

Review

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Structural biology of retron-mediated immunity against phage

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Abstract: Retrons recently emerged as widespread anti-phage systems in which a dedicated reverse transcriptase (RT) produces multicopy single-stranded DNA (msDNA) that regulates associated effector proteins. Once regarded as peculiar retroelements of unknown function, retrons are now understood to operate as modular defense platforms in which the RT–msDNA module maintains nucleases, hydrolases or other effector domains in an inactive state until a phage-trigger perturbs the complex. Current biochemical and cryo-EM structural studies across different retron types have begun to define a unifying mechanism for retron-mediated immunity. Despite substantial variation in architecture and effector types, these studies converge on a common principle: msDNA acts as a structural and functional hub that scaffolds multimeric assemblies, occludes catalytic sites or stabilizes inactive effector conformations. Phage infection is sensed through perturbation of the msDNA, including cleavage, modification or binding by phage-encoded factors, thereby activating the effector to trigger an immune response. Here, we discuss recent structural and mechanistic advances of retron immune systems. We detail how distinct retron types deploy different effectors and phage-factor-sensing strategies while preserving a shared msDNA-centered regulatory paradigm. We further place retrons in the broader landscape of bacterial defense, comparing their regulatory logic with that of toxin–antitoxin modules and other immune systems.

Keywords: bacterial immune system; cryo-EM; msDNA; phage; retron; reverse transcriptase

1 Introduction

Retrons were discovered several decades ago, yet for much of their history they remained among the most enigmatic genetic elements in bacteria. Early studies identified retrons by their ability to produce multicopy single-stranded DNA molecules, abbreviated msDNA, by reverse transcription of a structured non-coding (nc)RNA precursor (Dhundale et al. 1985; Lampson et al. 2005; Yee et al. 1984). Subsequent work repurposed retrons for genome engineering applications, underscoring their utility for biotechnology (Buffington et al. 2025; Cattle et al. 2025; Fishman et al. 2025; González-Delgado et al. 2024; Khan et al. 2025; Lim et al. 2020; Lopez et al. 2022; Schubert et al. 2021; Simon et al. 2019). Only recently was the long-standing mystery of retron function clarified by the demonstration that retrons act in anti-phage defense (Bobonis et al. 2022; Gao et al. 2020; Millman et al. 2020).

Although possible links between retrons and anti-phage activity had been proposed earlier (Rychlik et al. 2001), their general defensive role was only conclusively demonstrated in the context of bacterial immune system discovery pipelines (Gao et al. 2020; Millman et al. 2020). Since then, a rapidly growing body of structural, biochemical and genetic studies has begun to unravel the mechanisms of how retrons detect infection of the cell and convert phage-derived signals into an immune response (Dai et al. 2026).

Retrons are typically composed of three functional elements: a reverse transcriptase (*ret*, RT), a ncRNA that serves as the precursor of the msDNA (Inouye and Inouye 1992), and one or more accessory effector genes (Gao et al. 2020; Lampson et al. 2005; Mestre et al. 2020; Millman et al. 2020; Rychlik et al. 2001; Sun et al. 1991). In many bacterial genomes, retrons are often associated with prophages or present on other mobile genetic elements (Dodd and Egan 1996; Lampson et al. 2005), consistent with their participation in host–phage conflict and frequent horizontal transfer (Paul and Eren 2022; Rocha and Bikard 2022). According to RT phylogeny and associated effector composition, retron systems are currently classified into 13 types (I–XIII) and additional subtypes (Mestre et al. 2020).

Their genetic operon organization is broadly conserved in that the ncRNA is usually encoded upstream of the *ret*

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gene, whereas the effector gene may lie either upstream or downstream of the *ncRNA-ret* unit, or in some cases is fused directly to *ret* (Figure 1A). This arrangement suggests a conserved mode of retron system expression and effector complex biogenesis.

A unifying mechanistic model of retron function is now emerging, despite extensive sequence and architectural divergence across retron types. In this model, the RT synthesizes an msDNA molecule that remains stably associated with the RT and serves as both scaffold and sensor (Figure 1B). The msDNA promotes assembly of a phage-surveillance complex and simultaneously suppresses the activity of an immune effector. Phage infection perturbs this inhibited state through factors that act on the msDNA, thereby releasing or activating the effector and triggering a response that may result in abortive infection or another defensive outcome (Bernheim and Sorek 2020; Lopatina et al. 2020; Millman et al. 2020). Although architectures and activities differ between systems, the msDNA-centered regulatory logic appears to be broadly conserved.

In this review, we first discuss retron biogenesis, including gene expression, msDNA synthesis and maturation, before examining the architectures of retron complexes and the molecular principles by which the msDNA regulates diverse effector types. Finally, we present phage anti-defense strategies and discuss unresolved questions.

2 Retron biogenesis

Retron biogenesis can be viewed as a multistep process encompassing gene expression, msDNA synthesis and

maturation, as well as assembly of the immune complex (Figure 1B). Although these stages are conceptually separable, they are likely coordinated *in vivo* and comparable to other complex formation processes within bacterial cells (Williams and Dichtl 2018). The retron operon must first be transcribed and translated, yielding the RT, the effector protein or proteins, and the ncRNA. The RT then recognizes the ncRNA and catalyses reverse transcription from a conserved “branching G” to generate the msDNA, which undergoes additional maturation steps, including template removal and, in some systems, further processing. Finally, the RT-bound mature msDNA assembles with the effector proteins to form the retron immune complex, which entails oligomerization and often structural confinement of the effectors for maintenance of a regulated and immune-competent surveillance state.

2.1 Retron gene expression

Despite increasing mechanistic insight into retron function, surprisingly little is known about the regulation of retron expression in their native bacterial hosts (Lampson et al. 2001). Quantitative data are sparse, and it remains unclear under which environmental or physiological conditions retron loci are transcribed. Traditional identification of retron-containing strains often relied on detection of abundant msDNA, which can accumulate to hundreds of copies per cell (Dhundale et al. 1985; Khan et al. 2025; Nakamura et al. 2025; Yee et al. 1984). This observation suggests either that some retrons are constitutively expressed or that standard laboratory growth conditions coincide with states permissive for retron expression. msDNA-loaded retron

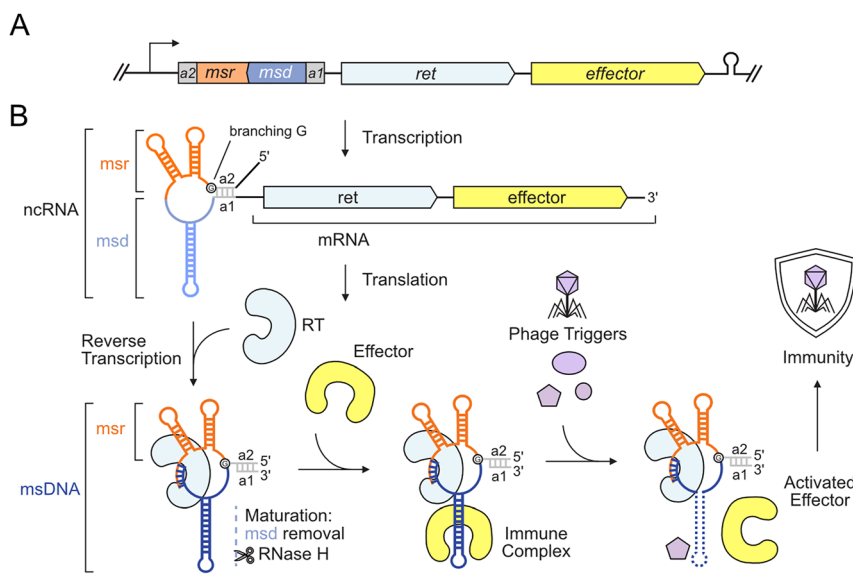


Figure 1: Retron locus architecture and defense mechanism. (A) Schematic of the retron operon illustrating a common gene arrangement, with the *ncRNA* (*a2-msr-msd-a1*) located upstream of the RT-encoding *ret* (light blue) and *effector* (yellow) genes. (B) Schematic illustrating retron biogenesis and defense. Note that the mature msDNA (dark blue stem-loop) typically retains the covalently linked *msr* segment (orange stem-loops) and *a2/a1* stem (grey duplex) of the non-coding msRNA after reverse transcription.

