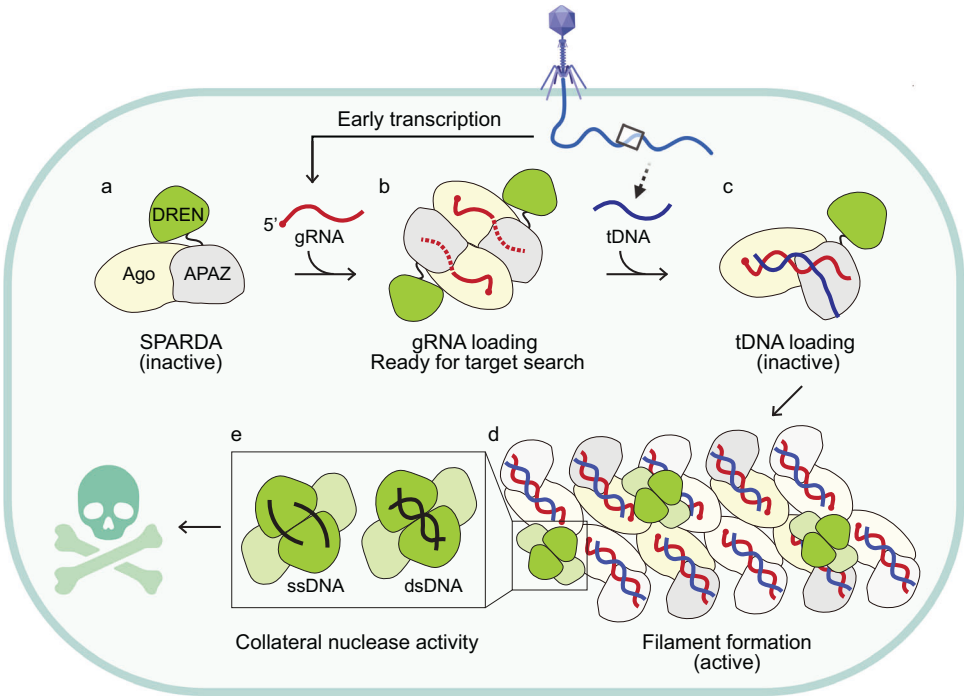

Fig. 1 RNA-guided, tDNA-dependent nuclease activation mechanism in *Nba*SPARDA systems. a *Nba*Ago and APAZ-DREN form a heterodimer in the apo state. **b** Upon gRNA loading, *Nba*SPARDA undergoes dimerization. **c** Target recognition of complementary invading ssDNA leads to dissociation of the *Nba*SPARDA dimer into monomers. **d** Extension of the guide–target heteroduplex triggers the assembly of *Nba*SPARDA into a helical filament, accompanied by DREN domain tetramerization required for activation. **e** In the tetrameric DREN assembly, two DREN domains cooperatively stabilize substrate ssDNA or dsDNA, enabling non-specific collateral cleavage. This widespread nuclease activity results in degradation of both invader and host genomes, ultimately leading to cell death.

with the APAZ domains and guide–target heteroduplexes arranged along the outer surface to anchor the filament structure. This structural arrangement positions the DREN domains within the filament core, facilitating their tetramerization in the inner layer and thereby activating their nuclease activities. Notably, the bridging loop is positioned between the DREN and APAZ domains, and is essential for nuclease activity. Within the tetrameric DREN domains, two catalytic pockets (DREN.2 and DREN.3) face towards the filament, while the other two (DREN.1 and DREN.4) are oriented towards the solvent and together stabilize two substrate ssDNA molecules (Fig. 1e), underscoring the requirement of tetramerization for DREN activation.

Collectively, Wang et al. characterize detailed structural and functional dynamics of the *Nba*SPARDA defense system, revealing an RNA-guided, DNA-dependent "monomer-dimer-monomer-filament" transition accompanied by large-scale domain rearrangements during gRNA loading, tDNA engagement, and the guide–target heteroduplex propagation. Notably, a homologous system from *Xanthobacter autotrophicus* Py2 (*Xau*SPARDA, clade S2B) also forms helical filaments upon gRNA and tDNA loading.[9] Its DREN domains assemble into a tetramer, with two domains engaging substrate dsDNA[9] (Fig. 1e). Similar filament assembly has been observed in *Enhydrobacter aerosaccus Eae*SPARDA and *Thermocrispum municipal Tmu*SPARDA,[9] suggesting a conserved activation mechanism across SPARDA systems. Supramolecular assemblies are increasingly recognized in bacterial immunity,[10] resembling immune complexes in plants and animals. Yet, their evolutionary origins, the in-cell benefits of filament formation in native hosts, and the reversibility of these structures after infection remain unclear. Deeper investigation into these supramolecular assemblies could inform future therapeutic and biotechnological advances.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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