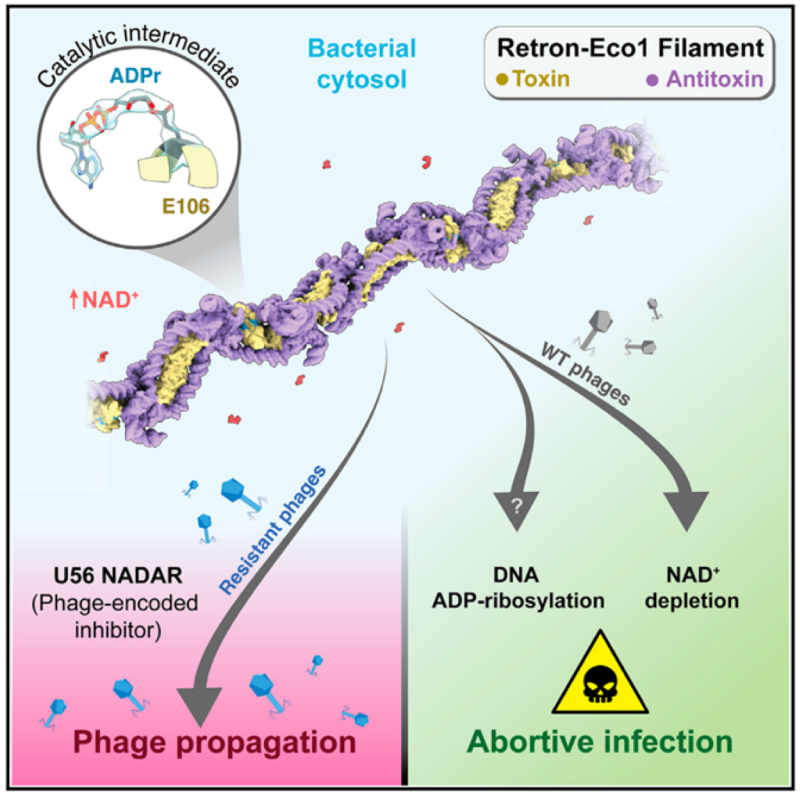
Molecular Cell

Article

Retron-Eco1 assembles NAD^+-hydrolyzing filaments that provide immunity against bacteriophages



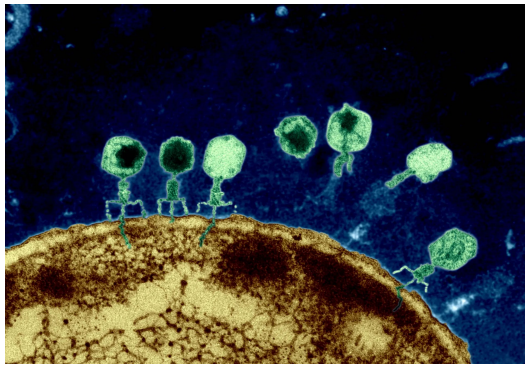
Arturo Carabias, Sarah Camara-Wilpert, Mario Rodrıguez Mestre, Blanca Lopez-Mendez,Ivo A. Hendriks, Ruiliang Zhao, Tillmann Pape, Anders Fuglsang, SeanHoi-ChingLuk, MichaelL.Nielsen, RafaelPinilla-Redondo and Guillermo Montoya.

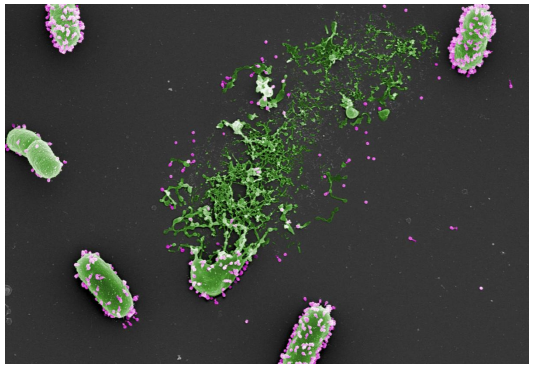
Presented by:

Lara Poluzzi, **Sara Radonic** and **Nicole Rizzetto**

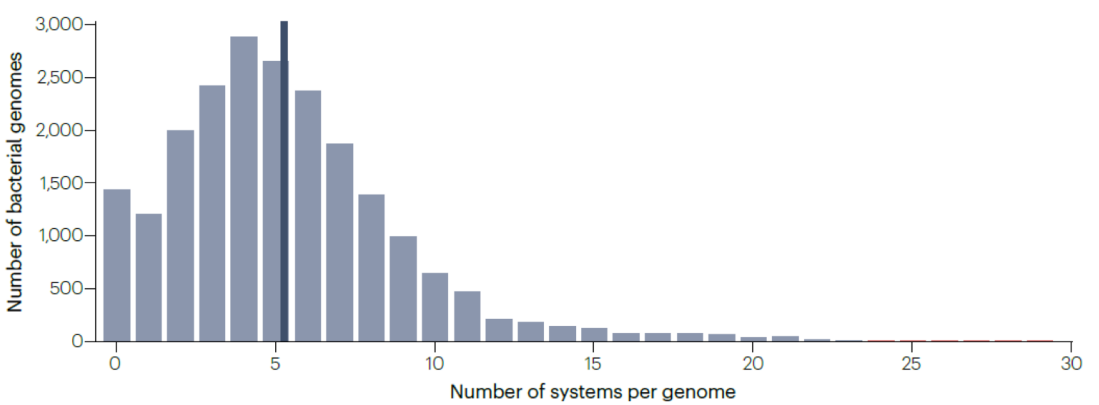
Bacterial immunity: the highly diverse antiphage systems

Estimates suggest that phage infection could account for 20–40% of bacterial daily mortality. As such, phage infection represents a **major evolutionary driver for bacteria.**





More than a hundred antiphage defense systems have already been discovered. They are combined in unique ways into an antiviral arsenal that is specific to a given strain.



Georjon, H., Bernheim, A. The highly diverse antiphage defence systems of bacteria. *Nat Rev Microbiol* **21**, 686–700 (2023). https://doi.org/10.1038/s41579-023-00934-x

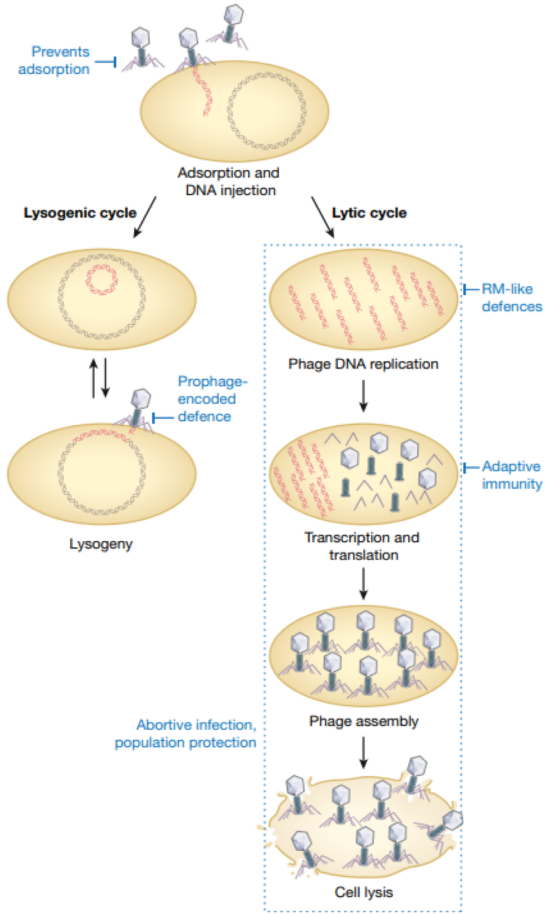
Features of antiphage defence systems

Most defence systems combine **two elements**:

- A **sensor** that detects the infection
- An **effector** involved in resolving phage infection

The antiphage defense mechanisms could take place at various stages of phage replicative cycle:

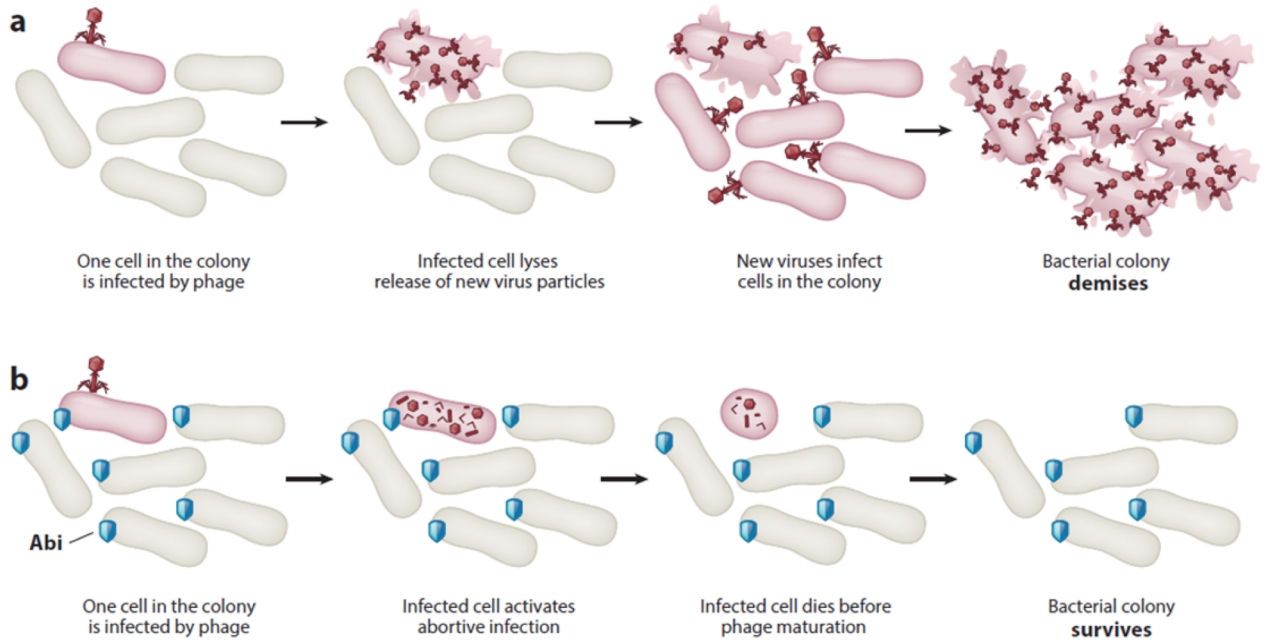
- phage **adsorption**, phage **DNA replication**, **expression** of viral genes, phage **assembly** (lytic cycle)
- **integration** in host genome (lysogenic cycle)



Abortive infection (Abi)

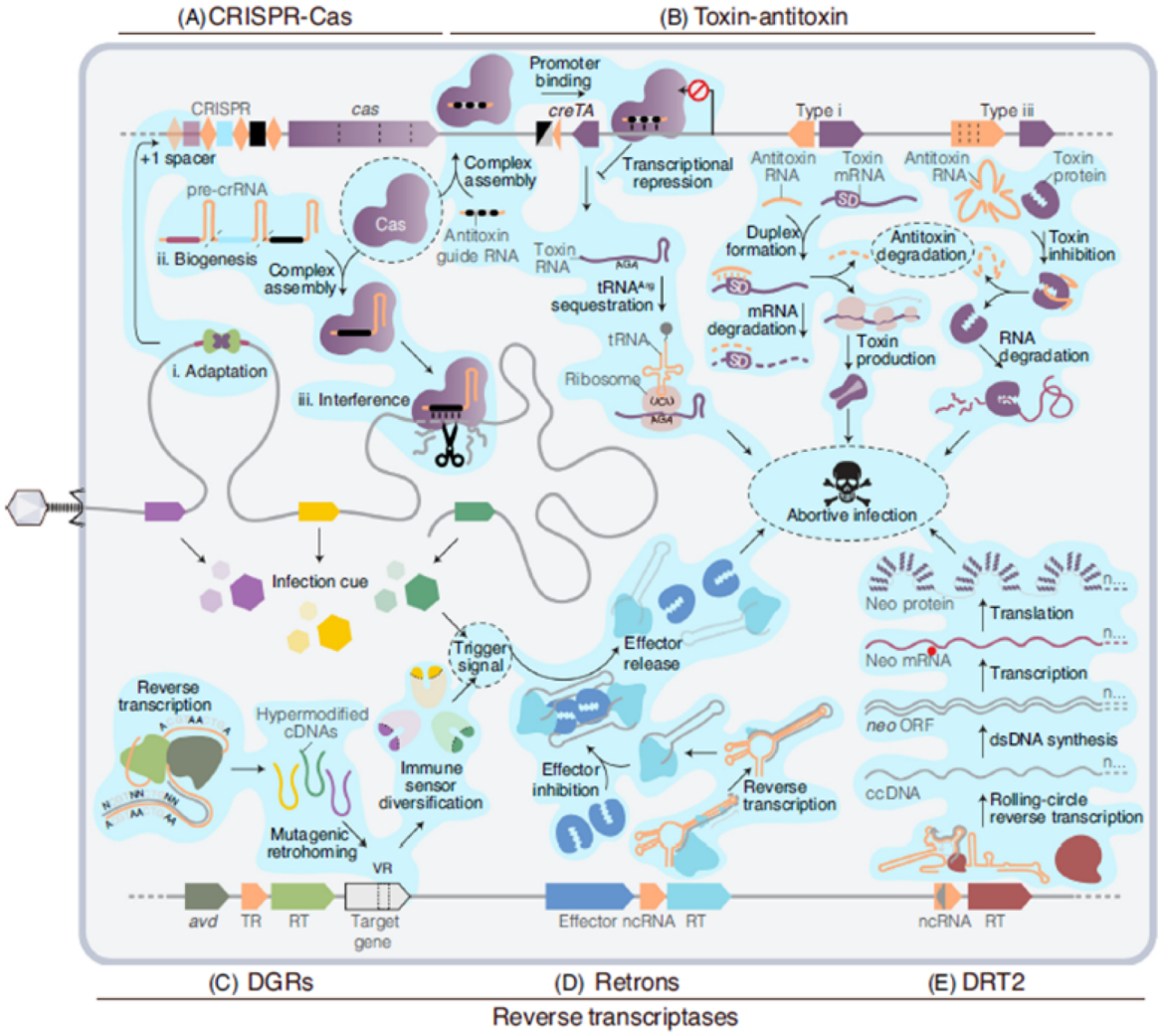
Effectors in bacterial immunity can either directly target the phage or **kill the infected host** before the phage can complete its reproduction cycle, through **Abortive infection** (Abi)

Abi is an **immune strategy** that can act through **many different mechanisms**, **protecting** the rest of the population from newly released virions



Lopatina A, Tal N, Sorek R. Abortive infection: bacterial suicide as an antiviral immune strategy. https://doi.org/10.1146/annurev-virology-011620-040628

The role of ncRNAs in bacterial immunity



Different ncRNA families play critical roles in bacterial defense against infection by phages underscoring the **dynamic properties of RNA for rapid adaptation to continually evolving parasites**

Examples include:

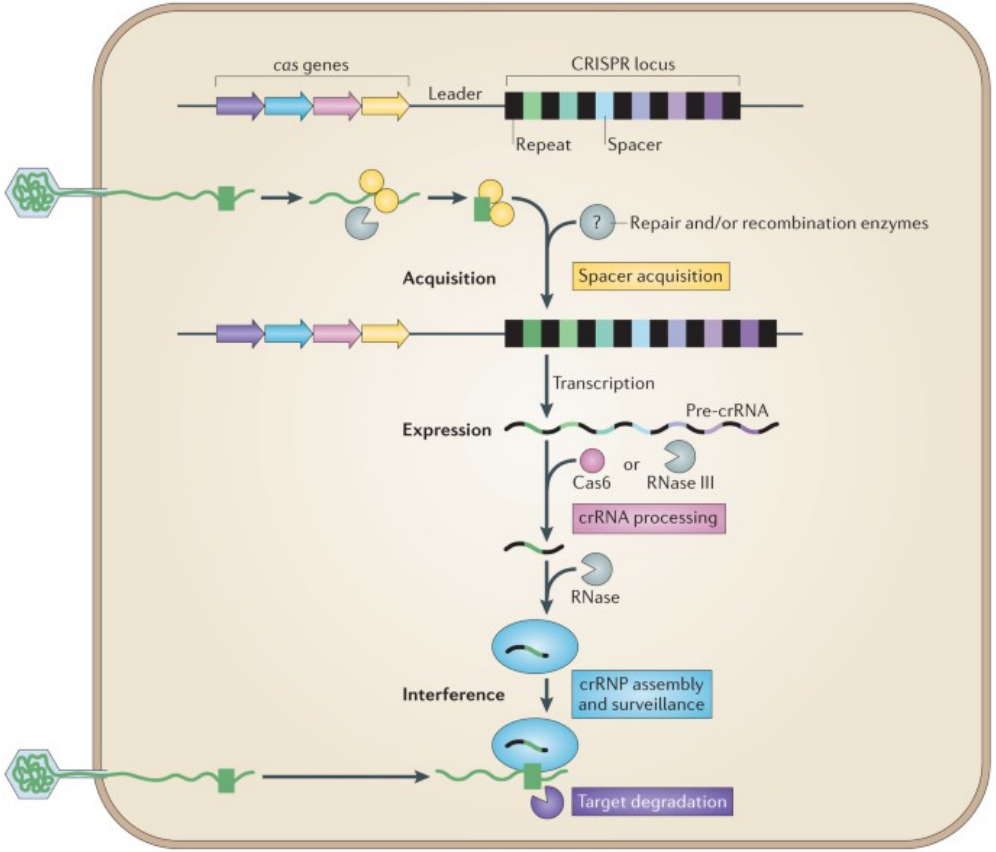
- **CRISPR-Cas systems**
- **Toxin-Antitoxin (TA) systems**
- **Retrons**

David Mayo-Muñoz, Huijuan Li, Mario Rodríguez Mestre, Rafael Pinilla-Redondo. The role of noncoding RNAs in bacterial immunity. Trends in Microbiology,2024. https://doi.org/10.1016/j.tim.2024.09.006.