complexes might also be unusually stable, such that high abundance does not necessarily reflect high levels of transcription. It is possible that expression in their native host may be regulated as a trade-off between immune readiness, metabolic burden and self-toxicity, but this remains poorly understood relative to other bacterial immune systems (Vander Zee et al. 2026). A study on retron Ec107 suggested growth-phase-dependent expression patterns in *Escherichia coli*, involving induction in the stationary phase during nutrient starvation (Herzer 1996), but a generalizable model has not yet emerged.

Given the retron operon architecture (Figure 1A), transcription produces a single polycistronic RNA that serves two distinct purposes: it encodes the RT and effector proteins as mRNA, and it contains the ncRNA precursor of the msDNA (Figure 1B). Thus, retron expression inherently couples production of the protein components with synthesis of the regulatory msDNA scaffold. How precisely this transcript is utilized for both translation and msDNA production remains unresolved. Notably, a study on retron Eco2 revealed that the ribosomal protein S1 associates with the msDNA-template region (msd) within the ncRNA (Jasnauskaitė et al. 2026). S1 is a multifunctional factor involved in RNA chaperoning and translation initiation (Duval et al. 2013; Qu et al. 2012; Sørensen et al. 1998; Webster et al. 2024), suggesting that retron protein production may be linked to msDNA formation by an as-yet-unknown mechanism. Once translated, the RT must specifically recognize the structured ncRNA and initiate msDNA synthesis.

2.2 RT structure and the mechanism of msDNA synthesis

Retron-associated RTs belong to the broader reverse transcriptase superfamily and share the canonical polymerase right-hand architecture encompassing palm, fingers and thumb subdomains (Lampson et al. 2001, 2005; Mestre et al. 2020; Simon et al. 2019; Steitz 1999; Wang et al. 2022b) (Figure 2A). The palm contains the RT active site, the fingers mediate nucleotide substrate recruitment, and the thumb engages the RNA-DNA hybrid during synthesis. Of note, segments of the fingers subdomain are occasionally attributed to the palm subdomain (Wang et al. 2022b and referencing literature). Although structural information is currently limited to terminated and matured product-bound states, a mechanistic view of msDNA synthesis already emerged from early biochemical studies (reviewed in Lampson et al. 2001), and is now supported by structural analyses.

2.2.1 Reverse transcription – initiation

To initiate reverse transcription, the RT recognizes the folded ncRNA and primes DNA synthesis from a conserved branching guanosine residue (Hsu et al. 1989; Shimamoto et al. 1995a) (Figure 2B). In this reaction, the first deoxynucleotide is linked to the 2'-OH of the ribose, generating the characteristic branching 2'-5' phosphodiester bond that distinguishes msDNA from linear DNA products. The ncRNA is typically described as comprising four segments: the structured msr (multicopy single-stranded RNA) region, the msd (msDNA RNA) region that serves as the DNA template, and the complementary a1 and a2 sequences, which are derived from inverted repeats within the genomic locus (Lampson et al. 2001). These elements form a characteristic secondary structure in which a1 and a2 pair up to form a stem, while the msr and msd regions fold into multiple additional stem-loop structures (Lampson et al. 2001) (Figure 2B). Although the precise secondary structure topology varies among retrons (Lampson et al. 2001), a conserved fold provides the structural launch pad for RT recognition, priming and subsequent reverse transcription.

Direct structural information on the priming state is still lacking and AlphaFold 3 predictions remain uncertain for most nucleic acid structures (Abramson et al. 2024). Nevertheless, inferences can be drawn from mature retron complexes. In all currently available structures, an msRNA hairpin located downstream of a2 engages the thumb domain of the RT (Carabias et al. 2024; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li et al. 2025; Wang et al. 2022b, 2024; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025), consistent with previous biochemical evidence (Inouye et al. 1999; Shimamoto et al. 1993). This interaction may anchor the ncRNA in an orientation that facilitates placement of the branching G into the active site (Inouye et al. 1999). Because the branching G lies near the base of the a1-a2 stem, this geometry positions the template-containing msd region into the active site for initiation of reverse transcription. In support of this hypothetical priming mode, AlphaFold 3 models of the Eco Forc82 (type I-A) RT and ncRNA suggest that the thumb-associated ncRNA stem-loop may indeed provide a handle to feed the branching G and msdRNA template into the RT active site (George et al. 2025) (Figure 2C). Thus, the branching G may be initially positioned near the conserved and retron RT-specific VTG and NAXXH motifs, as well as the universally conserved Mg^{2+} -cofactor-coordinating YADD motif (George et al. 2025; Inouye et al. 1999; Steitz 1999). It further suggests that the a1-a2 stem may be recruited to the primer binding site at the thumb to position the branching G. In this configuration, the fingers

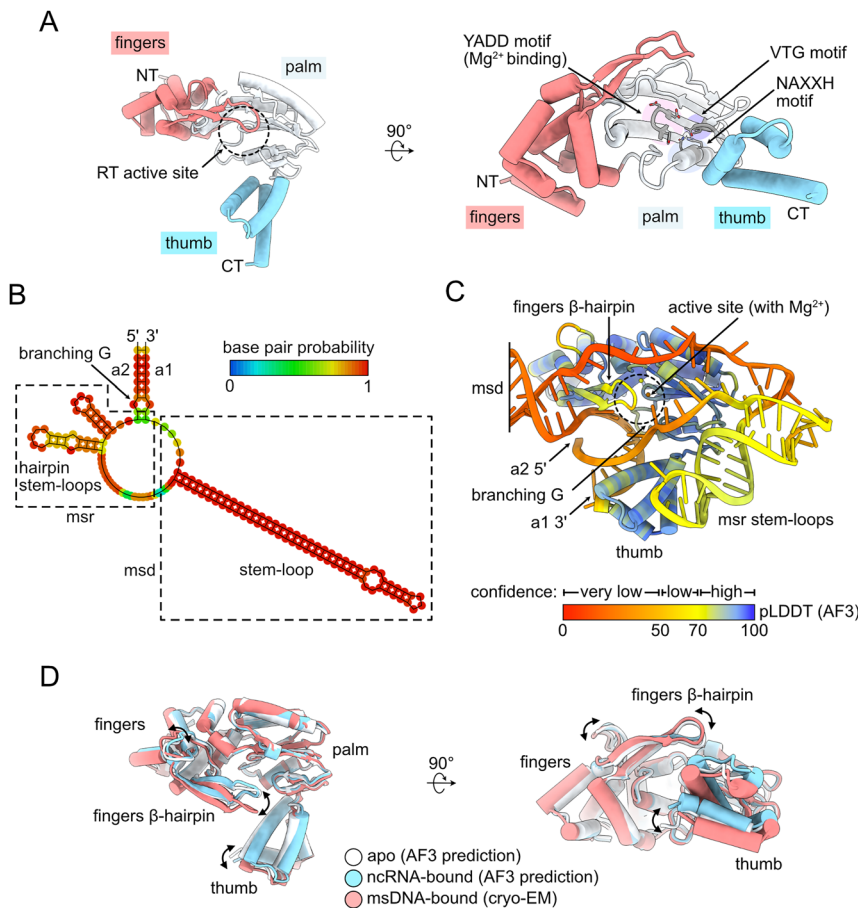


Figure 2: Retron reverse transcriptase. (A) Experimental cryo-EM structure of the *E. coli* Eco1 RT shown in two orientations rotated by 90° (PDB 7V9U; Wang et al. 2022b); RT subdomains are colored and conserved motifs are highlighted. (B) Exemplary ncRNA schematic illustrating conserved segments (labels) and base pairing probabilities (color scale; RNAfold WebServer) of the *E. coli* Forc82 ncRNA. (C) AlphaFold 3 prediction of the ncRNA-bound *E. coli* Forc82 RT, colored according to pLDDT confidence (multicolor scale). (D) Superposition of predicted and experimental Forc82 RT structures in two orientations rotated by 90°, colored according to the legend. Arrows highlight differences in subdomain conformations.

subdomain may assist in msdRNA recruitment, with the fingers β-hairpin motif potentially acting as a wedge to assist in unwinding of the template (Figure 2C).

It is evident that priming requires conformational rearrangements within both the RNA template and the RT itself. Consistent with the idea of a highly dynamic protein, hydrogen-deuterium exchange mass spectrometry has revealed substantial conformational dynamics across the RT domain of retron Eco2, when compared in the apo and msDNA-bound states (Jasnauskaitė et al. 2026). Comparison of AlphaFold 3-predicted conformations of Eco Forc82 apo RT, RT-ncRNA and the experimentally determined mature RT-msDNA state suggests that both the thumb subdomain and fingers β-hairpin motif may assume different conformations, providing support for this idea (Figure 2D).

Together with recruitment of the ncRNA, the incoming dNTP and catalytic ions are coordinated in the RT active site. By analogy to other DNA polymerases (Steitz 1999), catalysis proceeds via a two-metal-ion S_N2-type mechanism, typically involving Mg²⁺. During processive extension of the DNA, the msdRNA-msDNA hybrid must move away from the catalytic core while the fingers and active-site motifs negotiate recruitment of incoming dNTPs. In currently resolved

mature structures, the a1-a2 stem is displaced from the active site or appears flexible, showing that the priming configuration is not preserved after elongation (Carabias et al. 2024; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li et al. 2025; Wang et al. 2022b, 2024; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025).

2.2.2 Reverse transcription – elongation and termination

The elongation state remains structurally unresolved but can be approximated by structure prediction using AlphaFold 3. Prediction of retron Eco2 RT-msDNA in the final stage of elongation supports the view that the thumb domain simultaneously engages the msRNA hairpin and RNA-DNA hybrid to position the growing strand for extension, while residues in the fingers and palm coordinate the incoming nucleotide and catalytic metals (Figure 3A and B). These features are consistent with a conventional RT-mediated DNA-polymerization mechanism (Steitz 1999).

Template removal is a key maturation step, as it eventually allows the single-stranded msDNA to fold into its

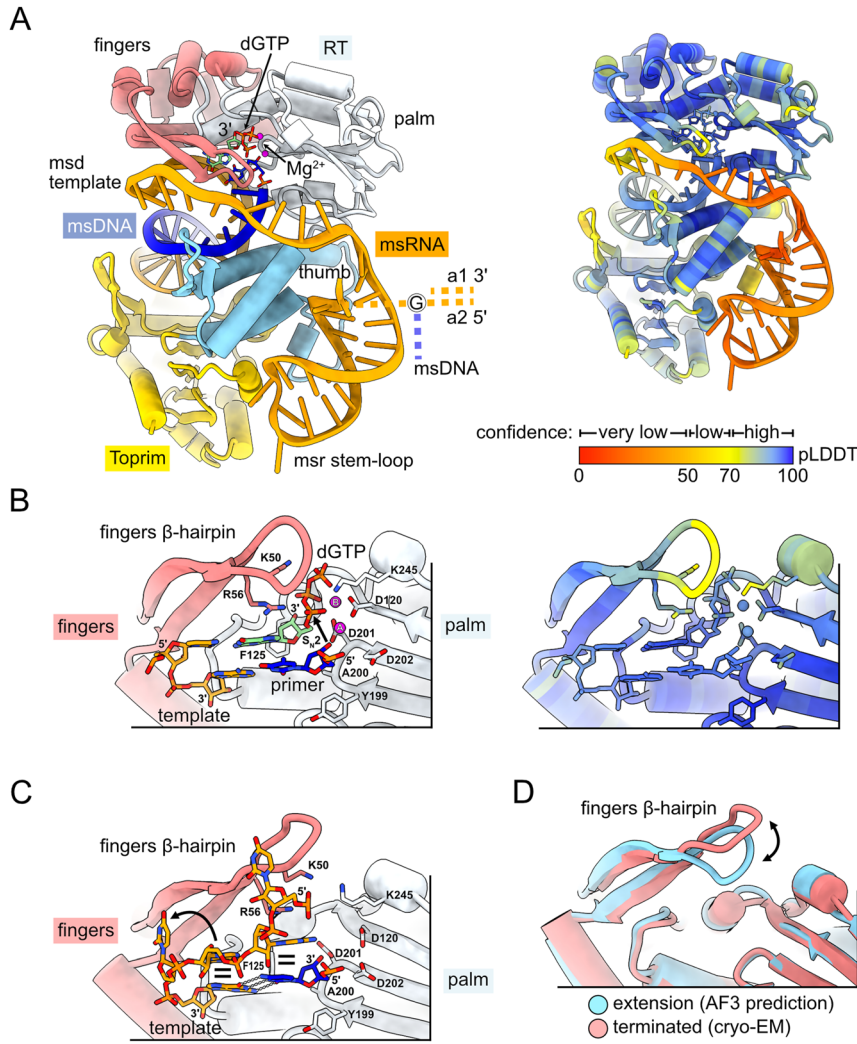


Figure 3: Reverse transcription elongation and termination. (A) Left: AlphaFold 3 prediction of the *E. coli* Eco2 RT-Toprim in a final stage of msDNA extension. The RT and Toprim domains are colored red/white/cyan and yellow, respectively. The msDNA and msRNA are shown in blue and orange. The substrate (dGTP) and cofactors (Mg²⁺) are colored green and magenta, respectively. Right: the same model colored according to pLDDT. (B) Left: Close-up of the fingers (red) and palm (white) subdomains from the AlphaFold 3 prediction in panel (A). The template (orange), primer (blue), substrate (green), cofactors (magenta), and RT active site residues are shown as sticks. Right: the same view colored according to pLDDT. (C) Experimentally determined structure of Eco2 RT in the terminated state (PDB 9I2G; Jasnauskaitė et al. 2026); coloring as in panel (B). (D) Close-up of the superimposed extension and terminated Eco2 RT structures, illustrating different conformations of the fingers β-hairpin motif (arrow).

mature configuration. For almost all tested retron systems, degradation of the RNA template depends on RNase HI, and loss of RNase H function (cleavage of RNA in RNA/DNA hybrids) disrupts msDNA maturation, impairing proper termination of reverse transcription (Lampson et al. 1989a; Palka et al. 2022; Shimamoto et al. 1995b). The molecular details of the template removal stage remain to be characterized.