The role of ncRNAs in bacterial immunity: CRISPR-Cas

CRISPR array is transcribed into a long precursor ncRNA, which is processed into **short CRISPR RNAs (crRNAs)**

The mature crRNAs form a complex with one or more **Cas proteins** and **guide** it to interfere with **complementary** nucleic acids, typically through nuclease-mediated **cleavage**



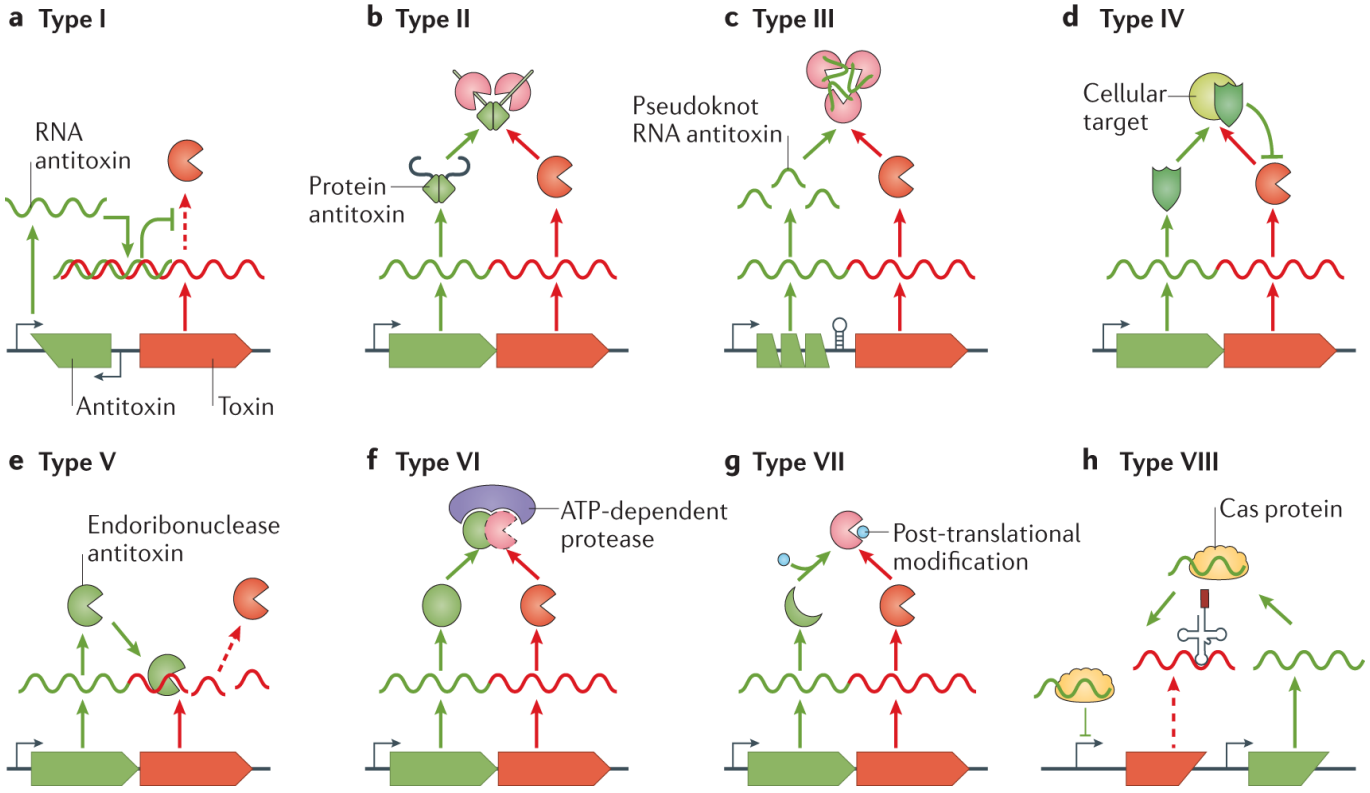
Nature Reviews | Microbiology

van der Oost, J., Westra, E., Jackson, R. *et al.* Unravelling the structural and mechanistic basis of CRISPR–Cas systems. *Nat Rev Microbiol* **12**, 479–492 (2014). https://doi.org/10.1038/nrmicro3279

The role of ncRNAs in bacterial immunity: TA systems

Toxin-Antitoxin (TA) systems consist of two **genetically associated** components:

- a **toxin** that disrupts an essential cellular component or process
- an **antitoxin** that neutralizes the toxin



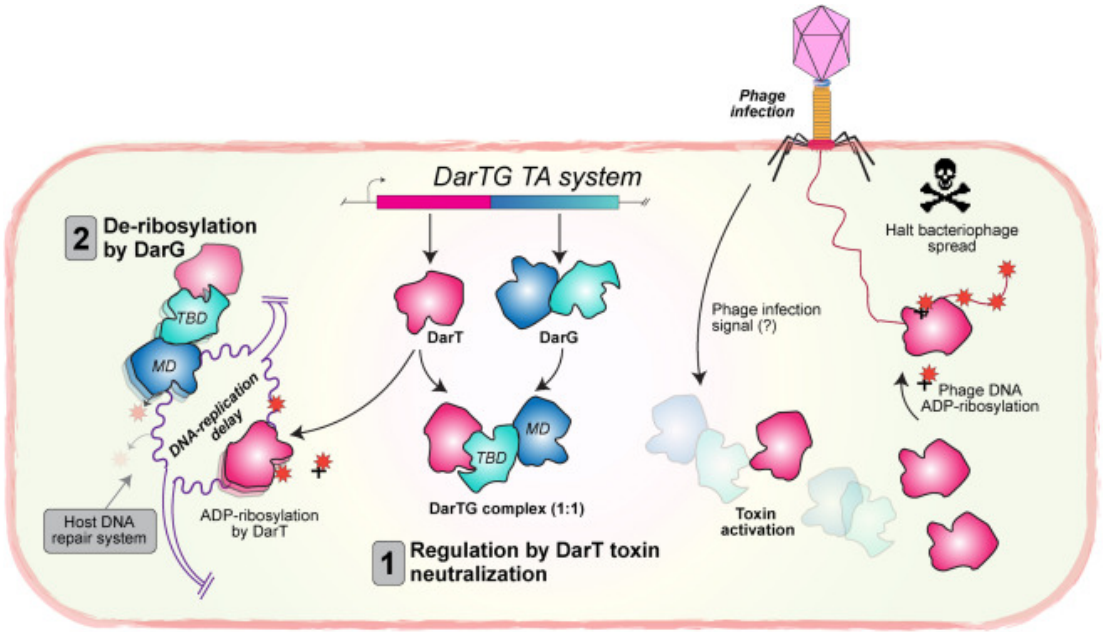
Jurėnas, D., Fraikin, N., Goormaghtigh, F. *et al.* Biology and evolution of bacterial toxin–antitoxin systems. *Nat Rev Microbiol* **20**, 335–350 (2022). https://doi.org/10.1038/s41579-021-00661-1

Abortive infection can act through Toxin-Antitoxin systems, to induce toxicity in the infected bacteria.

TA systems: DarT/G

- **Toxins**: Dart1 and Dart2 ADP-ribosyl transferases (ARTs) transfer ADPr to G and T bases, respectively. This modification induces DNA damage response and NAD+ depletion, with a cytotoxic effect

- **Antitoxins**: DarG1 and DarG2 neutralize the toxins, hydrolyzing the ADPr-base N-glycosidic bond



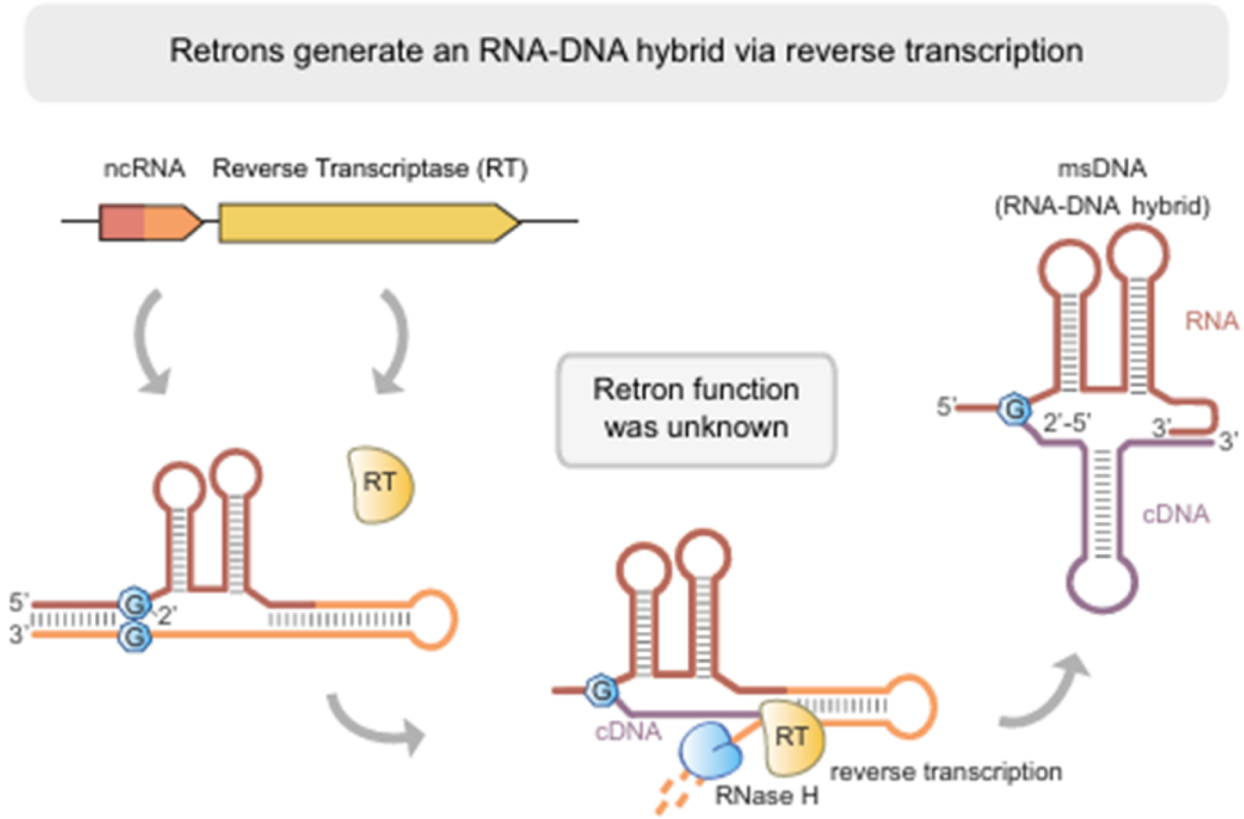
Deep, A. et al. Structural insights into DarT toxin neutralization by cognate DarG antitoxin: ssDNA mimicry by DarG C-terminal domain keeps the DarT toxin inhibited. *Structure* **31**, 780–789.e4 (2023)

Retrons systems

Retrons are genetic elements composed by:

- **non-coding RNA (ncRNA)**
- **Reverse Transcriptase (RT)**

The RT recognizes the folded ncRNA and starts the reverse transcription creating a RNA/DNA hybrid called **msDNA**.



Retrons were discovered in 1984 but their function remained unknown for many years…

Retrons are anti-phage systems acting via Abi

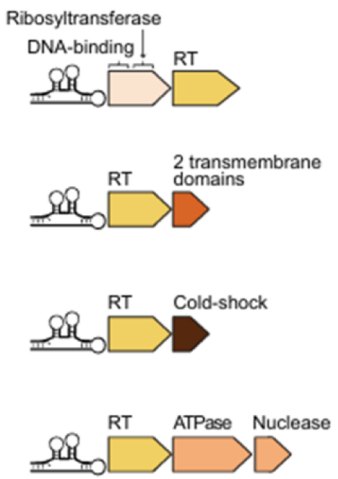
Retrons can present an **additional effector gene** which is responsible for the toxic effect in Abi.

Article

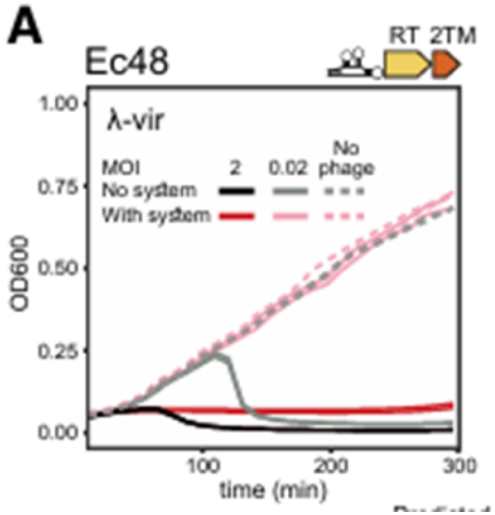
Bacterial Retrons Function In Anti-Phage Defense

Adi Millman,[1,3] Aude Bernheim,[1,3] Avigail Stokar-Avihail,[1,3] Taya Fedorenko,[1] Maya Voichek,[1,2] Azita Leavitt,[1] Yaara Oppenheimer-Shaanan,[1] and Rotem Sorek[1,4,*]

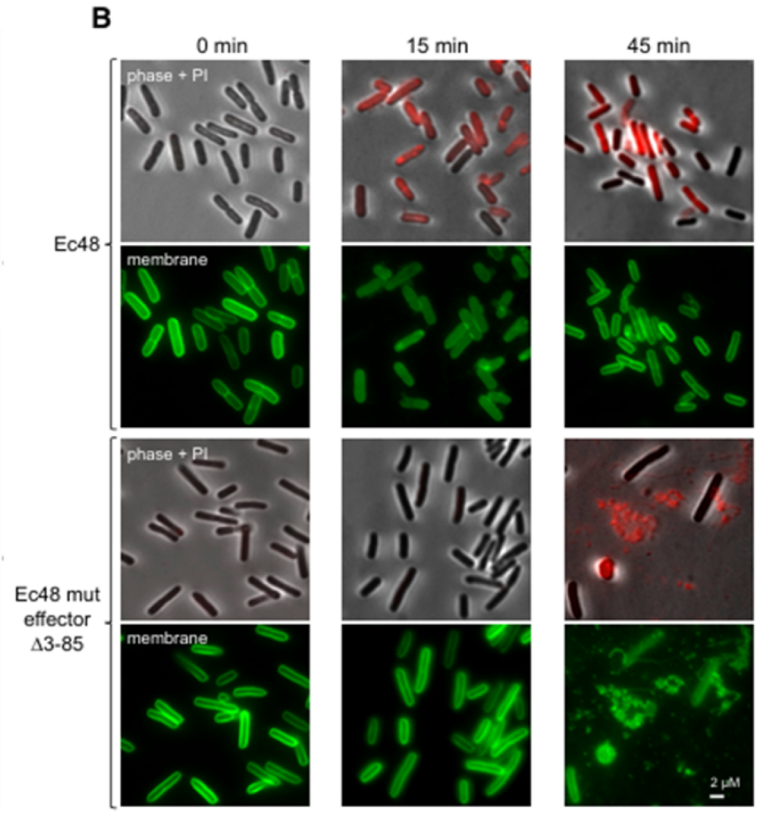
Retrons appear in an operon with additional "effector" genes



Retron Ec48 contains a gene with two trasmembrane spanning helices that impair membrane integrity.



After phage infection, the Ec48-expressing cell line doesn't collapse → anti-phage effect.



Ec48-expressing cells show propidium iodate staining after phage infection → effector protein action disrupted membrane integrity leading to Abi.

Molecular Cell

CellPress

Article

Retron-Eco1 assembles NAD^+-hydrolyzing filaments that provide immunity against bacteriophages

Arturo Carabias,[1,*] Sarah Camara-Wilpert,[2] Mario Rodríguez Mestre,[2,6] Blanca Lopéz-Méndez,[3,6] Ivo A. Hendriks,[4,6] Ruiliang Zhao,[2] Tillmann Pape,[1,5] Anders Fuglsang,[1] Sean Hoi-Ching Luk,[1] Michael L. Nielsen,[4] Rafael Pinilla-Redondo,[2,*] and Guillermo Montoya[1,7,*]

Retron-Eco1 structure

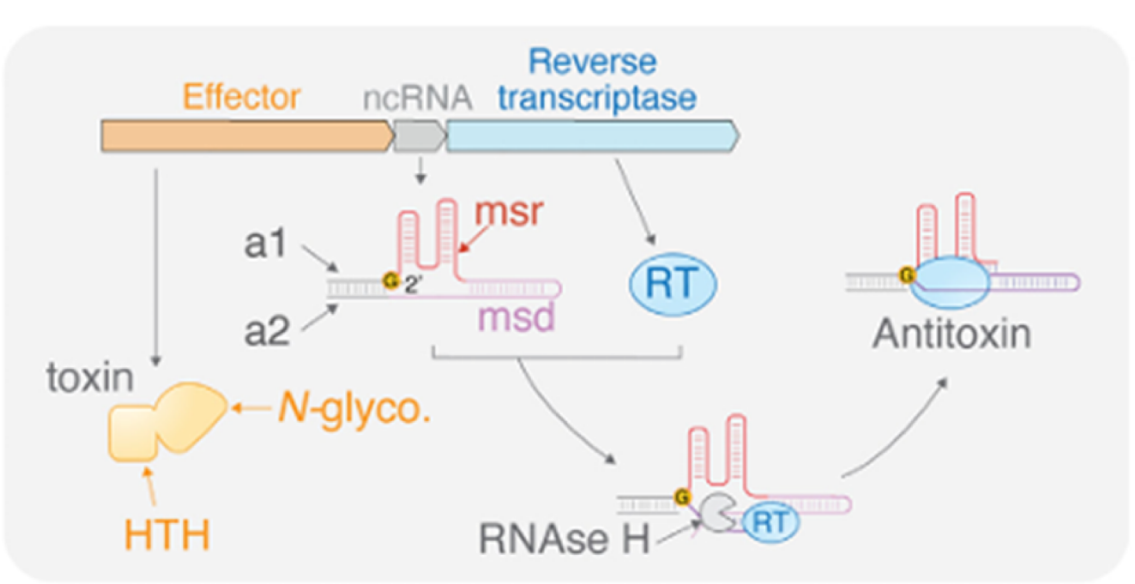
Retron-Eco1 is constituted by three components:

- Reverse Transcriptase (RT)
- Non-coding RNA (ncRNA)
- **Effector protein** with a **N-glycosidase domain**

Retron-Eco1 is a retron which works with a **Toxin-Antitoxin system**:

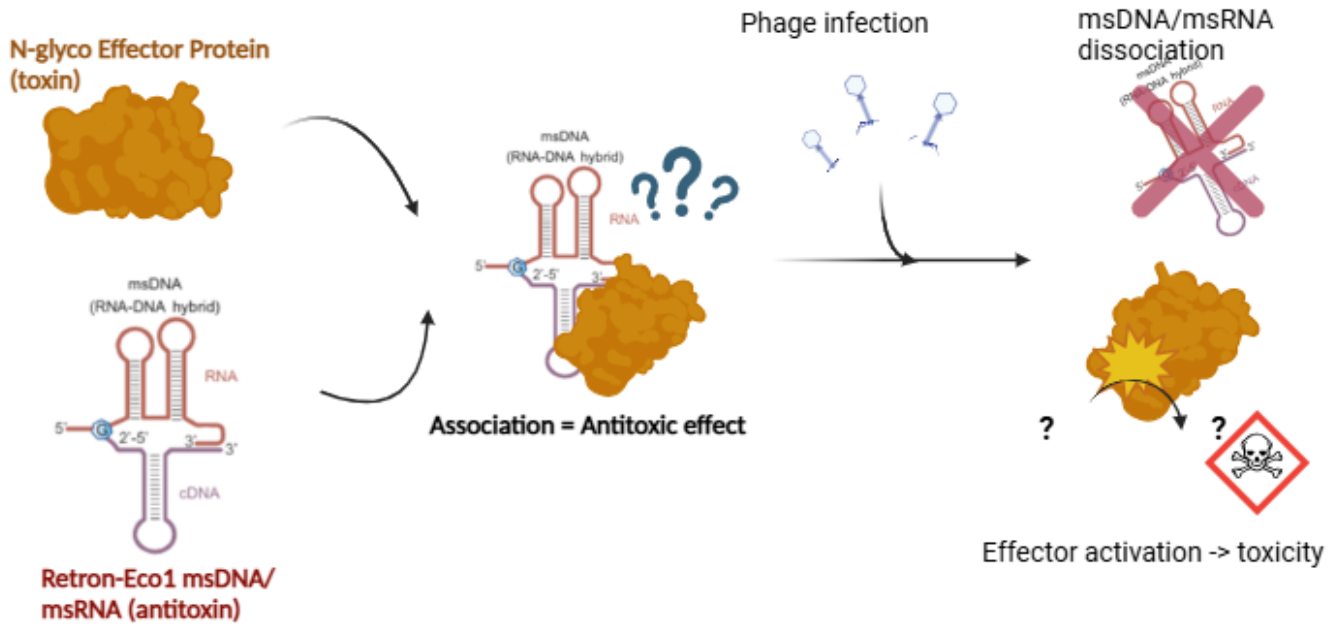
→N-glyco effector protein = **Toxin**

→msRNA/msDNA = **Antitoxin**



The TA system induce cells killing upon phage infection **via Abortive infection**.