Termination occurs at a defined position and yields mature msDNAs with lengths and folds varying between retrons (Khan et al. 2025; Lampson et al. 2001). Recent studies show that the msd-upstream msRNA hairpin helps limit further template entry into the active site, while local rearrangements near the template terminus prevent further extension (Carabias et al. 2024; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li et al. 2025; Wang et al. 2022b, 2024; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025). In most studied retron types, the terminal template base is flipped away

from the active site (Figure 3C), and the RT fingers β-hairpin is repositioned relative to the predicted catalytically competent polymerase state (Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li et al. 2025; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025) (Figure 3D). Mature msDNA contacts the palm and fingers subdomains in all RT-msDNA structures (Carabias et al. 2024; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li et al. 2025; Wang et al. 2022b, 2024; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025), raising the possibility that the product contributes allosterically to termination and stabilization of the RT-inactive post-synthetic state. A full understanding of how retron RTs transition from priming to elongation to termination will require experimental capture of intermediate states.

Once synthesis terminates, the RT remains bound to the mature msDNA (Carabias et al. 2024; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Jasnauskaitė et al. 2026; Li

et al. 2025; Lampson et al. 2001; Wang et al. 2022b, 2024; Wang et al. 2025a,b,c; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025). This product-bound state has now been visualized by cryo-EM for several distinct retron types in the context of their effectors and emerged as the central sensory and regulatory unit within the immune-competent complexes (Supplementary Table 1).

3 Structural and mechanistic diversity across retron complexes

3.1 Type I-A

Type I-A retrons, exemplified by structurally characterized *E. coli* Eco4/Ec83 (Wang et al. 2025b), Eco7/Ec78 (Dai et al. 2025; Ishikawa et al. 2025; Li et al. 2025; Wang et al. 2025a), and Eco Forc82 (George et al. 2025) assemble into higher-order complexes in which a long stem-loop msDNA and RT associate with a Septu-like effector module: four ATP-binding cassette (ABC) ATPase subunits (PtuA) and one or two HNH nickase-nuclease subunits (PtuB) (Figure 4A). Different from most other retron systems, the msDNA is further processed by nucleolytic degradation of the RNA-DNA branching site by unknown cellular factors, after the synthesis of the branched RNA-DNA molecule is terminated. In these systems, the ATPase bridges the RT-msDNA subunit and the nuclease effector. RT-msDNA interactions with the ATPase are required for complex formation, regulation and defense, indicating that the mature msDNA is central to the immune-competent state. These studies further demonstrate that ATP binding and hydrolysis are likewise critical for complex formation and phage defense.

In the RT-msDNA-bound inhibited state, each HNH nuclease subunit is held in place by both C-terminal extensions of the ATPase dimer, comparable to the Septu defense system (Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Li et al. 2025; Wang et al. 2025a,b). Upon msDNA decay, initiated by phage nucleases, and release of the ATPase and HNH, the ATPase nucleic acid binding site may become available for substrate placement and HNH-mediated nucleic acid destruction, either targeting specific tRNAs or DNA *in vivo* (Azam et al. 2023; Dai et al. 2025; George et al. 2025; Ishikawa et al. 2025; Li et al. 2025; Wang et al. 2025a,b). An alternative model posits that the HNH becomes liberated from the ATPase to potentially dimerize prior to targeting tRNA, as suggested by a low-confidence AlphaFold 3 prediction (George et al. 2025). Curiously, tRNA cleavage has not been demonstrated *in vitro*, unlike DNA nicking (Wang et al. 2025a). Thus, it is possible that tRNA turnover might be an

indirect immune response outcome, rather than tRNAs being directly targeted by type I-A retrons (Wang et al. 2025a).

The molecular basis by which the HNH is controlled is not fully resolved, although direct interaction with the RT may facilitate its regulation (Li et al. 2025). This is a key unresolved feature of type I-A systems: the overall architecture is increasingly well defined, but the mechanistic route from phage sensing to nuclease activation remains to be fully understood.

3.2 Type I-B

Type I-B retrons are composed of the RT-msDNA module, encoded either upstream (type I-B1) or downstream (type I-B2) of an OLD (overcoming lysogenization defect) family helicase-nuclease effector (Mestre et al. 2020) (Figure 4B). Type I-B2 retrons assemble into RT-msDNA-OLD complexes in which the effector is a tetrameric OLD protein, containing N-terminal ATPase and C-terminal Toprim nuclease domains (Ji et al. 2025; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025).

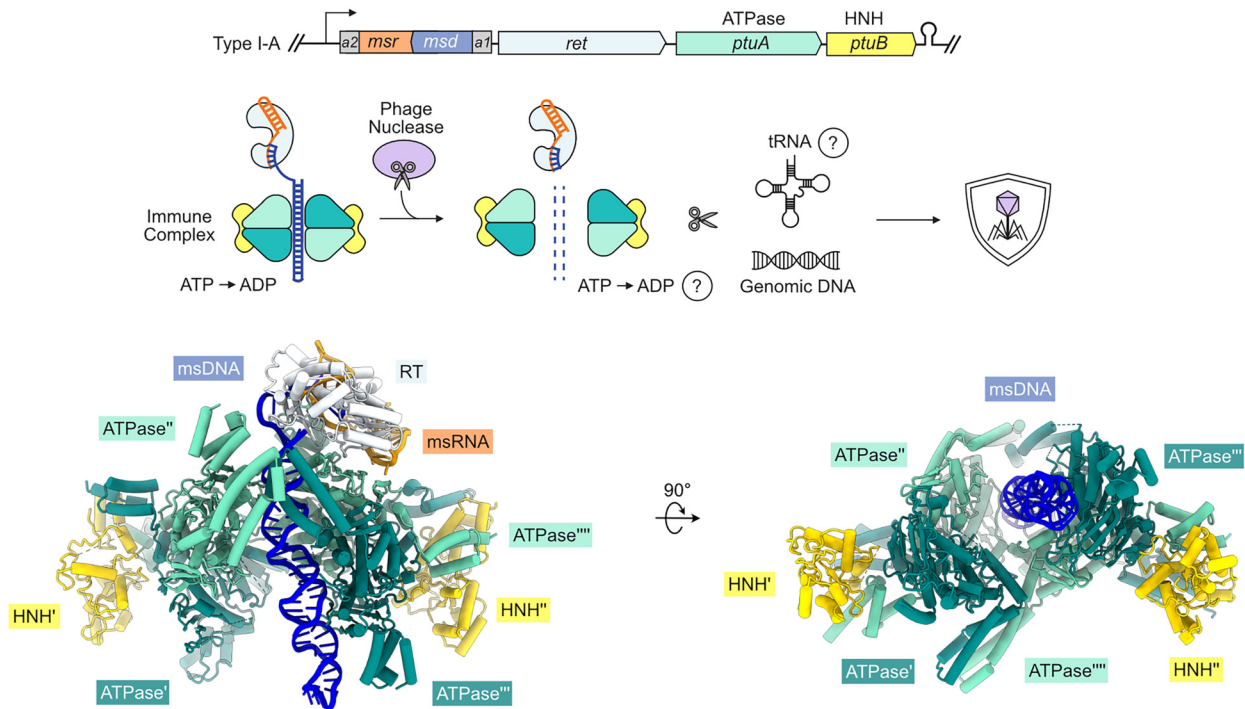
The complex architecture can be compared to a turtle, with the OLD tetramer forming the central shell and the four RT-msDNA subcomplexes projecting from it. Overall, the *E. coli* Eco8 effector compares well to the Gabija immune system component GajA (Ji et al. 2025; Yang et al. 2024; Yu et al. 2025). Tetramerization depends on interactions involving the ATPase-fused dimerization domain together with direct contacts between the ATPase and Toprim domains (Ji et al. 2025; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025). Within this architecture, the msDNA wraps across the OLD surface to control the effector.

Phage ssDNA-binding proteins activate the system by oligomerizing along the msDNA, leading to displacement from the OLD effector (Ji et al. 2025; Stokar-Avihail et al. 2023; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025). Displacement of the msDNA in turn activates the OLD effector for DNA degradation, possibly leading to abortive infection.

Current data indicate that both OLD active sites (ATPase and DNase) are essential for defense (Ji et al. 2025; Xiong et al. 2026; Yu et al. 2025; Yuan et al. 2025). However, the mechanistic role of ATP binding and hydrolysis remains opaque with studies reporting ATP-independent DNA cleavage (Xiong et al. 2026), ATP-dependent DNA degradation (Yuan et al. 2025), or no ATPase activity (Ji et al. 2025) of the OLD effector.

Together, this shows that type I-B2 retrons detect occupation and distortion of the msDNA rather than decay or modification, expanding the repertoire of retron trigger mechanisms and emphasizing the broader sensory

A



B

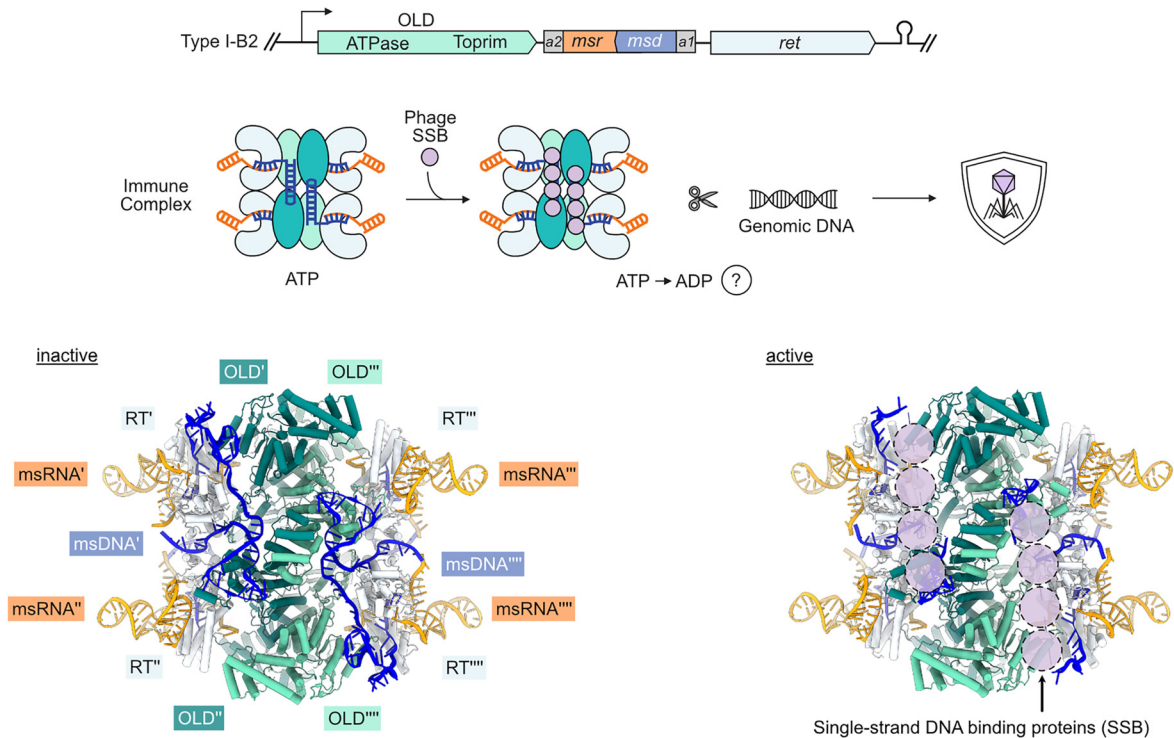


Figure 4: Type I-A and I-B2 effector complexes. (A) Top: Type I-A operon architecture. Middle: Schematic of the type I-A immunity mechanism. Bottom: Cryo-EM structure of the Eco4 immune complex shown in two orientations rotated by 90° (PDB 9E8Z; Wang et al. 2025b); subunits are colored according to the operon scheme. (B) Top: Type I-B2 operon architecture. Middle: Schematic of the type I-B2 immunity mechanism. Below: Cryo-EM structures of the Eco8 immune complex in its inactive (left) and SSB-bound active (right) states (PDB 9P4J and 9P4K; Yu et al. 2025); subunits are colored according to the operon scheme. Flexible SSB-subunits are indicated as circles with dashed outlines.

capabilities of msDNA that can detect multiple distinct DNA-processing activities. Conceptually, the use of a nucleic acid guide for direct and specific molecular recognition and activation resembles *bona fide* immune systems like CRISPR-Cas and argonautes (Bobadilla Ugarte et al. 2023; Wang et al. 2022a), rather than canonical toxin-antitoxin systems, which are primarily passively activated by disruptions in toxin-antitoxin homeostasis (Jurėnas et al. 2022; Seo et al. 2026).

3.3 Type I-C

Type I-C retrons differ from multi-component retrons in that the effector is fused directly to the RT, yielding a bipartite RT-Toprim protein that operates together with msDNA in a compact three-fold symmetric assembly, as shown for *E. coli* retron Eco2 (Jasnauskaitė et al. 2026; Wang et al. 2025c) (Figure 5A). Type I-C has been subtyped into types I-C1 to I-C3 according to the phylogeny of the RT-Toprim fusion protein (Mestre et al. 2020). As also observed in other retrons, the msRNA engages the thumb and palm surfaces of the RT and contributes to oligomerization (Jasnauskaitė et al. 2026; Wang et al. 2025c). However, the DNA component displays an unusual configuration. Rather than adopting a simple stem-loop (Lampson et al. 1989b; Wang et al. 2025c), the msDNA appears to assemble into a three-way junction that cages and controls the Toprim domain (Jasnauskaitė et al. 2026).

Structural comparison of the msDNA-bound inhibited state with msDNA-depleted activated state showed that inhibition involves both steric occlusion and allosteric regulation (Jasnauskaitė et al. 2026) (Figure 5A). The three-way junction blocks access to the Toprim active site, while additional distal contacts between the junction and Toprim may bias the active site toward an inactive configuration (Jasnauskaitė et al. 2026). Once the msDNA is degraded, the Toprim active site rearranges and the RNase activity is unleashed (Jasnauskaitė et al. 2026). This process is initiated by phage-encoded nucleases such as DenB and A1 (Azam et al. 2023; Jasnauskaitė et al. 2026; Stokar-Avihail et al. 2023; Wang et al. 2025c), although additional unknown host factors may further degrade the inhibitory msDNA (Jasnauskaitė et al. 2026). The activated Toprim then specifically cleaves a subset of tRNAs and promotes non-specific RNA turnover, which has been shown to halt translation and thereby terminate productive infection (Jasnauskaitė et al. 2026).