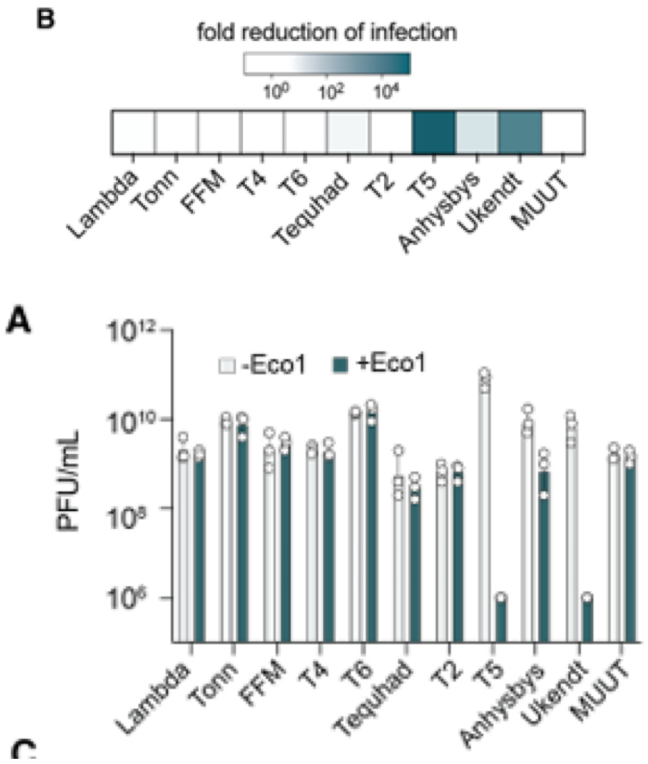
Overview of the hypothesis

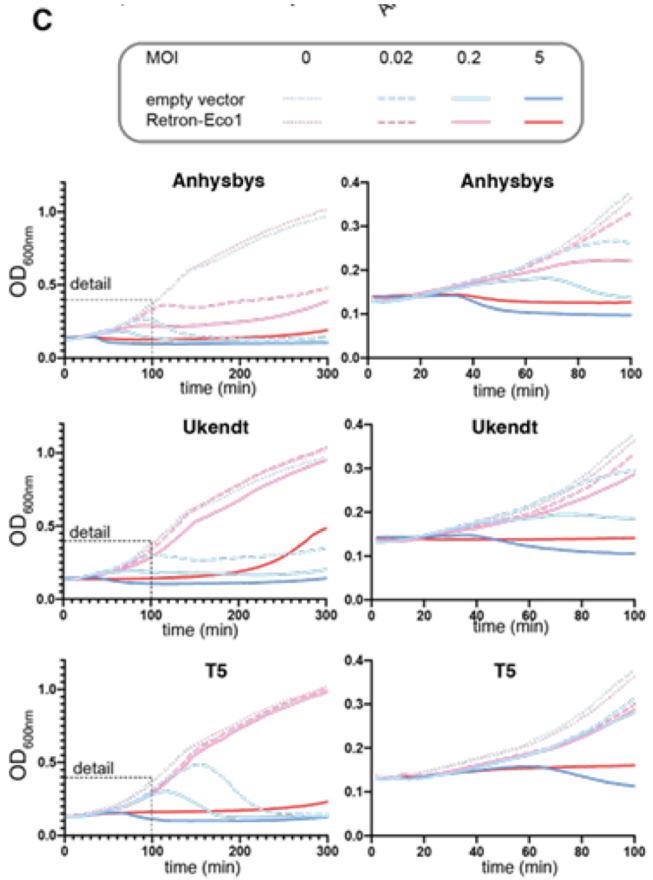


Question answered in the paper:

1. What's the substrate of the N-glycosidase domain?
2. What's the structural architecture of the toxin-antitoxin complex?
3. What's the role of msDNA in complex stability and Abi toxicity?
4. Are there phage encoded proteins that work as Retron-Eco1 inhibitors?

Retron-Eco1 provides immunity against T5 and phi-92 like phages

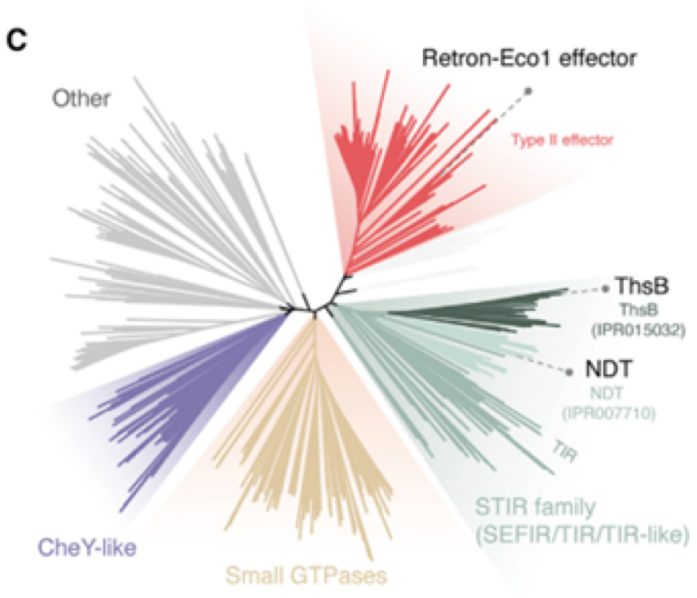




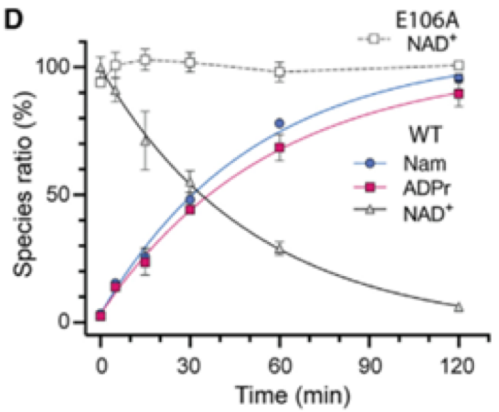
Retron-Eco1 provides **immunity against T5 and Ukendt phages**

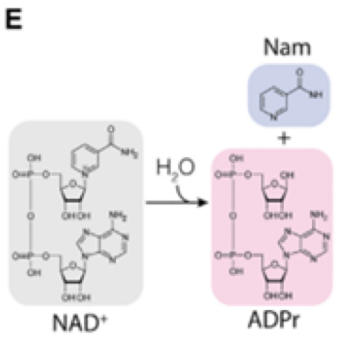
At high MOI the Retron-Eco1 containing lines collapse **because of abortive infection**

The Retron-Eco1 effector protein hydrolyzes NAD+

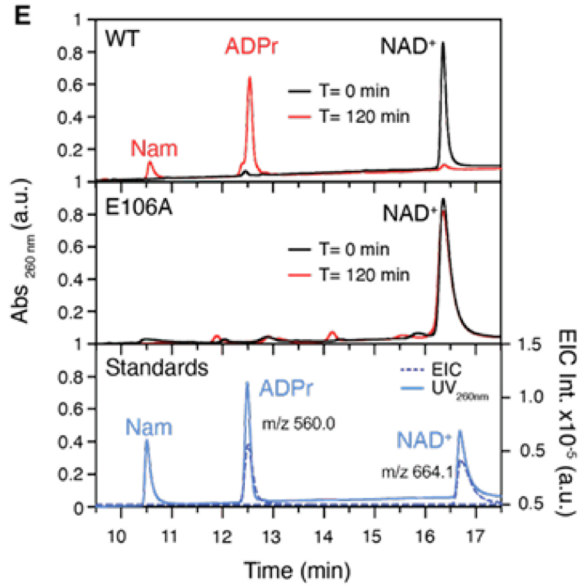


The N-glyco effector protein constitutes a separate **group within a superfamily of NAD+ processing enzymes.**





N-glyco protein **hydrolyzes the nicotinamide-ribosyl bond of NAD+** releasing Nicotinamide and **Adenosine Diphosphate Ribose**.



No other products of the NAD+ - ADPr network were observed.

Kinetic activity characterization:

NAM

V_{max} = 10.7 ± 0.9 µM min-1

K_m = 954 ± 150 µM

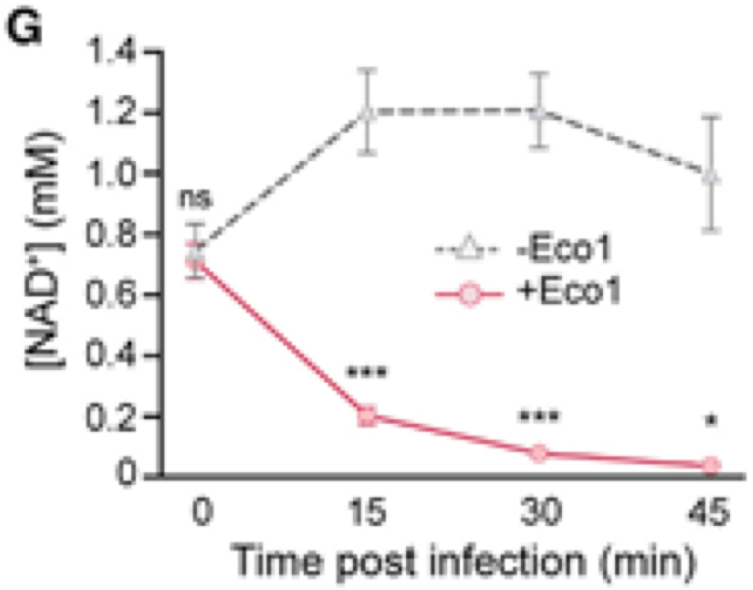
ADPr

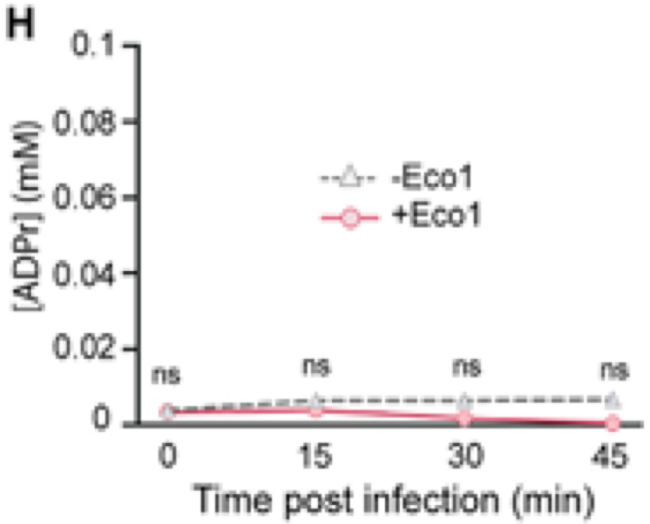
V_{max} = 8.8 ± 0.9 µM min-1

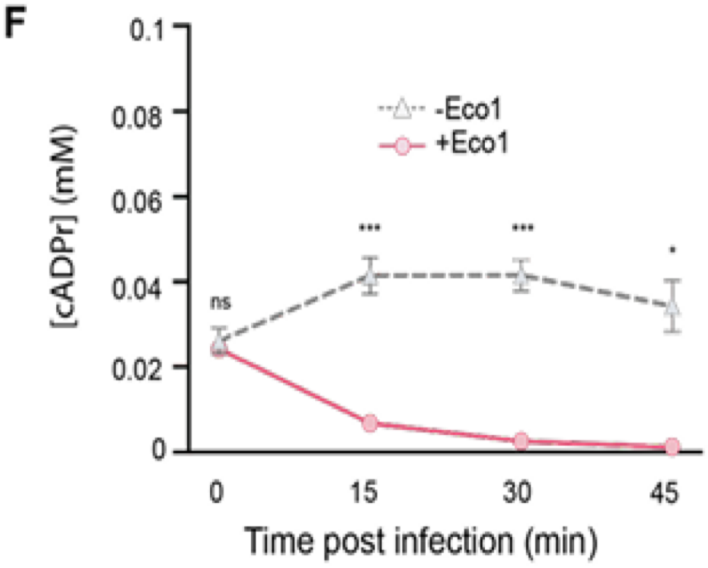
K_m = 883 ± 180 µM

Km is consistent with NAD+ concentration estimated in E.Coli → **Retron-Eco1 can hydrolyze NAD+ under cellular conditions**

Retron-Eco1 activity during active infection causes NAD+ depletion in bacterial cells







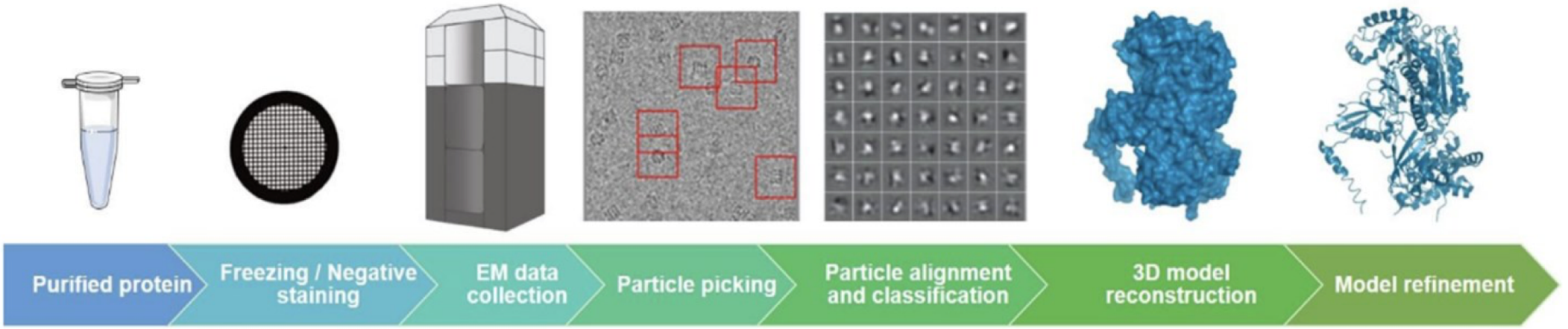
Retron-Eco1 containg cells show a **decrease in cellular NAD+ concentration** during infection

Interestingly, neither of the two ADPr or cyclic ADP ribose were detected → **ADPr and its metabolites are further processed or transferred to other molecules.**

Key points – NAD+ hydrolysis

1) Retron-Eco1 is a retron that provides immunity against T5 and Ukendt phages.
2) The immunity mechanism is an toxin-antitoxin system where the effector ia a protein containing a N-glycosidase domain.
3) During infection, the **N-glyco protein hydrolyzes NAD+ causing its depletion** in the cellular environment.
4) **This process induces bacterial death** interrupting phage replication (Abortive Infection defense system).

Cryo-Electron Microscopy



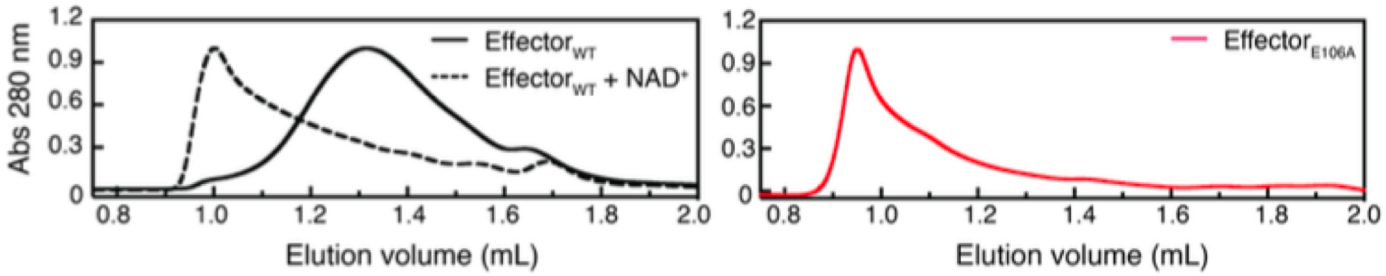
Cryo-EM

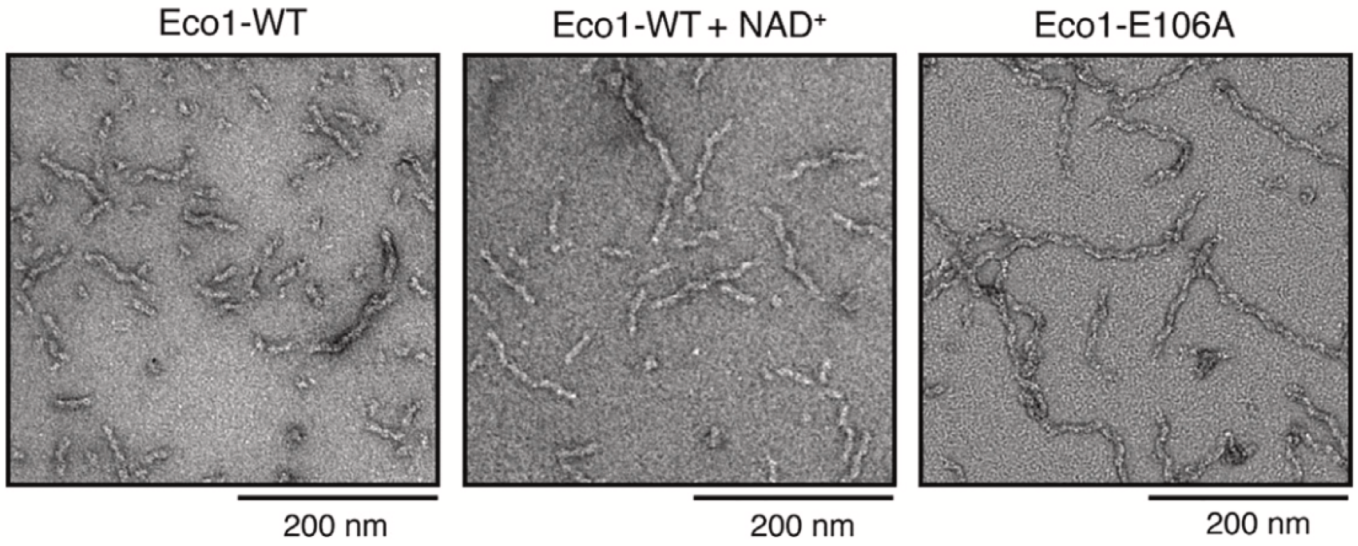
- Allows the determination of **high resolution** structures of macromolecules (3-4Å)
- **Does not require crystals**, therefore is suitable to study complexes that cannot be crystallized
- **Allows to detect different conformation** (in silico purification)