Among currently characterized retrons, type I-C has also provided most evidence for conformational regulation of both reverse transcription and nucleolytic effector activity (Jasnauskaitė et al. 2026). Hydrogen-deuterium exchange

mass spectrometry revealed that apo RT-Toprim is highly dynamic and that msDNA binding and trimerization induce widespread stabilization, with pronounced effects in the RT domain (Jasnauskaitė et al. 2026).

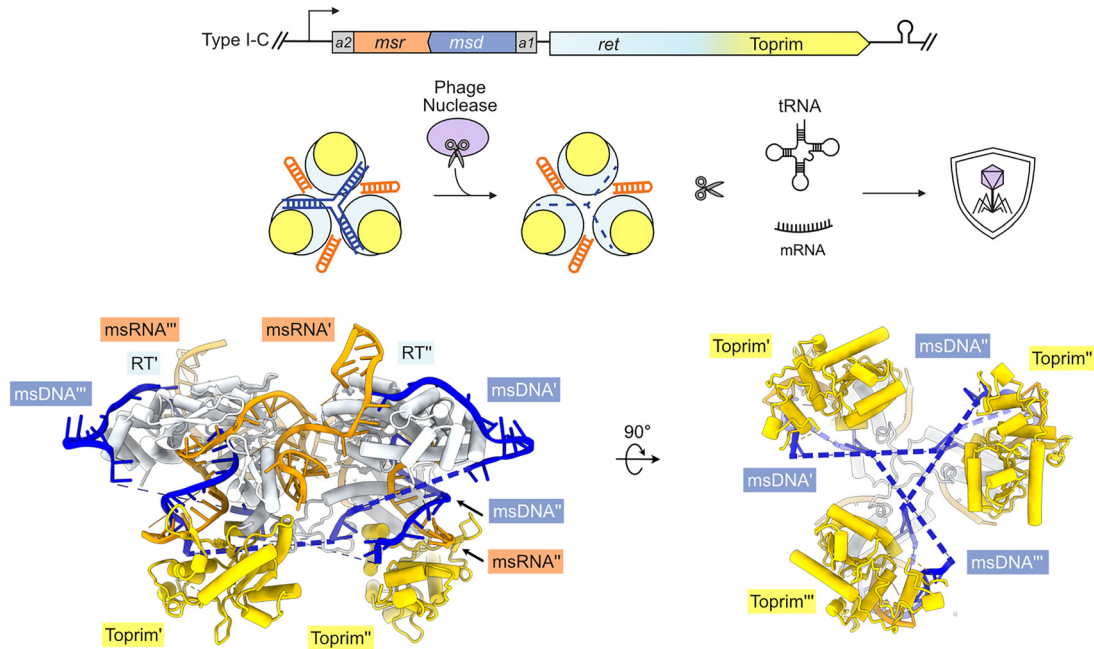
3.4 Type II-A

This type is characterized by the presence of an N-glycosidase effector. Type II-A retrons are further classified into three distinct subtypes (type II-A1 to II-A3), based on the locus architecture that either encodes the msDNA upstream or downstream of the effector (Mestre et al. 2020). Type II-A was the first system for which structural data became available, but its precise defense mechanism is not yet fully resolved. An early study characterized monomeric and dimeric assemblies containing RT, msDNA and effector (Wang et al. 2022b). More recent work showed that the type II-A retron from *E. coli*, Eco1/Ec86, can assemble into supramolecular filamentous oligomers that cage the N-glycosidase-HTH effector (Carabias et al. 2024; Wang et al. 2024) (Figure 5B). These filaments consist of repeating RT-msDNA-effector units that dimerize through effector-msDNA interactions, while neighboring repeating units are connected by RT-RT contacts positioned at the inflection points of the helical filament assembly.

The functional significance of supramolecular filament formation remains to be fully established, but the architecture suggests that higher-order organization may be central to regulation (Carabias et al. 2024; Payne et al. 2024; Wang et al. 2024). The effector-msDNA dimer interface seems directly linked to activity (Carabias et al. 2024; Wang et al. 2024). Structural analyses identified an effector state bearing a covalently attached ADP-ribosyl reaction intermediate, derived from NAD (Carabias et al. 2024; Wang et al. 2024). Yet, the effector remains in a catalytically inefficient configuration in the presence of msDNA, consistent with msDNA-mediated inhibition (Carabias et al. 2024; Wang et al. 2024).

Functional work indicates that the msDNA suppresses effector toxicity and that msDNA-truncated variants, mimicking the active state, can promote ADP-ribosylation of DNA together with NAD depletion (Carabias et al. 2024). These findings are suggestive of a dual defense mechanism in which genotoxic stress and metabolic collapse may jointly contribute to abortive infection. Methylation of the msDNA by phage-encoded methyltransferases and msDNA degradation by RecE have been shown to activate type II systems (Bobonis et al. 2022; Wang et al. 2024). Type II-A retrons thus highlight both the mechanistic diversity of retron effectors

A



B

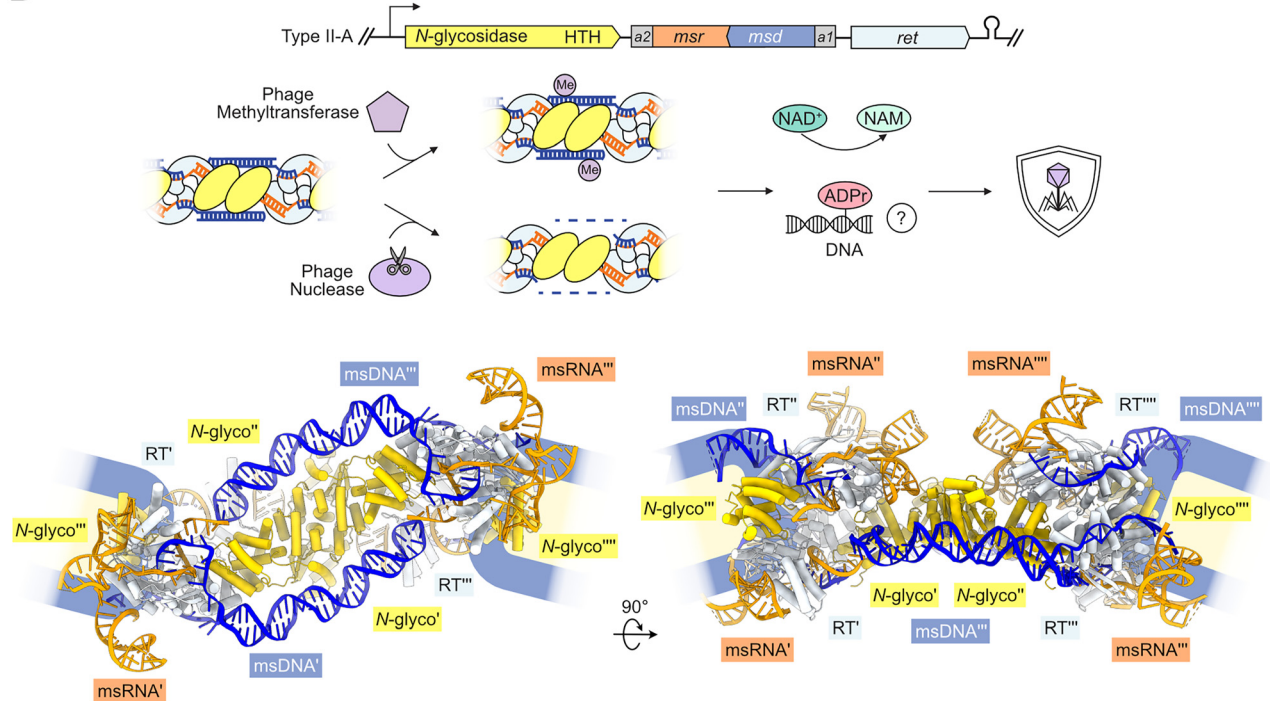


Figure 5: Types I-C and II-A effector complexes. (A) Top: Type I-C operon architecture. Middle: Schematic of the type I-C immunity mechanism. Bottom: Cryo-EM structure of the Eco2 immune complex shown in two orientations rotated by 90° (PDB 9I2G; Jasnauskaitė et al. 2026); subunits are colored according to the operon scheme. (B) Top: Type II-A operon architecture. Middle: Schematic of the type II-A immunity mechanism. Bottom: Cryo-EM structures of the Eco1 immune complex in two orientations rotated by 90° (PDB 8QBK; Carabias et al. 2024); subunits are colored according to the operon scheme.

and the possibility that chemical modification of msDNA may serve as a trigger.