Negative staining

- Preliminary and complementary technique to Cryo-EM
- The sample is coated with a **heavy metal** to enhance the contrast in electron microscopy images obtaining **low resolution** images (20Å)

Retron Eco1 assembles filaments





SEC chromatograms:

- **Eco1-WT** elutes with a wide peak
- **Eco1-WT + NAD+** and **Eco1-E106A** show a narrower peak, shifted towards the dead volume, indicating the formation of larger, more homogeneous structures

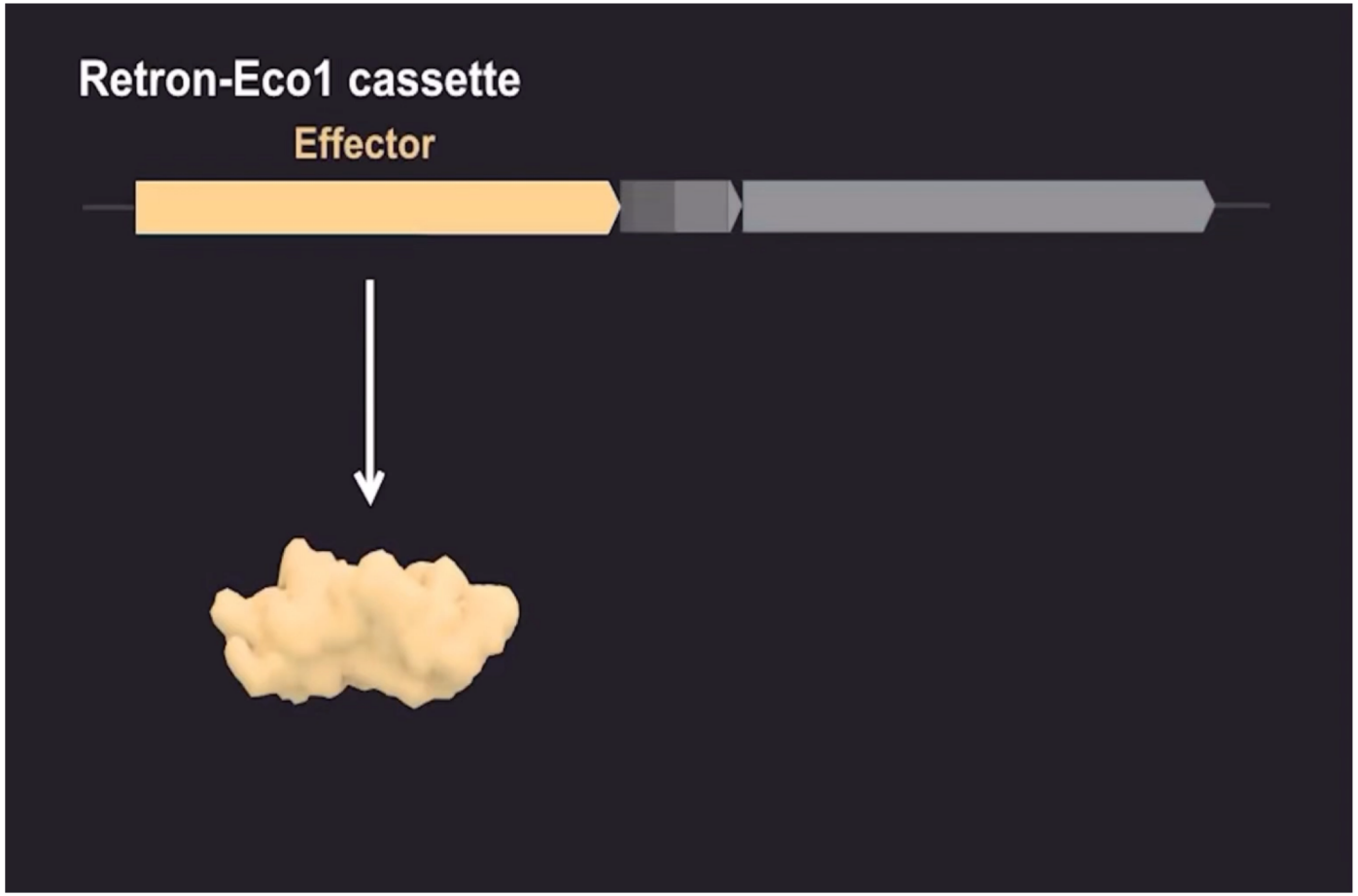
Negative staining images:

- **Eco1-WT** forms heterogeneous structures (dimers + filaments <100 nt)
- **Eco1-WT + NAD+** forms higher-order filamentous structures
- **Eco1-E106A** forms longer filaments (up to 400 nt) in absence of NAD+

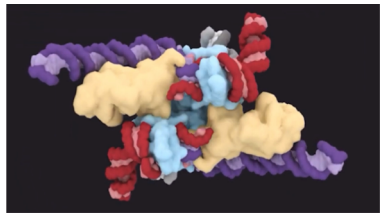
Eco1 filament architecture and formation

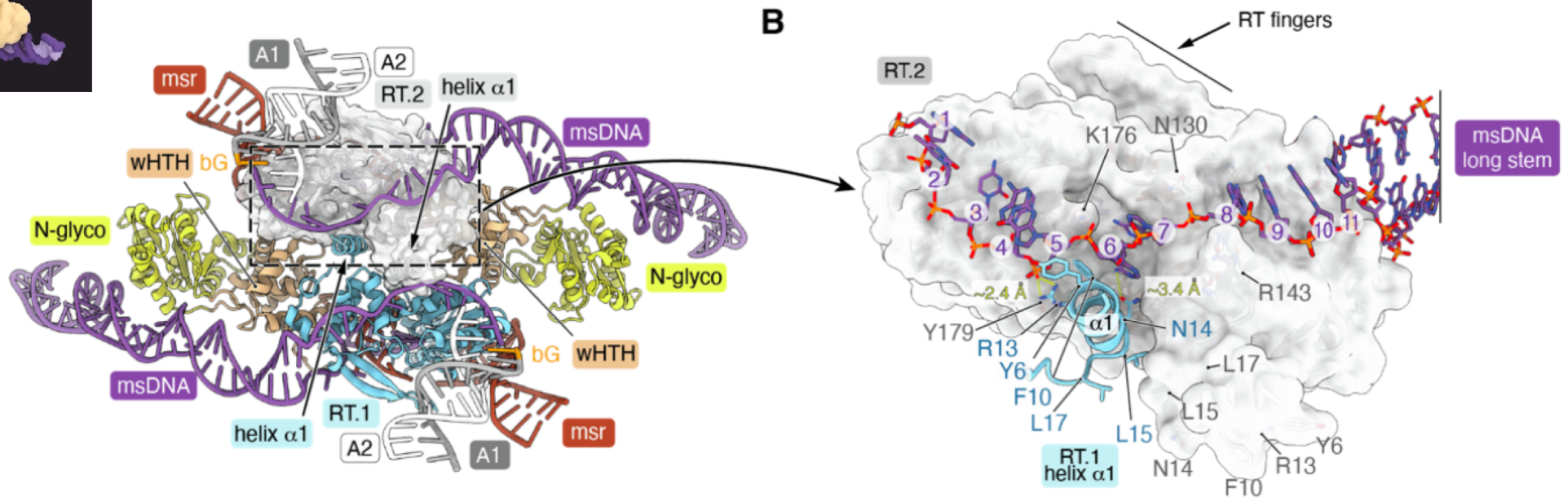
The Cryo-EM structures (Eco1-WT, Eco1-WT + NAD+, Eco1-E106A) reveal that the filament consists of an assembly of the previously reported Eco1 dimers

- Retron-Eco1 assembles into **dimers** that form the basic units of the filament
- The dimers associate to form a **helical filament**

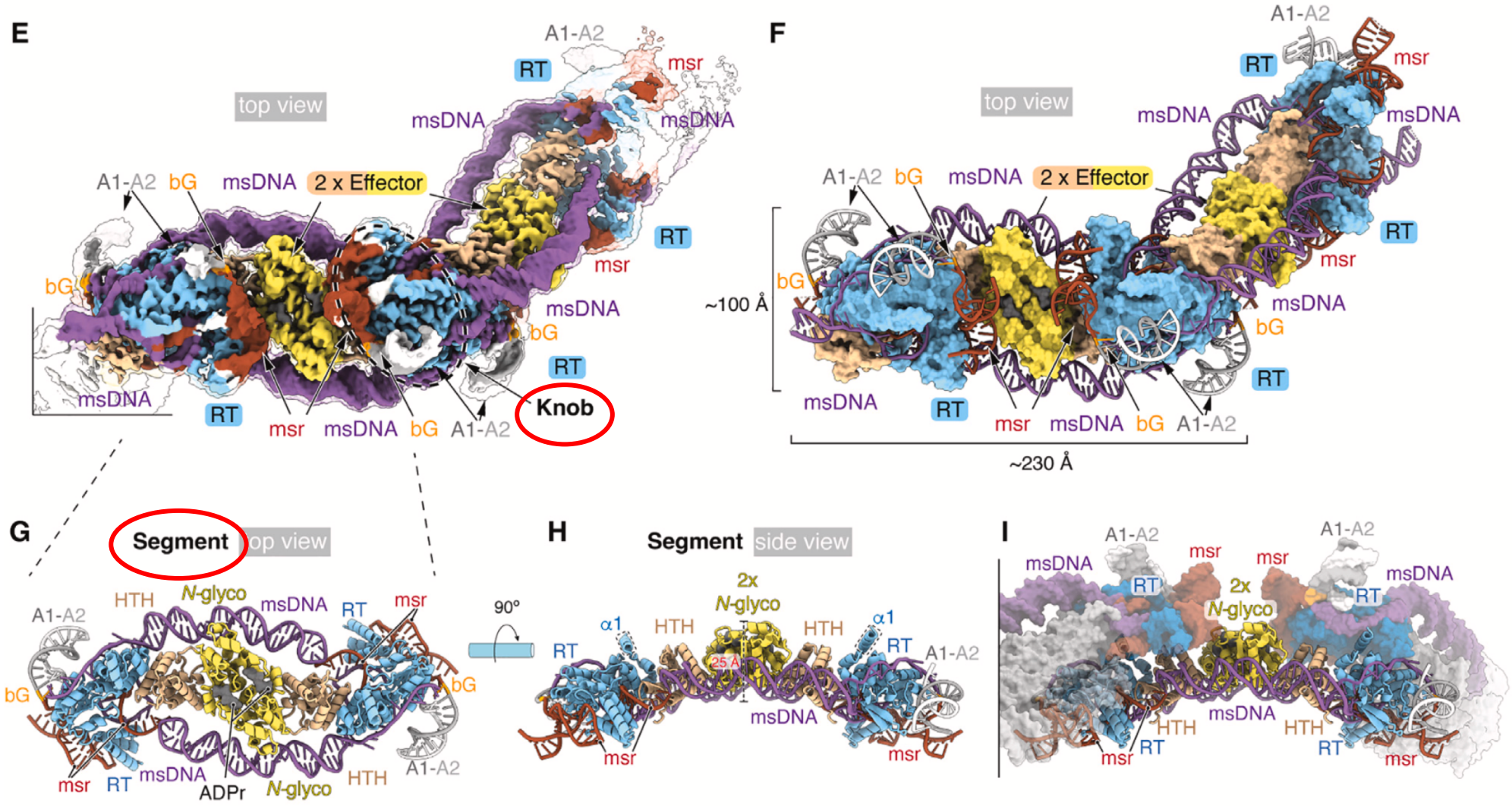


Eco1 dimer structure





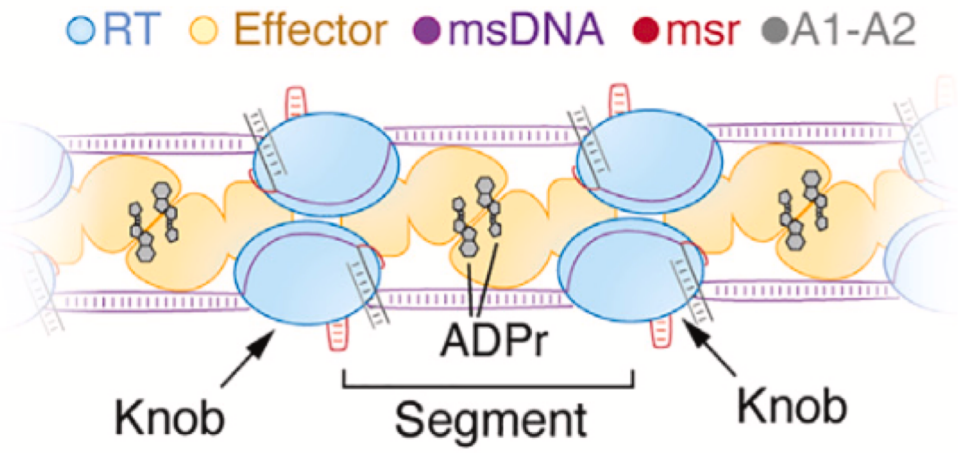
The retron dimer is stabilized by interactions of the **helix α1** of one RT protomer with the surface formed by the other and the nucleotides **dA4**, **dG5**, and **dA6** of the msr-msDNA hybrid



Cryo-EM map and atomic model of Eco1 (E106A) showing two segments of the filament

Architecture of a segment of the WT filament

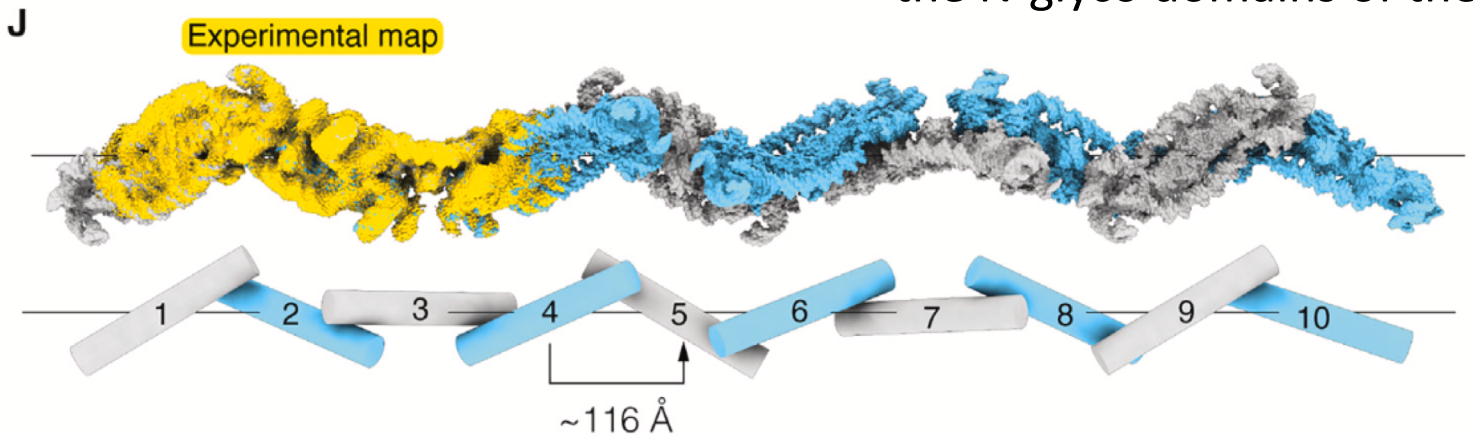
Helical filament model

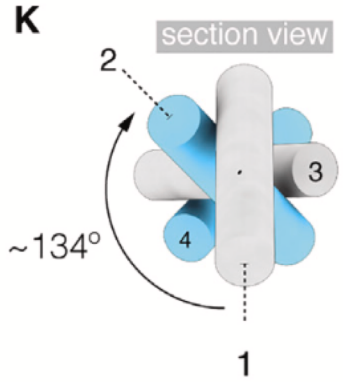


The **stem-loops of msDNA** are positioned in a staggered arrangement that builds a scaffold **encapsulating the effector dimers** along the axial axis of the filament.