3.5 Type IX

Type IX retrons encode a HEPN-family ribonuclease together with an accessory DNA-binding HTH/KH-domain protein (Mestre et al. 2020) (Figure 6A). Three independent preprints report the structures and mechanistic characterization of *Klebsiella variicola* Kva2 and related *Salmonella enterica* Sen3/*Yersinia intermedia* Yin1 systems (Carabias et al. 2026; Hayashi et al. 2026; Hibshman et al. 2026). These studies describe oligomeric RT-msDNA-effector assemblies, although the reported higher-order architectures differ. Yin1 was observed as closed rings containing 8–10 repeating units, in which the msDNA connects neighboring subunits and scaffolds an outer rim that contains the effector (Figure 6A) (Carabias et al. 2026), whereas Kva2 was reported to form sheet-like or asymmetric higher-order assemblies (Hayashi et al. 2026; Hibshman et al. 2026). Despite these architectural differences, the preprints converge on a common inhibitory principle of the individual RT-msDNA-effector subunits, in which the dimeric HEPN RNase is enclosed by msDNA, and the HTH/KH-domain protein restricts access of RNA substrates to the active site.

Two of the preprints propose that phage nucleases activate type IX retrons through msDNA cleavage. In Sen3/Yin1, the phage PD-(D/E)XK nuclease B14T was shown to cleave the exposed msDNA stem-loop, likely causing disruption of msDNA-mediated interactions and in turn HEPN dissociation (Carabias et al. 2026). Similarly, the Bas24 phage exonuclease was reported to cleave Kva2 msDNA and promote dissociation of the HEPN-HTH/KH subcomplex (Hayashi et al. 2026). A distinct mechanism was proposed for Kva2 activation by phage T5. Hibshman et al. suggest that the HTH-fold protein D5 structurally mimics the retron HTH/KH protein and binds the msDNA stem-loop, possibly leading to partial remodeling rather than complete disassembly of the complex (Hibshman et al. 2026).

The three preprints further implicate HEPN-mediated RNA cleavage in growth arrest for anti-phage defense. Sen3/Yin1 was reported to predominantly cleave tRNAs at the anticodon loop (Carabias et al. 2026), while Kva2 was shown to likewise target tRNAs and, in one study, possibly rRNAs (Hayashi et al. 2026; Hibshman et al. 2026). Together, these preprints suggest that type IX retrons follow the broader msDNA-centered regulatory logic observed in other retron families, while introducing distinct higher-order architectures and potentially multiple routes to HEPN activation. However, the physiological relevance and *in vivo* dynamics of the reported oligomeric states remain to be established.

3.6 Structurally uncharacterized types

Of the 13 distinct retron types, which differ primarily in their associated effector proteins (Mestre et al. 2020), only types I-A, I-B, I-C, II-A, and IX have been structurally and mechanistically characterized. As evidenced above, type I retron systems already exhibit substantial structural and mechanistic diversity. Given these variations, it may be appropriate to reconsider the current classification framework to better reflect the mechanistic and evolutionary distinctions among retron systems.

The remaining types (III–VIII, X–XIII) may be similarly diverse in both structure and function. These systems encompass a wide range of genomic architectures, with predicted effectors including nucleic acid- and nucleotide-targeting proteins (types III, V, VI, X, XIII), serine esterases and peptidases (types VIII and XI), proteins containing domains of unknown function (type VII), and even immune signaling domains such as TIR (type XII) (Mestre et al. 2020). Intriguingly, a recent preprint proposed a mechanism for type VI retrons in which phage infection induces production of msDNA that activates, rather than neutralizes, the effector (Zhang et al. 2025). Characterization of these systems is expected to uncover novel mechanisms of bacterial immunity and further expand our understanding of retron-mediated defense.

4 Anti-defense strategies

As retron-mediated immunity inhibits phage propagation, phages have evolved diverse strategies to evade or suppress this defense by either blocking biogenesis, effector activation, or downstream toxicity (Figure 6B).

To interfere with biogenesis, inhibition of retron component production or degradation of the msDNA precursor ncRNA could compromise immunity, as proposed in a recent preprint (Azam et al. 2023). However, given that retron complexes can accumulate to high copy numbers within bacterial cells (Dhundale et al. 1985; Yee et al. 1984), post-infection inhibition of their synthesis alone may be insufficient to fully neutralize the defense *in vivo*.

One of the most direct routes to escape defense systems involves random genetic variation within the phage itself. Spontaneous mutations in genes encoding retron-triggering factors can avoid activation of the system. For example, alterations in DNA-binding domains or catalytic residues of these triggers generate “escaper” phages able to replicate in retron-carrying hosts (Millman et al. 2020; Samson et al. 2013; Stokar-Avihaïl et al. 2023).

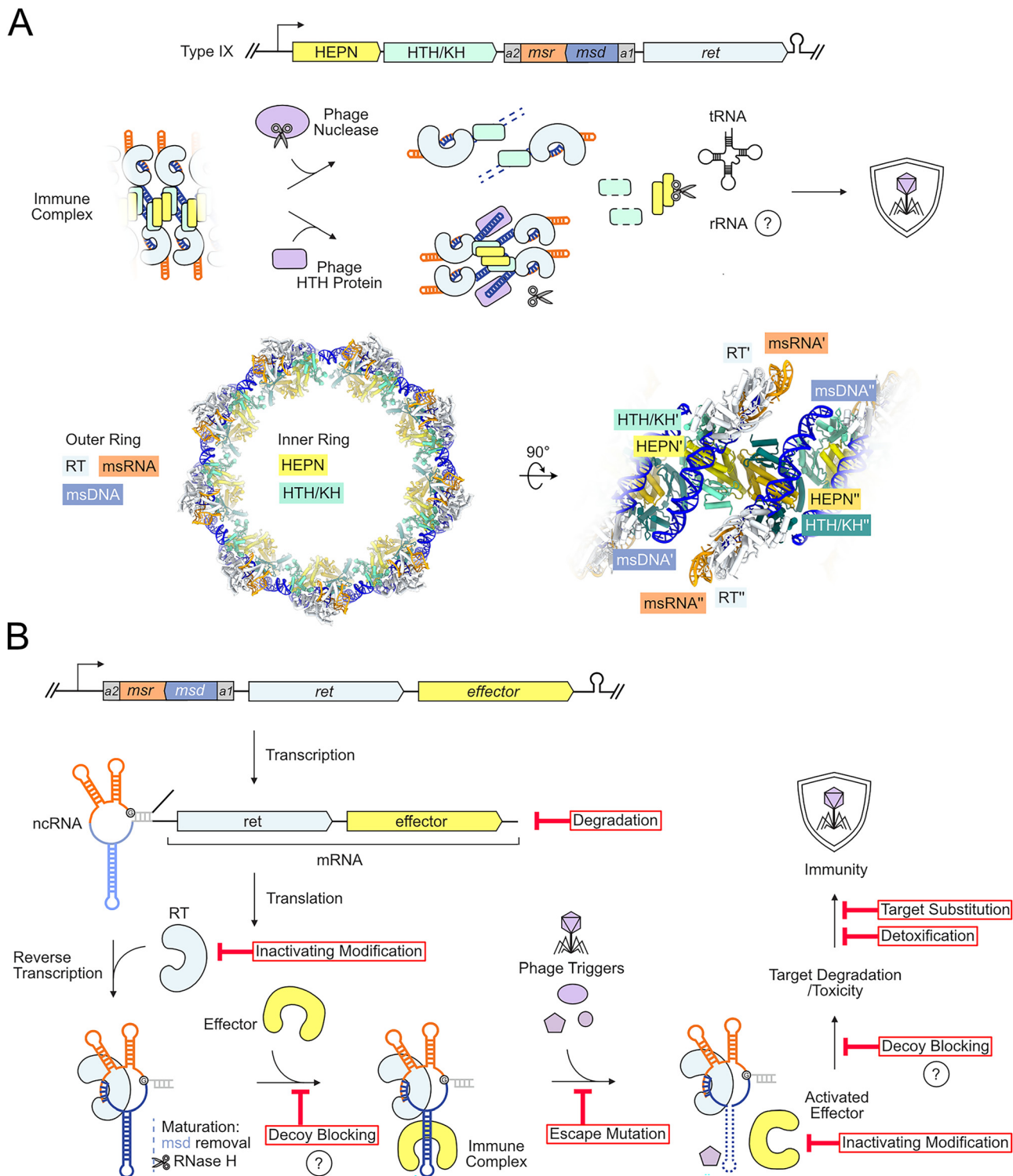


Figure 6: Type IX effector complex, and anti-retron strategies. (A) Top: Type IX operon architecture. Middle: Schematic of the proposed type IX immunity mechanism. Bottom: Cryo-EM structures of the Yin1 immune complex in two orientations rotated by 90° (model from preprint; Carabias et al. 2026); subunits are colored according to the operon scheme. (B) Schematic illustration of retron biogenesis and defense, analogous to Figure 1. Anti-retron mechanisms are highlighted in red; hypothetical mechanisms are indicated by question marks.

An alternative and more direct strategy may involve molecular mimicry (Blower et al. 2012). Phages could encode anti-retron nucleic acid or protein decoys that structurally resemble retron effector targets or msDNA intermediates. Such decoys could sequester retron components, prevent activation (e.g. by covering msDNA), or block access to target substrates. While analogous mechanisms are exceedingly well documented in other anti-defense contexts such as CRISPR-Cas (Jia and Patel 2021), experimental evidence for mimicry in retron systems is currently lacking.

In addition to sequestering retron components, phages may inactivate retron systems through chemical modification. A recent preprint reported that the phage T7 kinase broadly phosphorylates cellular host proteins, leading to suppression of multiple host defense pathways, including retron Eco9 (Bartolec et al. 2024).

Phages may also counter retron immunity by directly neutralizing its toxic outputs. For instance, NADAR (NAD and ADP-ribose) enzymes have been proposed to mitigate type II-A retron-mediated defense (Carabias et al. 2024; Carabias and Montoya 2024). Although a characterized NADAR enzyme does not appear to hydrolyze ADP-ribose, it may function by binding ADP-ribose or related conjugates, thereby reducing their effective toxicity (Carabias et al. 2024).

Finally, phages may bypass retrons by functionally replacing targeted molecules. In type I-A retron systems, defense involves degradation of specific tRNAs; to overcome this immune response, phages encode alternative tRNAs to supplement tRNAs available for translation (Azam et al. 2024; George et al. 2025).

Taken together, phages deploy a multifaceted arsenal of anti-retron strategies, ranging from stochastic mutational escape to detoxification and substitution of targeted molecules. However, precise molecular modes of action remain largely unresolved for many anti-defense factors (Bobonis et al. 2022). Future mechanistic studies are needed to fully understand how phages counter retron-mediated immunity.

5 Discussion

In conclusion, recent structural and mechanistic studies establish retrons as a major class of bacterial immune systems built around a conserved regulatory logic in which an RT-derived msDNA molecule functions as both scaffold and sensor to control diverse effector activities.

Across diverse retron types, msDNA engages in higher-order assemblies, enforces inactive effector conformations, and transmits phage-derived signals upon stimulation. Whether by cleavage, chemical modification, or competitive binding, disturbance of the msDNA sensor is the unifying

trigger that transforms a quiescent surveillance state into an activated defense response. This conceptual framework places retrons alongside other nucleic-acid-guided immune systems such as CRISPR-Cas and argonautes, yet highlights a distinct strategy in which the sensory nucleic acid is self-generated and sensitive to its chemical state, rather than derived from invading genetic material and responsive to the presence of complementary nucleic acids. Paralleling tRNA-targeting retron systems, some CRISPR-Cas12 and -Cas13 types degrade tRNAs upon nucleic acid-guided target recognition to trigger growth arrest for anti-phage defense (Dmytrenko et al. 2026; Jain et al. 2024). Importantly, this defense mode is conceptually different from toxin-antitoxin systems such as type III, which employs small ncRNAs to monitor changes in host transcription via disruption of toxin-antitoxin homeostasis (Guegler and Laub 2021; LeRoux and Laub 2022).

Despite this progress, several fundamental questions remain unresolved. First, retron biogenesis is still poorly understood. How transcription, translation, and msDNA synthesis are coordinated, and whether retron expression is actively regulated in response to environmental or infection-relevant cues, such as cell density, are key open questions. Second, structural snapshots have captured endpoint states; intermediate stages of priming, elongation, and termination during msDNA synthesis, as well as the transition from inhibited to activated effector states, remain to be investigated for many retron types.

Another major challenge lies in defining the precise molecular events for activation across retron families. While msDNA degradation, modification and displacement have emerged as common themes, the full range of phage-derived signals and the extent to which host factors contribute to activation are not yet completely understood. In parallel, the mechanistic basis of effector function, particularly for systems with poorly characterized or multifunctional domains, requires further dissection. The diversity of predicted effectors in uncharacterized retron types suggests that new modes of immunity remain to be discovered.

In summary, retrons, once regarded as obscure genetic curiosities, are now understood as a paradigmatic example of nucleic-acid-directed immune systems. Continued investigation promises not only to resolve outstanding mechanistic questions but also to uncover new principles of molecular sensing and defense in biology.

6 Methods: structure prediction

Structures were predicted using AlphaFold 3 (default settings) via the AlphaFold Server (<https://deepmind.google/>)

science/alphafold/). RNA base pair probabilities were predicted using the *RNAfold* WebServer (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>).

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Research ethics: Not applicable.

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning

Tools: Large Language Models and AI-based tools were used in this work. AlphaFold 3 was used for structure prediction. ChatGPT was used exclusively for proofreading and language refinement. All outputs were carefully reviewed by the authors.

Conflict of interest: The authors declare no competing interest.

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Data availability: Not applicable.

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