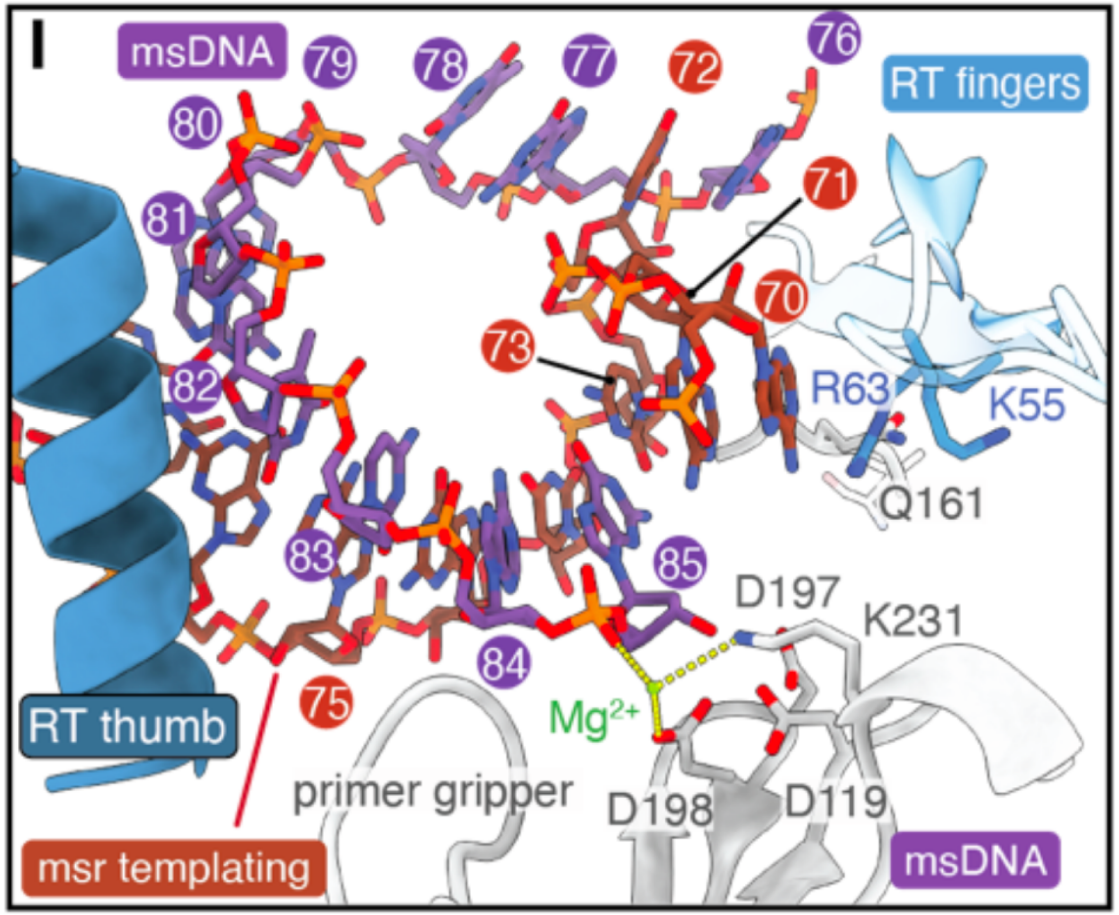
Helical filament model:

- helix pitch: 116Å along the longitudinal axis
- rotation angle: 134°
- a second axis of symmetry at the interface between the N-glyco domains of the effectors



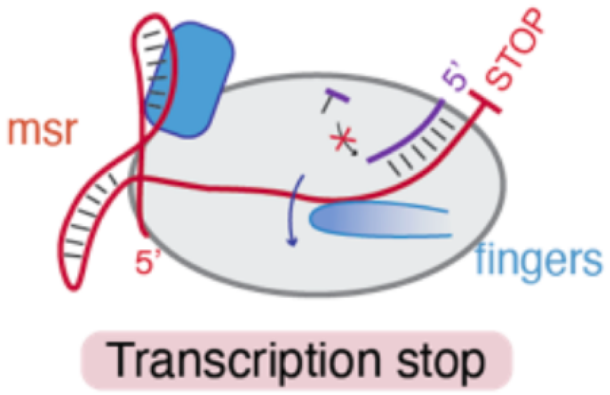


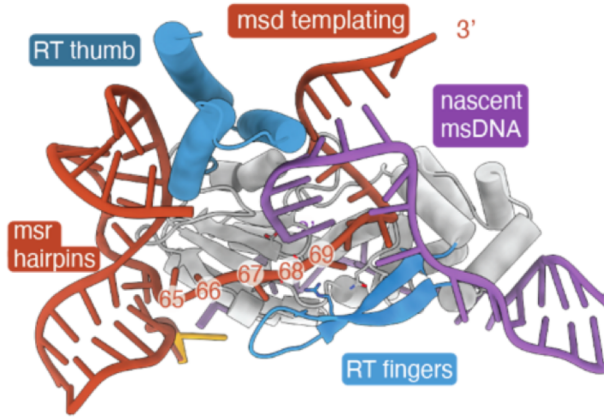
The RT is present in a post-catalytic state

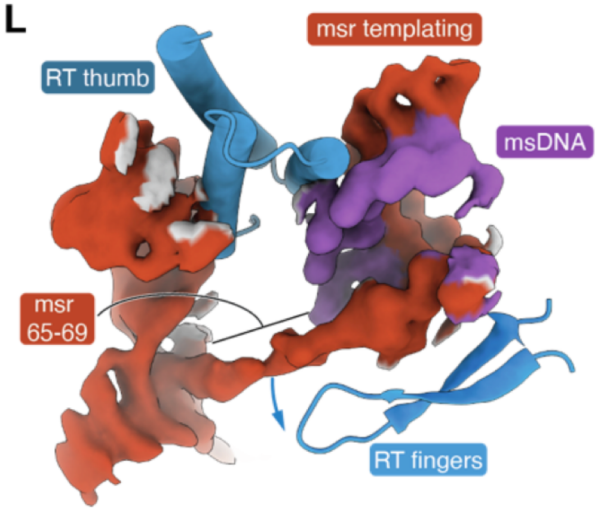


- the **duplex between C73/U82 and dA79/dG85** of the msr-msDNA hybrid (the last nucleotides to be transcribed) is accommodated in the catalytic groove
- **dG85** (the last incorporeted nucleotide) is located near the catalytic residues (D197, D198, D119), and its phosphate backbone is coordinated by Mg2+ and by D198 residues
- **U72** (the incoming nuclotide) is **flipped**, probably due to the conformation of the msr-msDNA hybrid. The base of nucleotide U72 is **stabilised by π-π stacking intera**ctions with nucleotides dA76 and dG77, which prevent U72 from entering the catalytic site.

Reverse transcription termination







- The **finger domain residues R63 and K55** are located 9Å away from the catalytic site and adopt an inactive conformation

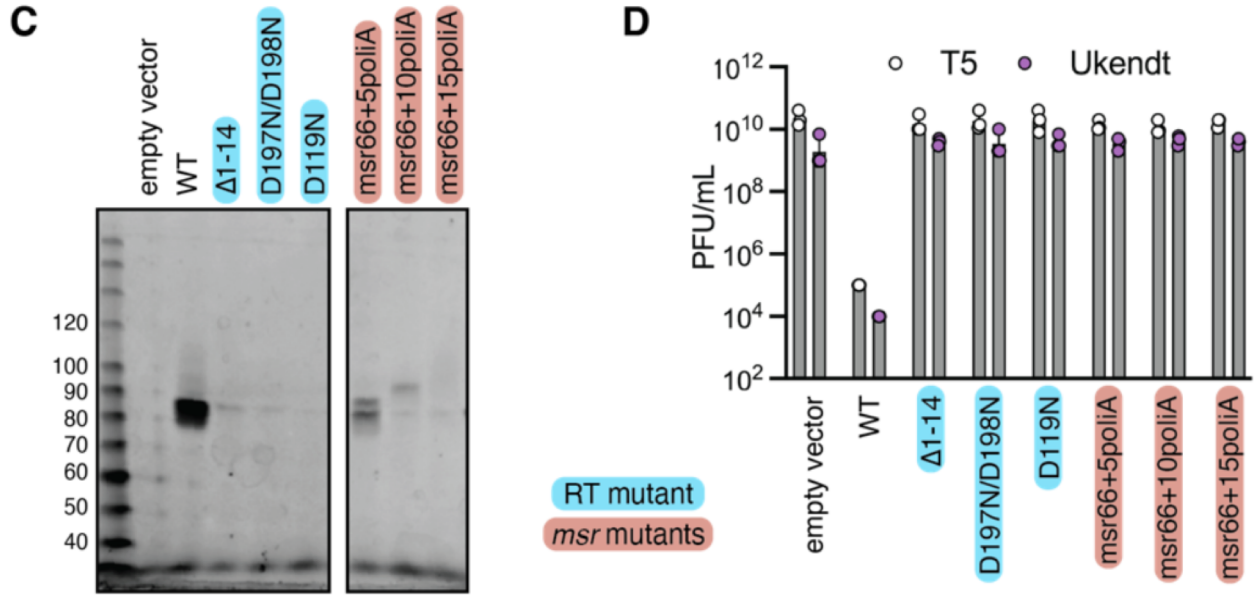
- the **msr region 66–71**, which connects the msr-msDNA duplex with the *msr* hairpins, likely displaces the b hairpin carrying the fingers in this state, preventing the coordination of the next incoming nucleotide

What is the role of the msr 66-71 region in transcription termination?

Role of *msr* region 66–71 in trascription termination

Mutants that include extra nucleotides after the msr nucleotide A66 (msr66+5poliA, msr66+10poliA, msr66+15- poliA):

- Produce msDNA less efficiently
- Produce longer msDNA (msr66+5poliA, msr66+10poliA)
- Abrogate the immunity against phages



- Reverse transcription termination is essential to allow the production of a correct amount of msDNA of the appropriate length, which are essential to confer immunity against phages

- Interfering with transcription termination results in fewer, longer msDNAs and the loss of defence agaisnt phages.

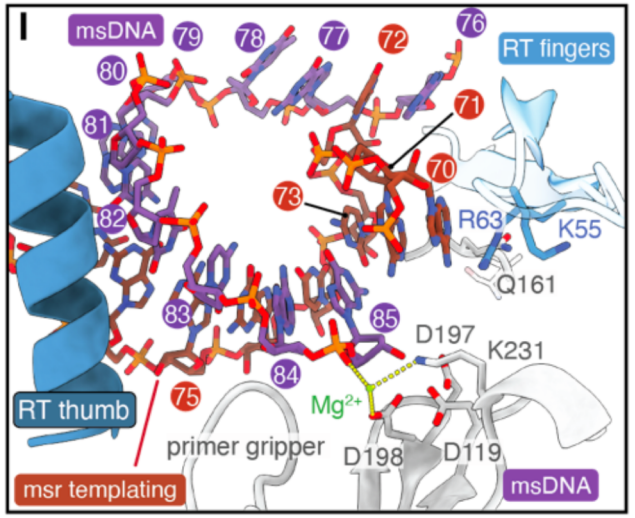
Key points – RT and reverse transcription termination

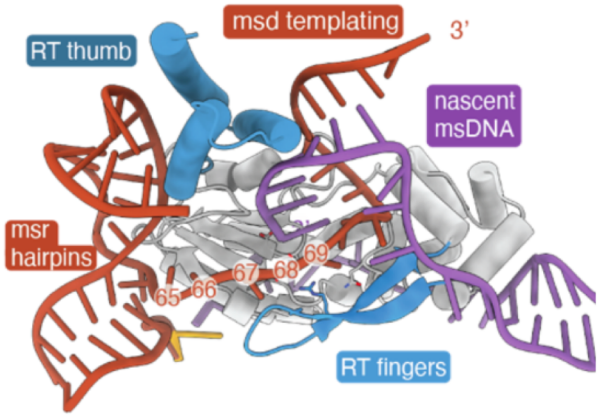
1. The **RT is present in a post-catalytic state**

2. **Reverse transcription termination** likely occurs for two reasons:
   - **Flipping and stabilization of the U72 residue**
   - **Finger dislocation**

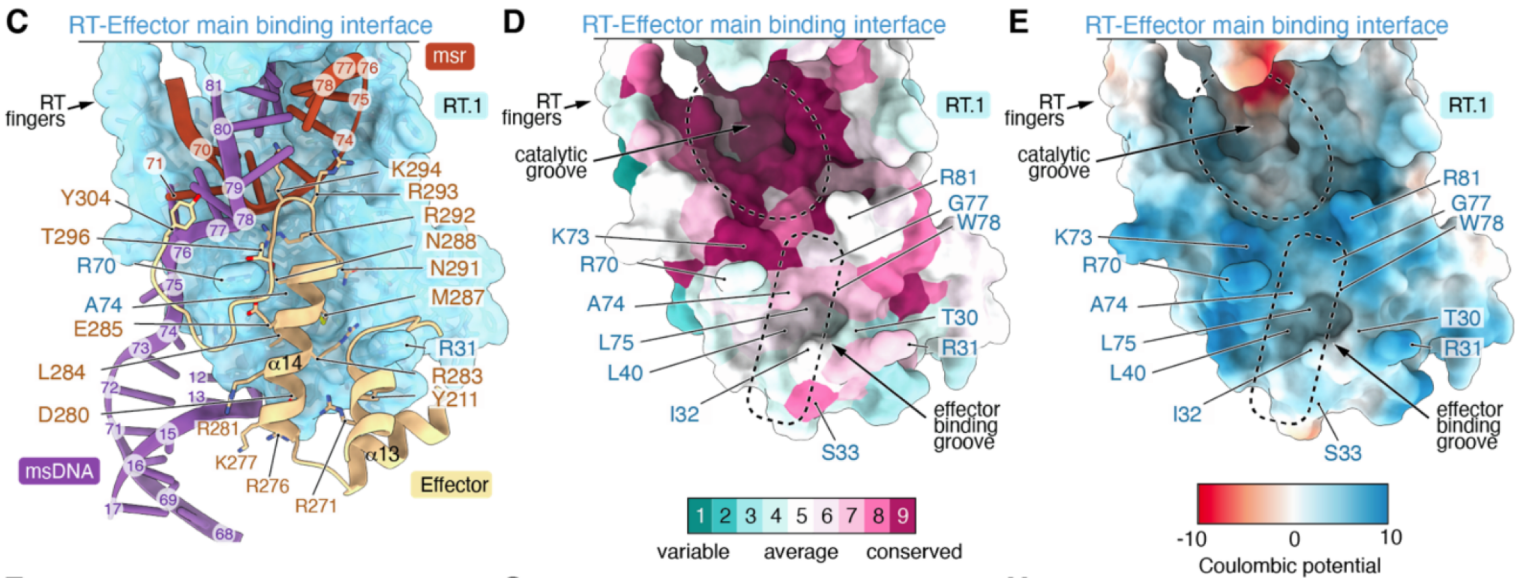
3. Reverse transcription termination is essential to allow the **correct production of msDNA**

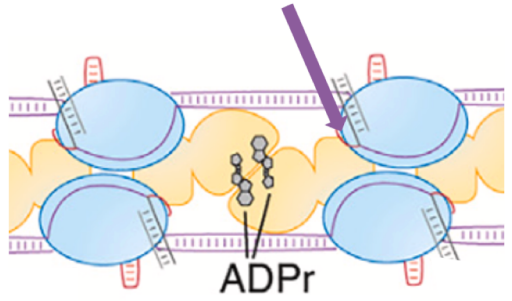
4. Mutants with additional retro-transcribed nucleotides produce msDNA less efficiently and lose immunity against phages





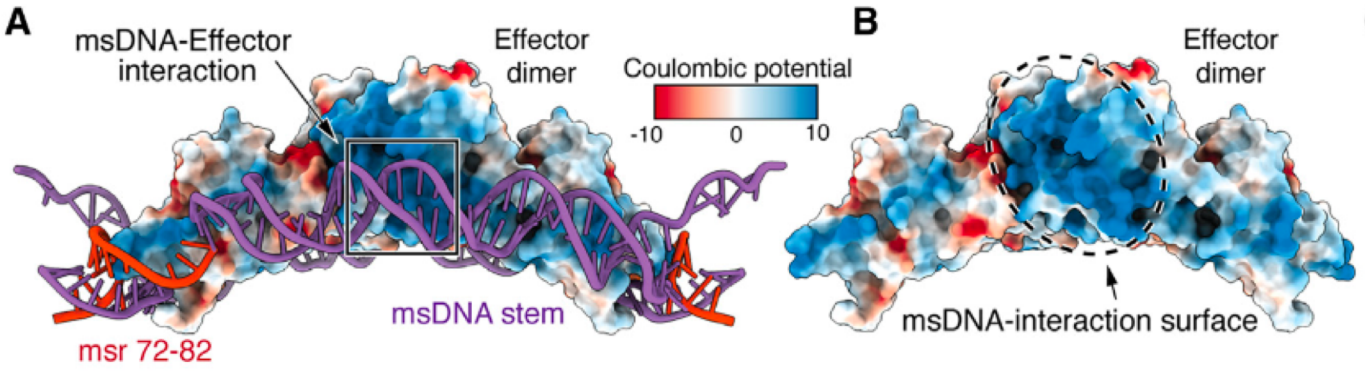
RT-msr-msDNA and effector interaction



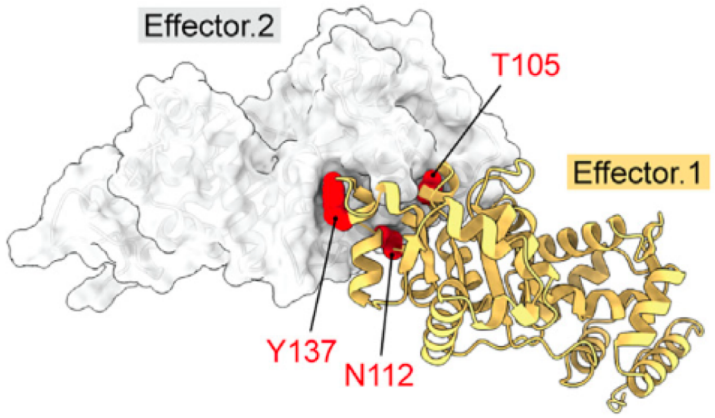


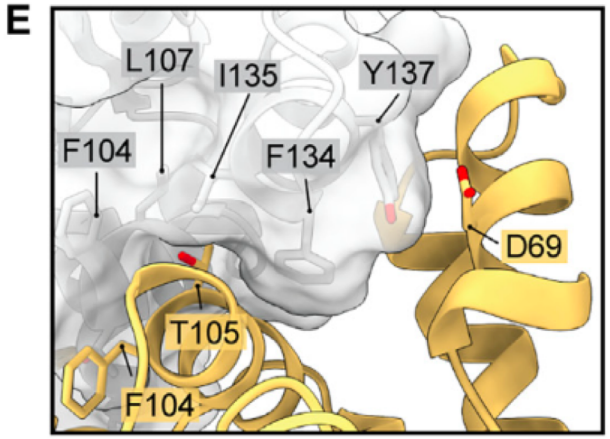
The effector mainly interacts with the RT-msr-msDNA module through the **helix-turn-helix (HTH) domain**.

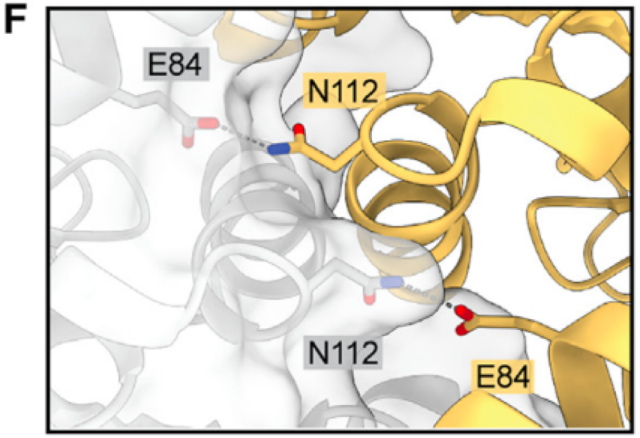
The two antiparallel msDNA stem-loops interact with the **positively charged surface of the effector** and the **final nucleotides of the *msr*** (A80, C81, and U82), encapsulating an effector dimer

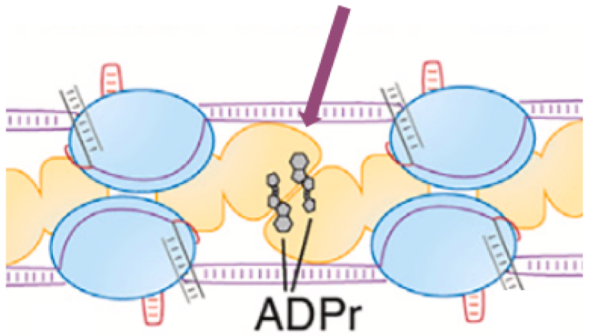


Dimerization of the effector protein







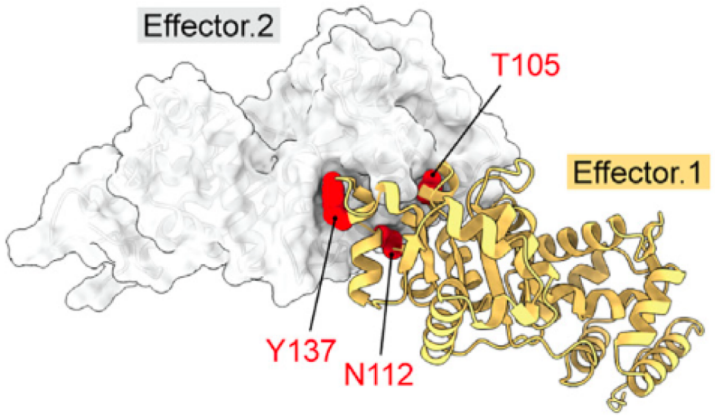


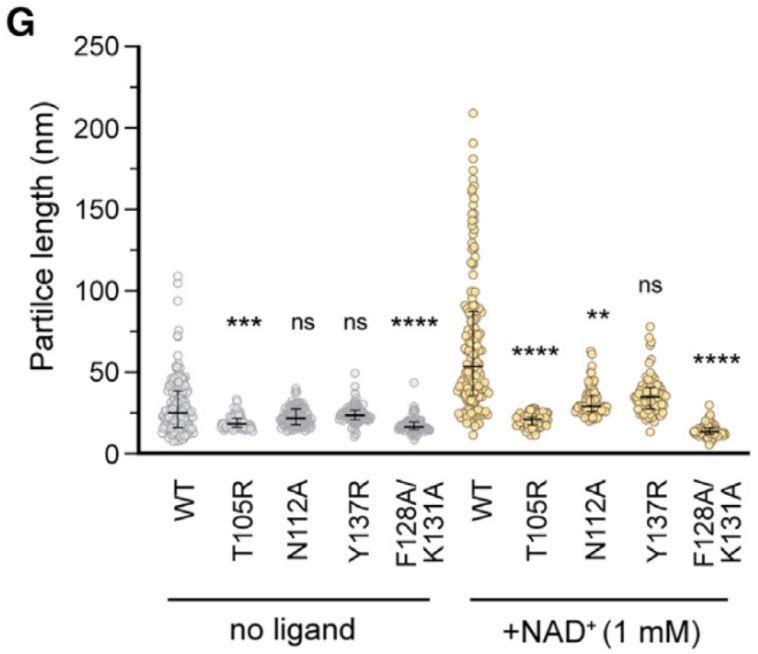
The two effector proteins dimerize at the level of the N-glycosidic domain, at the segment equator

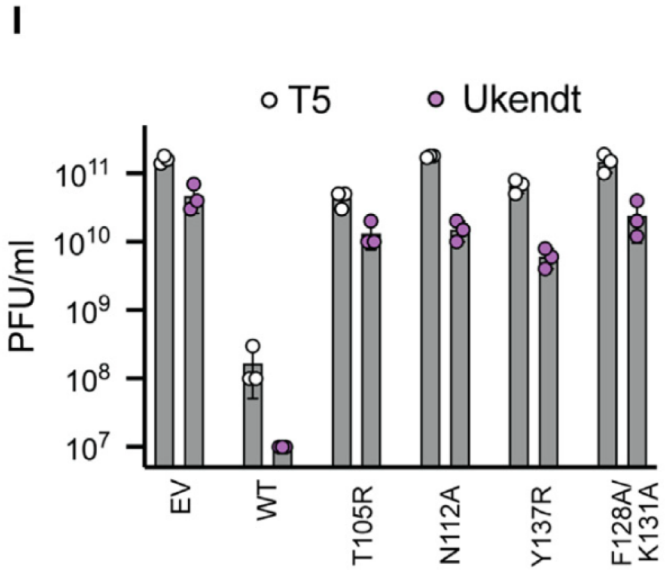
To characterize the essential interactions at the interface between the two effector proteins, different mutants were studied:

- T105R abrogates filament formation, leading to the production of dimers
- Y137R and N112A lead to the formation of shorter filaments

Dimerization is essential for filament assembly and defense







T105R, N112A and Y137R mutations impair correct filament formation and cause the loss of immunity against phages

Key points – effector's interactions

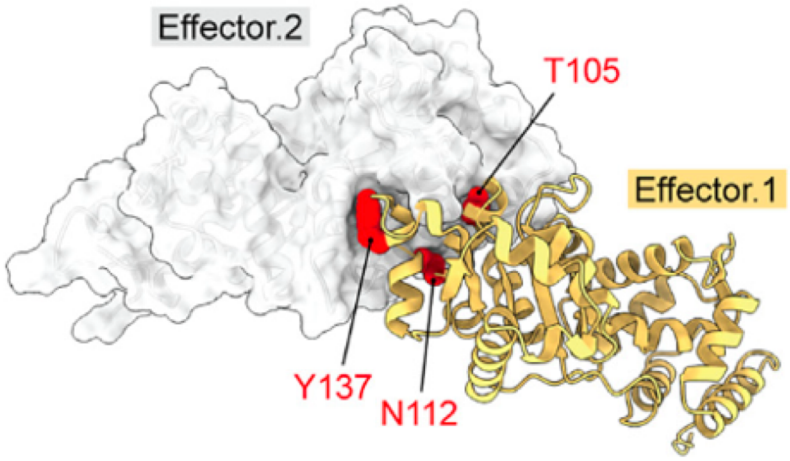
1) The effector interacts with the RT-msr-msDNA complex through a **HTH domain**

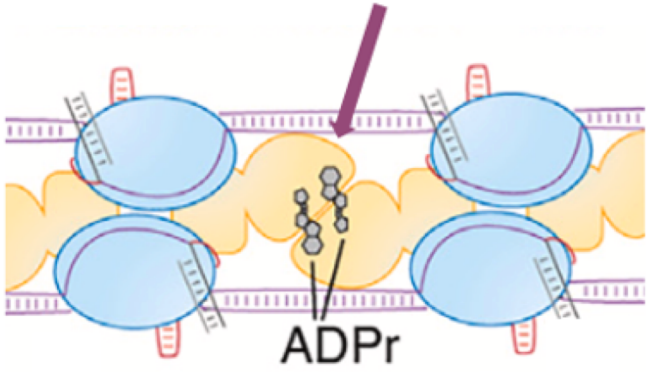
2) The **msDNA stem-loops** interact with the **positively charged amino acids** on the effector's surface, **encapsulating an effector dimer**

3) The **two effectors dimerize at the N-glycosidic domain**

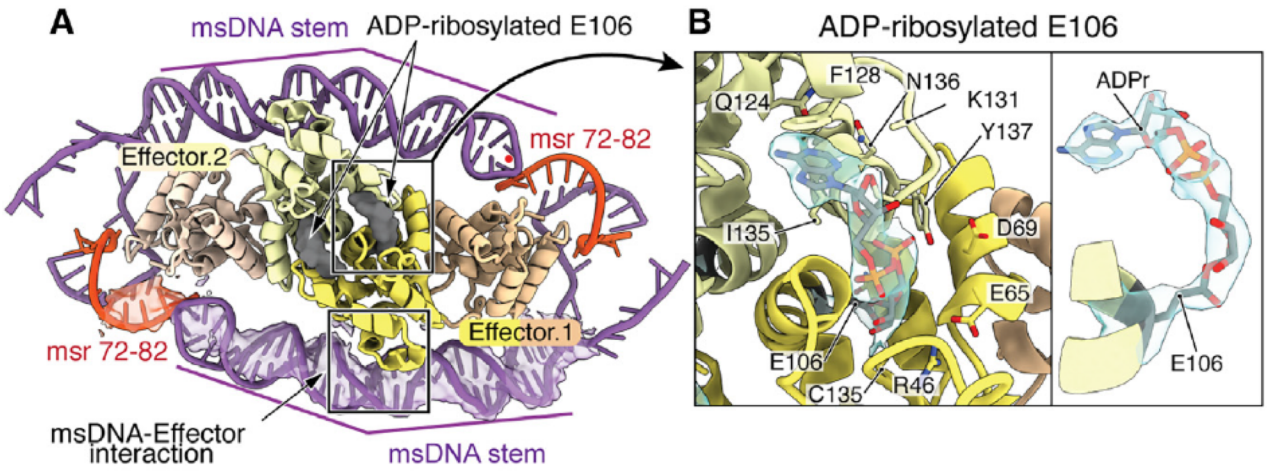
4) The effector's **dimerization is essential for filament assembly and defense**

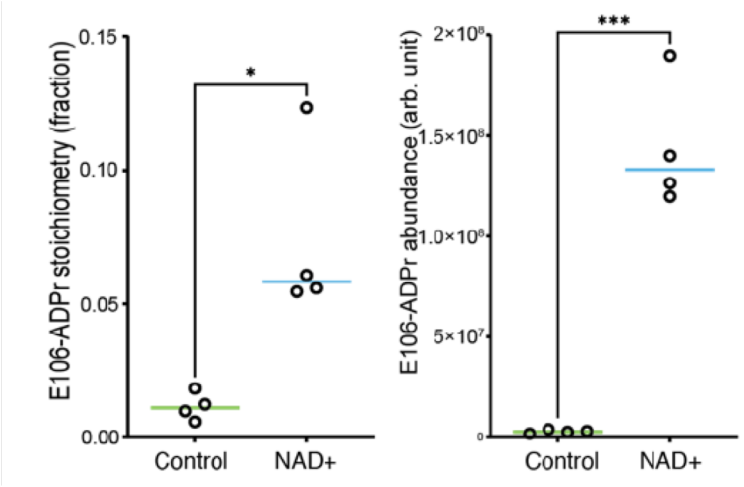
5) Mutantions in residues important for these interactions abrogate filament assembly and immunity against phages





ADPr is covalently bound to the catalytic residue

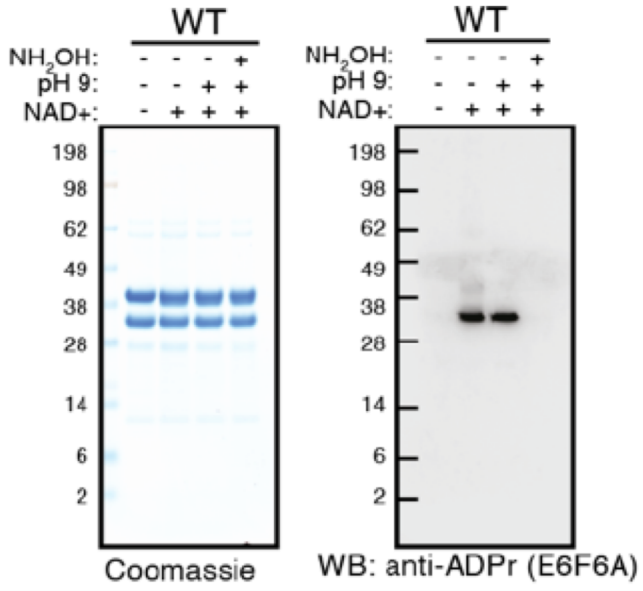


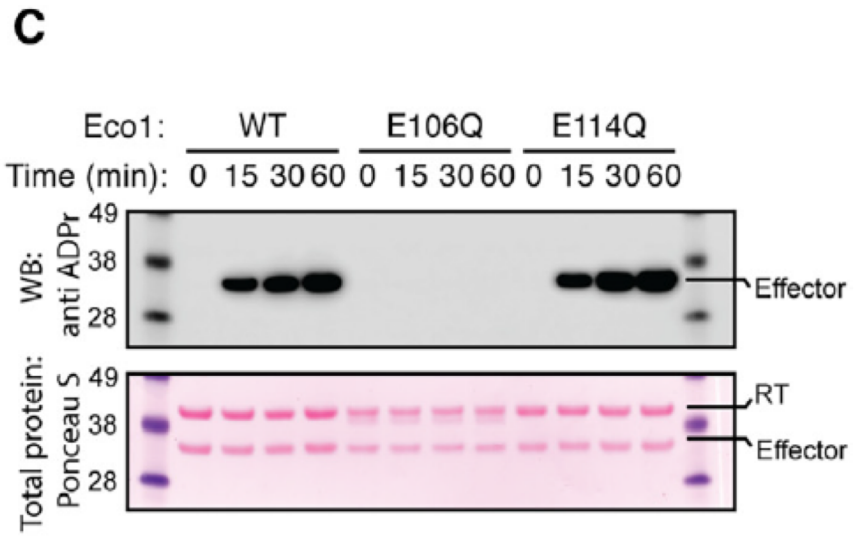


Additional **densities** in the effector dimer indicate the presence of two ligands. MS analysis reveal the ligand's chemical nature: **ADPr** covalently bound to the effector upon NAD+ addition.

Putative targets: **E106** and E114

ADPr is covalently bound to the catalytic residue



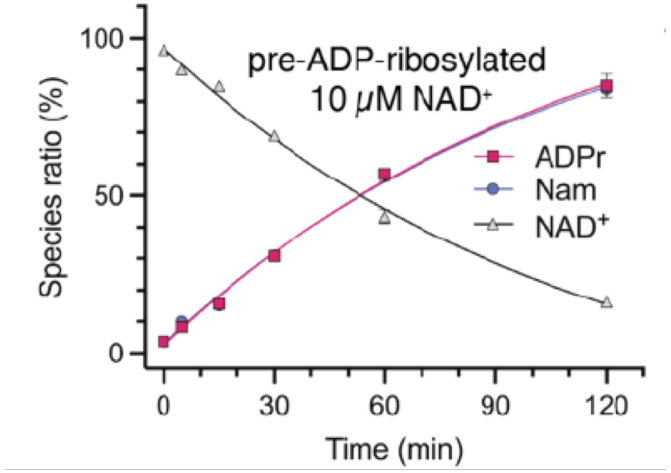


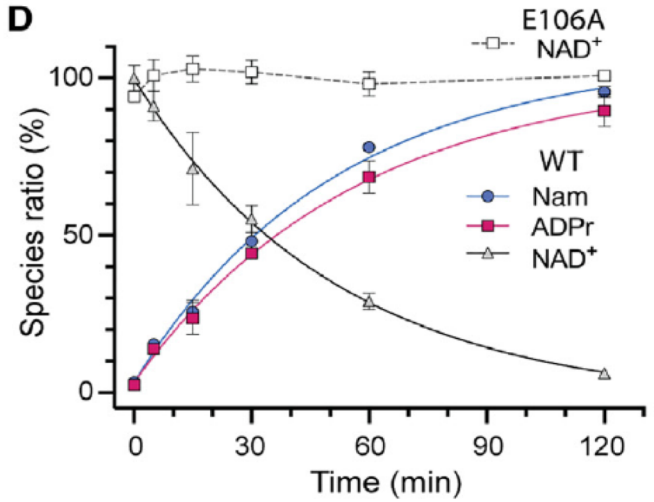
Hydroxylamine treatment **reverts** the ADP-ribosylation of E106. Effector ADP-ribosylation is abolished with **E106Q mutant** and no effects are observed with **E114Q mutant**.

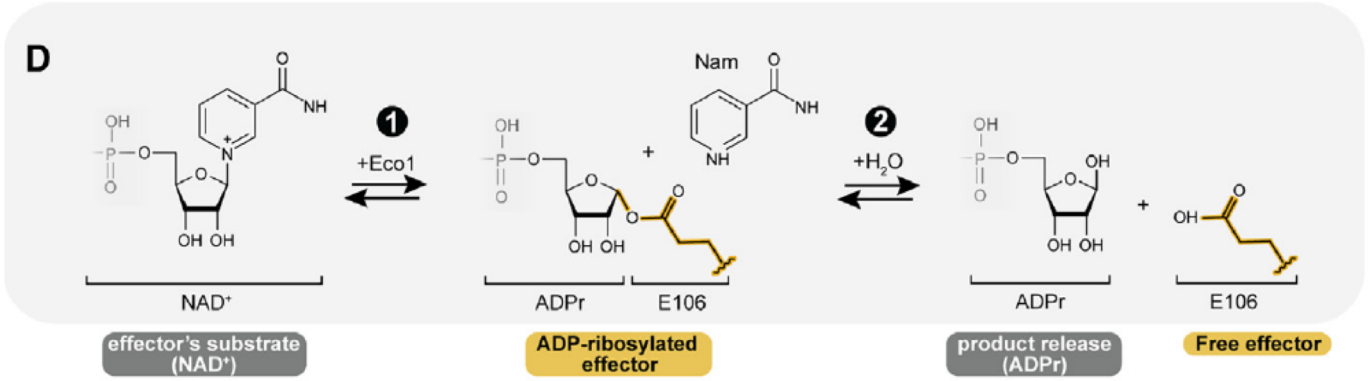
The side chain of **E106 is covalently bound to ADPr**, one of the NAD+ degradation products.

ADPr-E106 is an enzymatic intermediate

The effector is catalitycally competent in the filament form, but hydrolyzes the newly added NAD+ at a **slightly slower rate** than the non ADP-ribosylated form.



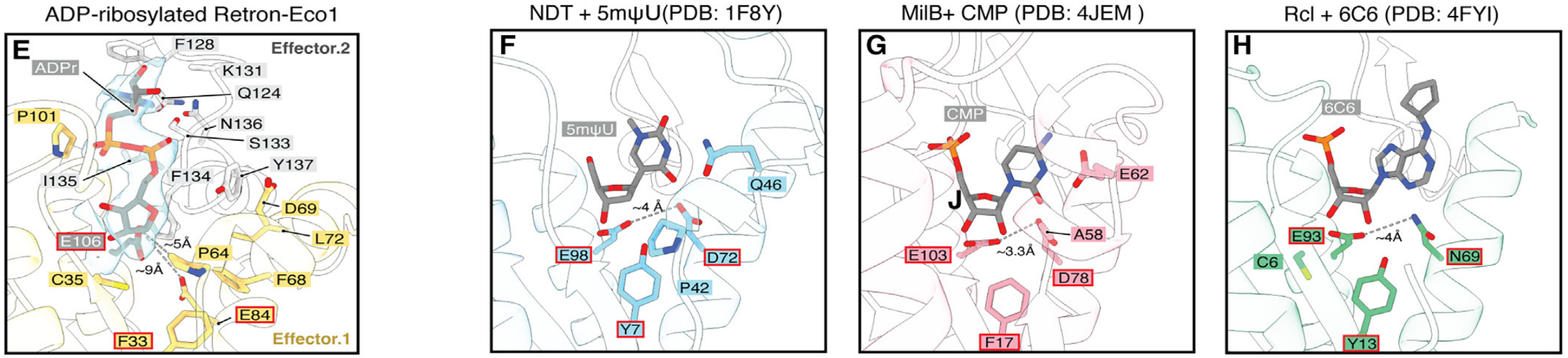




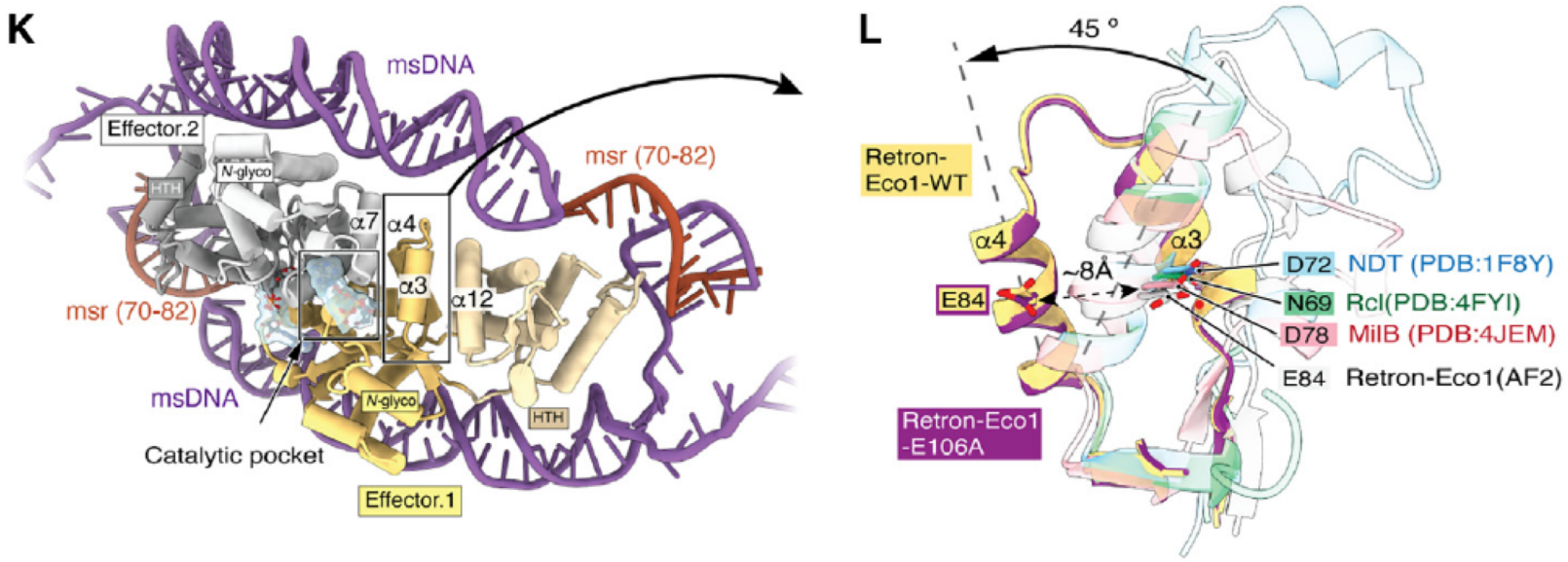
ADPr-linked E106 does not irreversibly inhibit NAD+ hydrolysis. ADPr-E106 is an **enzymatic intermediate** in a NAD+ hydrolysis **two-step mechanism.**

Two-steps NAD+ hydrolysis reaction: E106, F33, E84 catalytic residues

Like other NDT homologs, Retron-Eco1 has a **catalytic triad** in the active site. The three residues in Retron-Eco1 are **E106**, **F33** and **E84** and have different roles in the NAD+ hydrolysis reaction. The effector's **E106** works as a **nucleophile**. The specific roles of F33 and E84, on the other hand, are still hypothetical and based on comparison with their homologs.



The active site is in a catalytic inefficient configuration

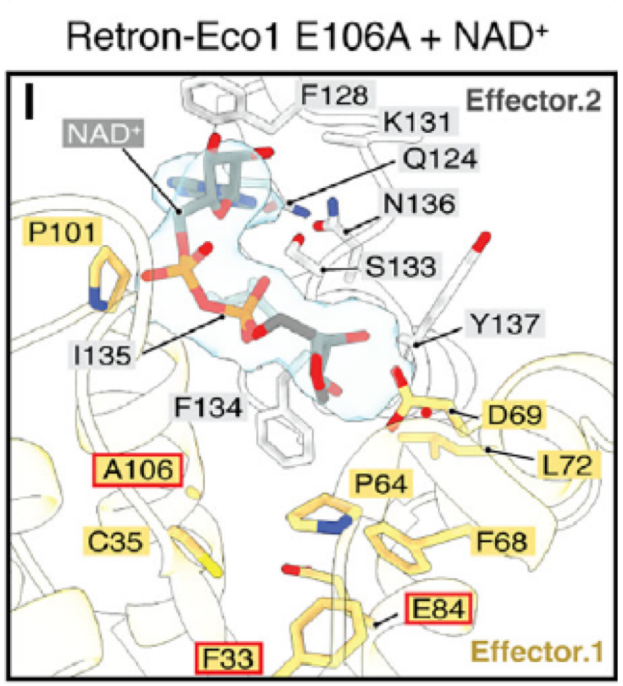


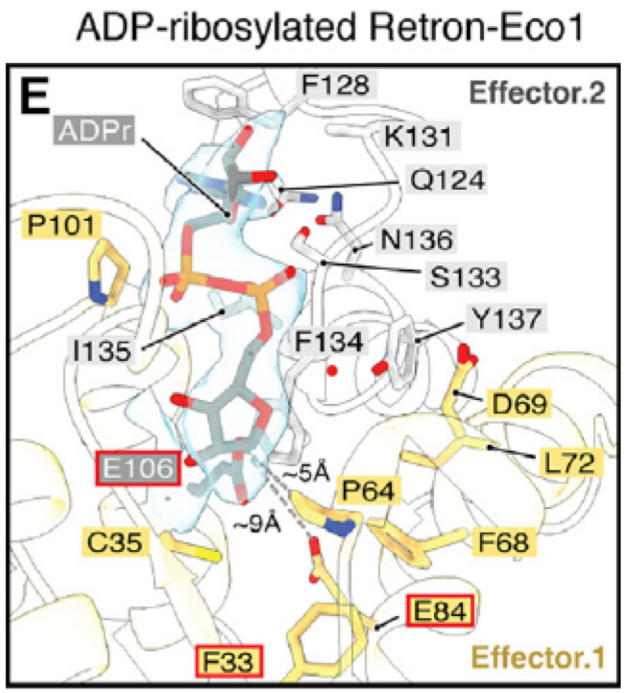
The segments undergo **rotational movements,** transmitted to the effector proteins. However, the effector's a3-a4 helical hairpin remains locked, resulting in a 45° shift compared with other NDTs.

Conformational analysis reveal that Retron-Eco1 filament stabilizes the effector protein in a **catalytically suboptimal state**. These observations explain the filament's **low NAD+ hydrolysis** activity and the **stabilization** of the ADP-ribosylated E106 **intermediate**.

The E106A mutant stabilizes a substrate-bound form

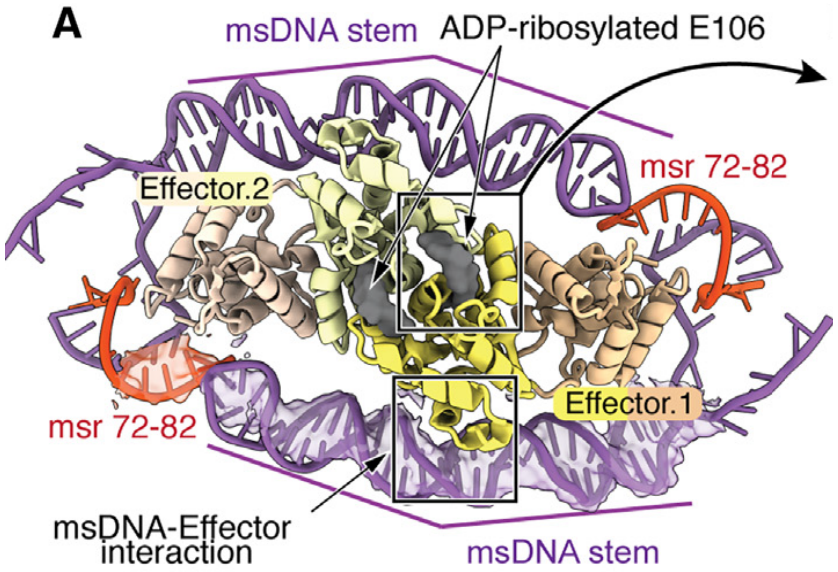
The density represents the **substrate** of the reaction (NAD+).
This explain the formation of long filaments observed in the Retron-Eco1-E106A mutant, as the bound NAD+ cannot be hydrolyzed, thus **stabilizing the assembly**.



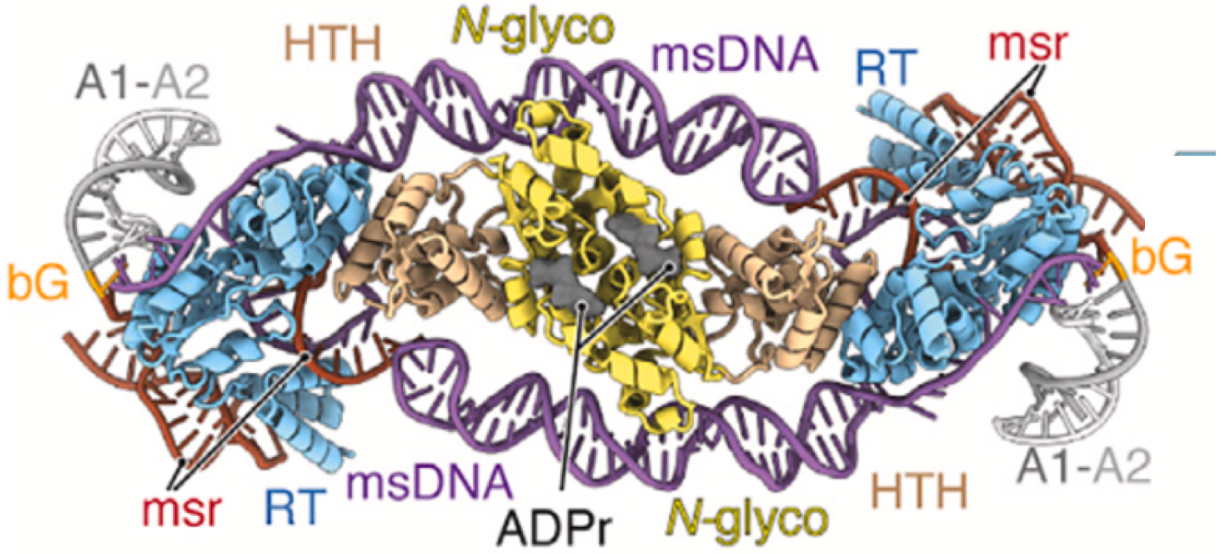


Key points – Effector protein active site

1) ADPr is **covalently bound** to the catalytic residue E106 in the **filament form**.
2) ADPr-E106 is an **enzymatic intermediate** in a two step reaction, with a low NAD+ hydrolysis activity.
3) Normally the ADPr release would occur with conformational changes of the enzyme.
4) In the filamentous structure, the **msDNA does not allow conformational changes,** blocking the reaction in the intermediate step and leaving the enzyme in a **catalytically suboptimal state**.



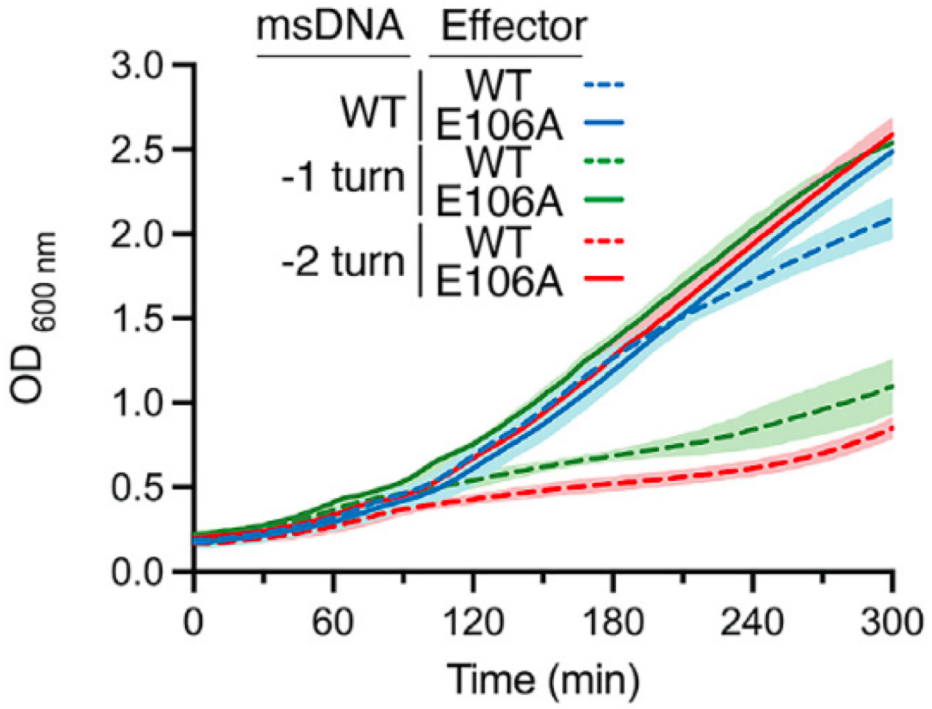
The role of msDNA



- Modifications of msDNAs has been reported as a common line for the activation of retrons, althroug the mechanisms by which changes in msDNA lead to retron-mediated toxicity are unclear
- In retron Eco1, **the msDNA stem-loops encapsulate the effector dimer**

Hypothesis → the msDNA stabilizes the filament structure

Shorter msDNA inhibits cell growth



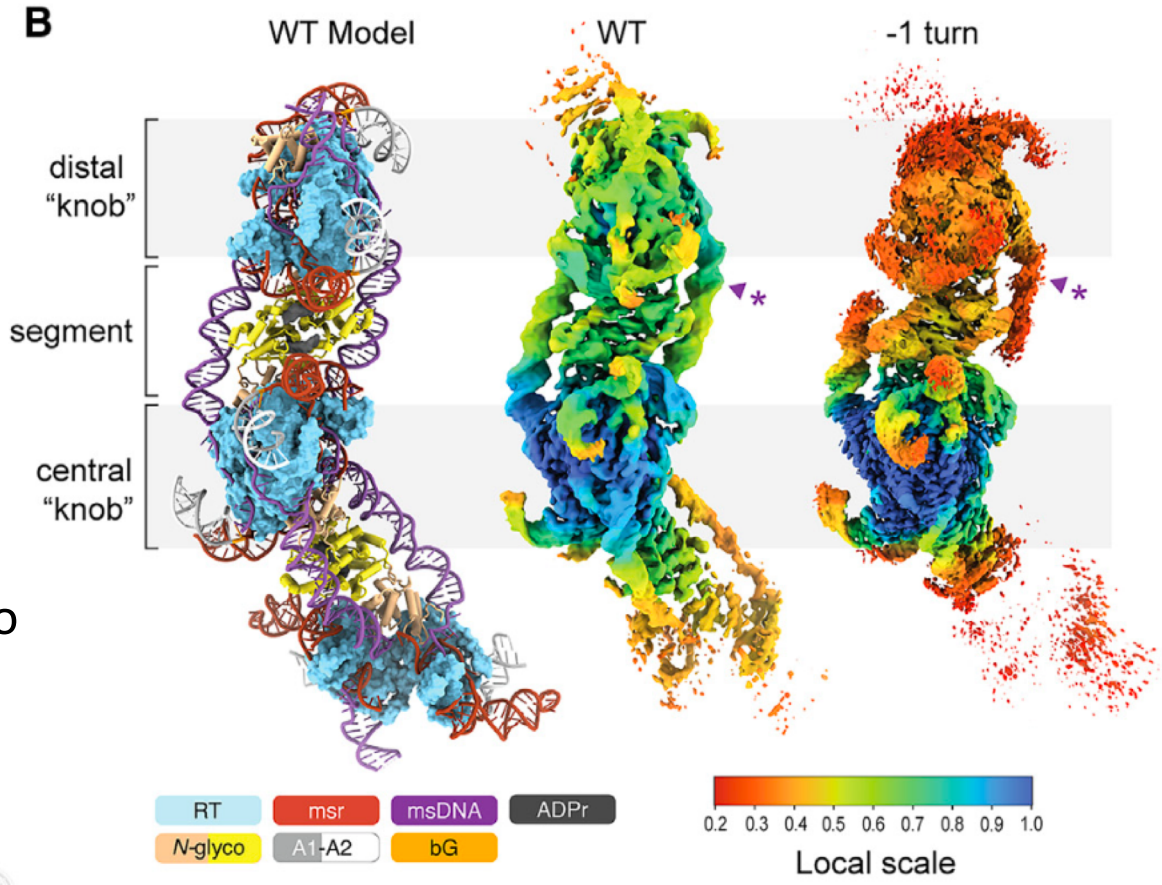
To test this hypothesis **2 mutants** were created: **-1 turn mutant** and **-2 turn mutant**, which shortern the msDNA stem-loops by one and two turns of the DNA duplex respectively

- The expression of mutants with shorter msDNA **reduces bacterial growth**
- The phenotype is reverted when the effector-inactivating E106A mutation is present suggesting **growth defect arose from *N*-glyco activity**

In the mutants expressing shorter versions of the msDNA the effector is present in a catalitycally active form, and its N-glico activity inhibits cell growth

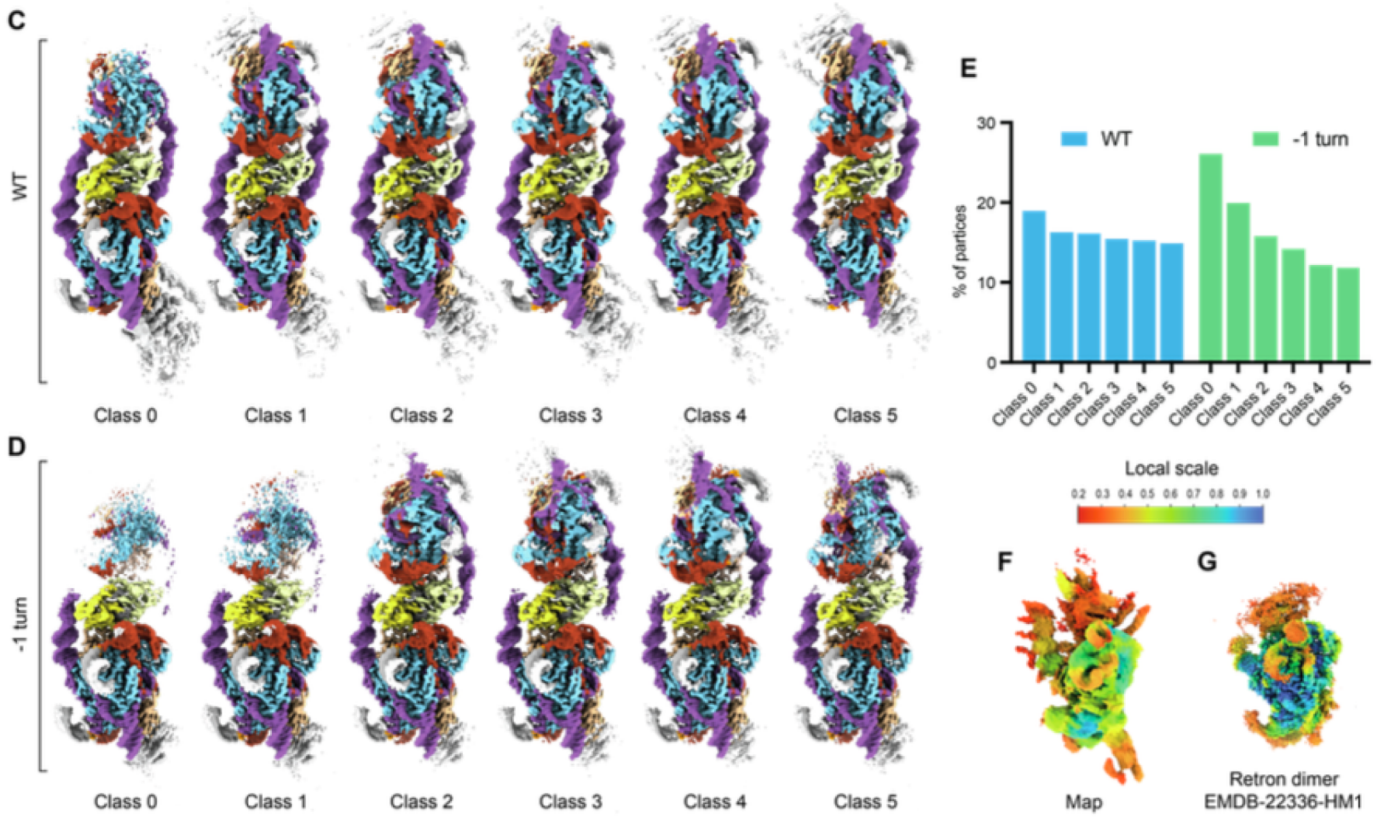
Charachterization of the catalitycally active complex

- In the mutants expressing shorter versions of the msDNA the effector is present in a catalitycally active form
- Attempts to purify the **-2 turn mutant** resulted in low yield due to its toxicity, limiting further characterization
- The **-1 turn mutant** structure is similar to the WT but with **lower local resolution of the distal knob region** suggesting the **flexibility and misalignment** of these components



Comparison between the consensus maps for Retron-Eco1-WT+ NAD+ and 1 turn mutant + NAD+. The local quality of the maps is plotted on their surfaces (**Blu** → high, **Red** → low)

3D classification analysis

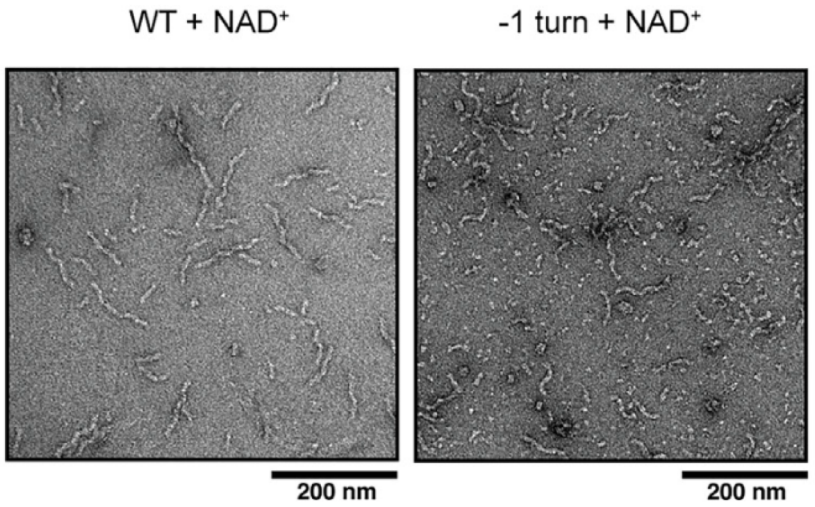


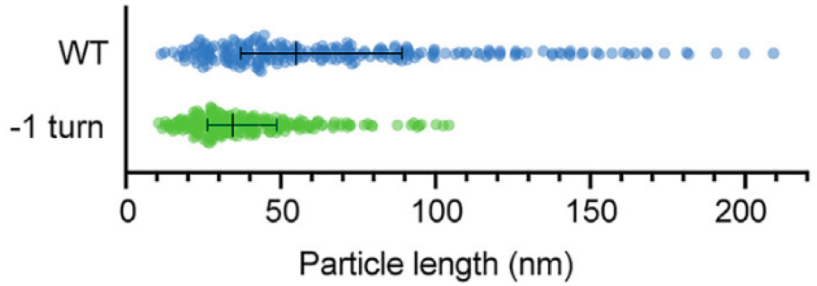
The **3D classification** of the CryoEM images revealed that:

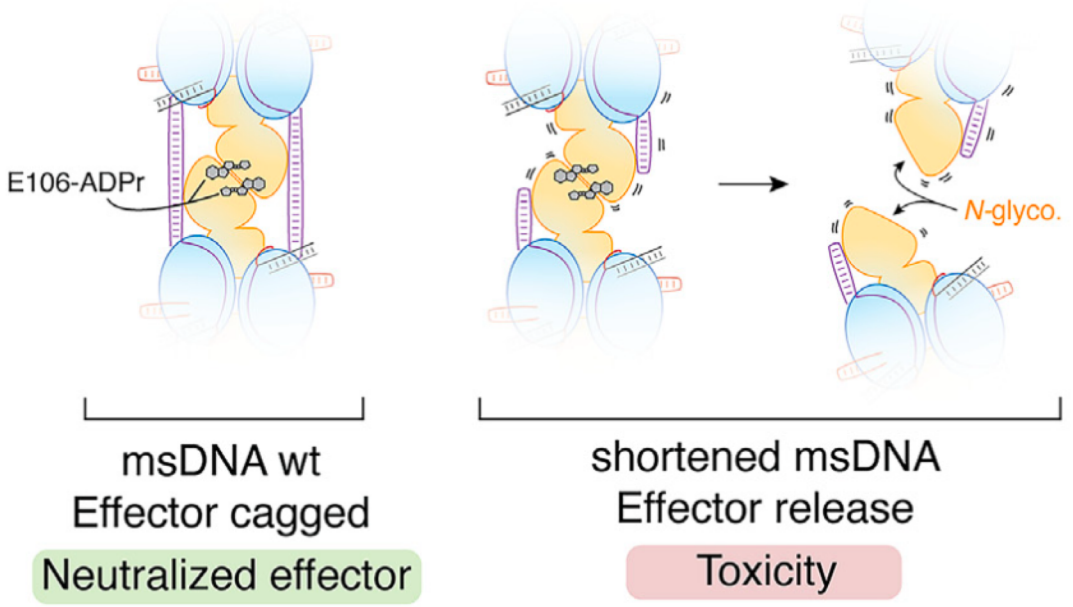
- **classes 2-5** assemble more heterogenous filaments compared to the WT
- **classes 0-1** show a weaker density at the distal knob indicating greater conformational changes than the WT
- **11% of the total particles** show low density at the catalytic domain level reminiscent of the **Eco1 dimer** which probably represents the **disassembled form,** in which the catalytic N-glycosidic domain has **greater flexibility** that allows it to **undergo all the conformational changes necessary for effector activity.**

The msDNA prevents the effector's toxicity

-1 turn mutant forms shorter fialments than Eco1-WT + NAD+



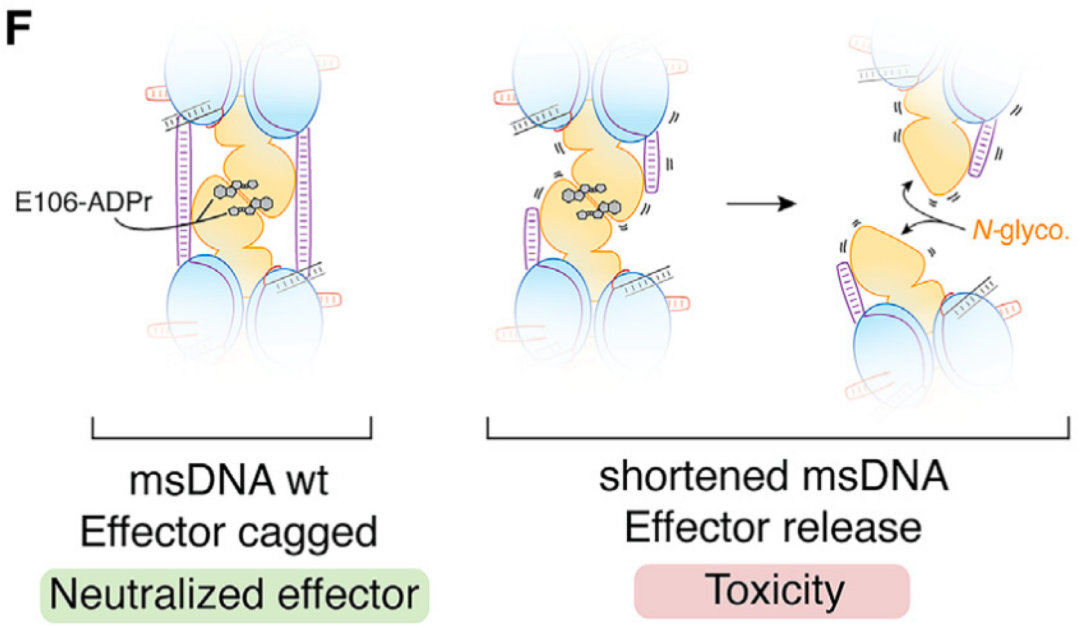




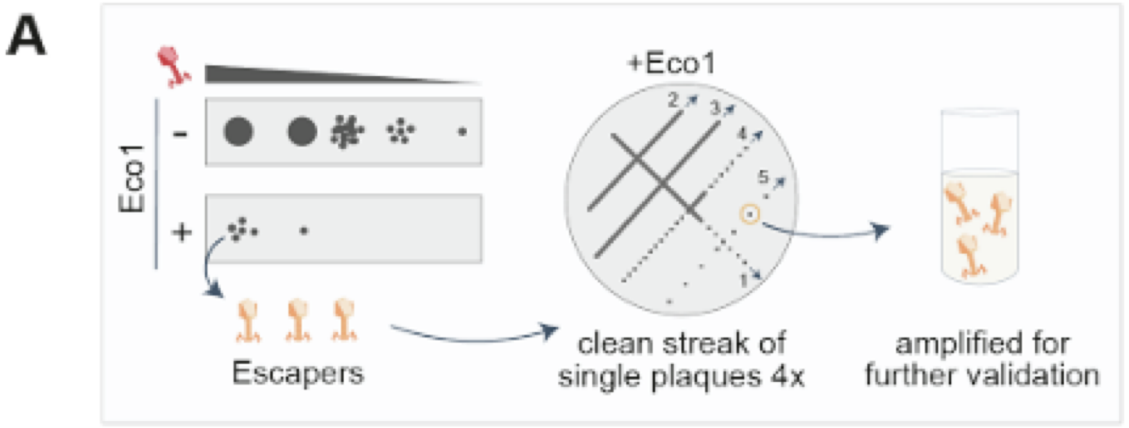
- The **msDNA stem-loops stabilizes the filament** structure caging the effector protein in a suboptimal state of activity
- The **shortening of msDNA stem-loops** causes the **release of the effector's N-glycosidase domain** from the cage that is normally created in the filament and thus induces toxicity

Key points – msDNA's role

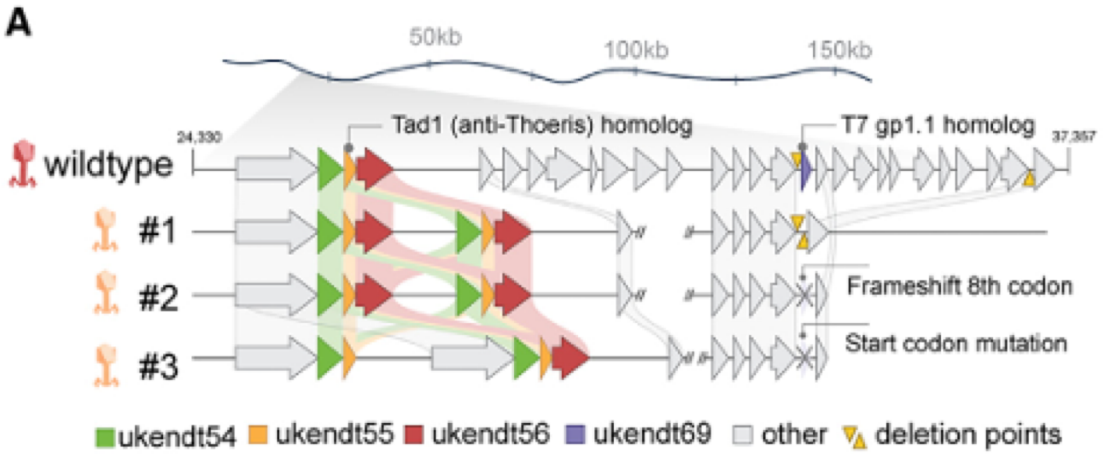
- The **msDNA stem loops encapsulate the effector dimer**, keeping it in a **suboptimal state**
- The **shortening of the msDNA** (-1 turn mutant and -2 turn mutant) causes the:
  1) **Destabilization of the filament**
  2) **Effector release,** which is no longer constrained by the msDNA and can switch through all the conformations necessary for N-glyco activity
  3) **Toxicity** due to N-glyco activity



Phages accumulate mutations to escape the Retron-Eco1 immunity

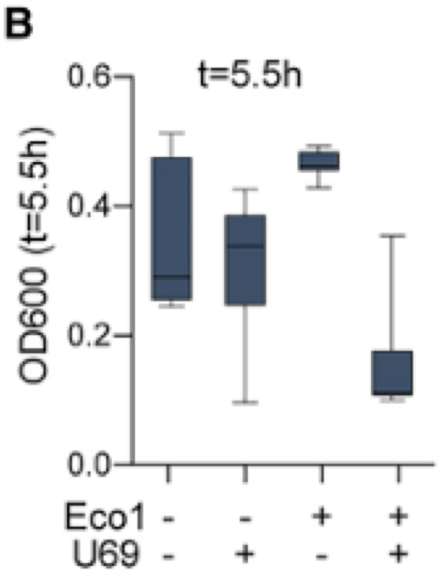


Selection of resistant phages in E. Coli cultures expressing Retron-Eco1.

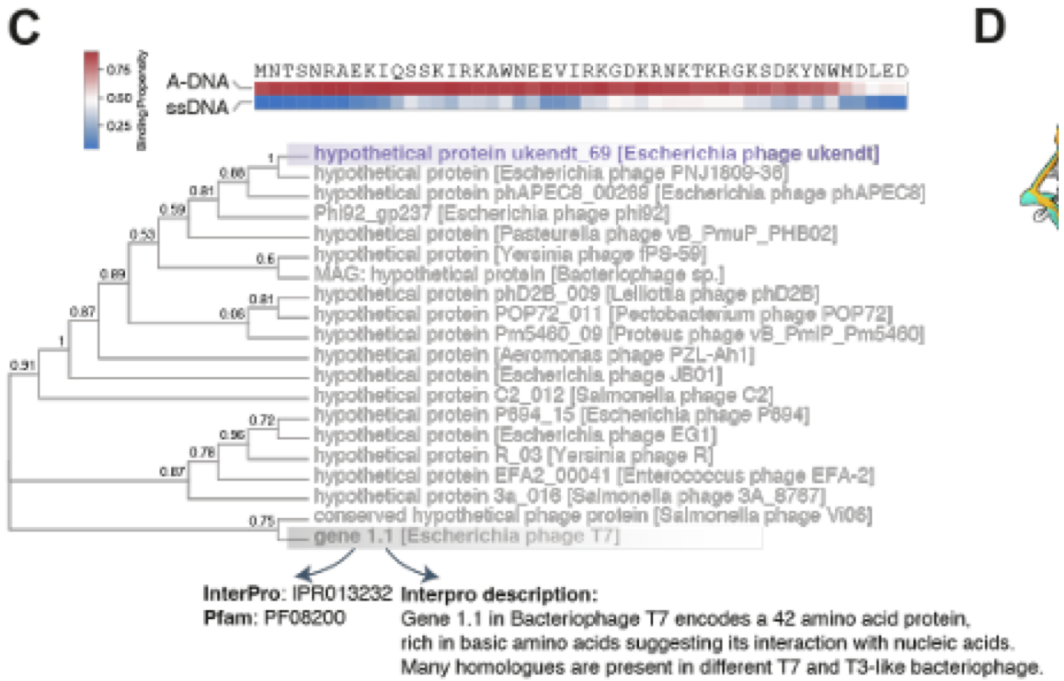


WG-seq and comparative genomic analysis between resistant Ukendt and WT phages showed the presence of mutations localized in two regions: **U54-U56 genes** and **U69 gene**

Role of U69 gene in the retron-escape mechanism

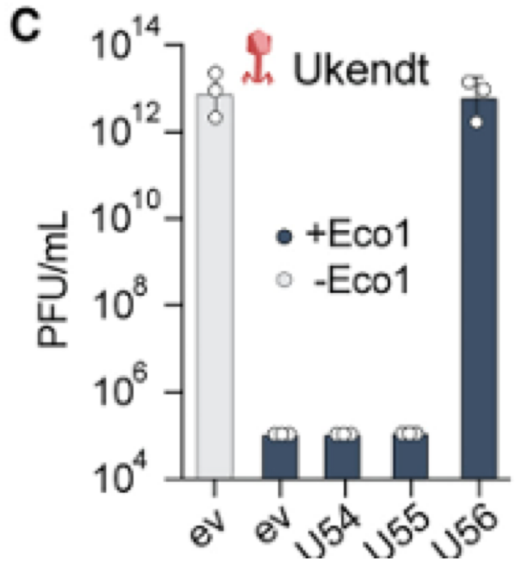


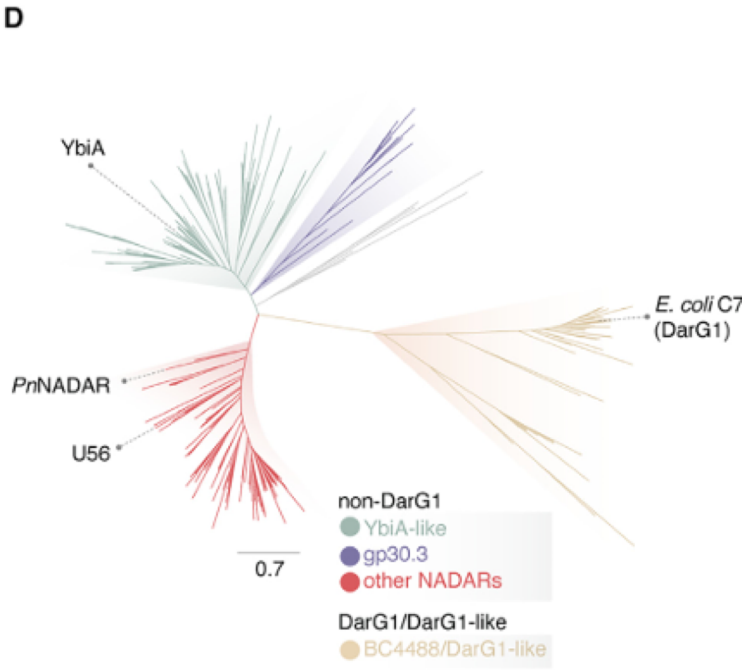
Expression of U69 in Retron-Eco1 encoding *E. Coli* **ehanced the toxic effect of the retron**



The highly basic character of U69 aligns well with the **DNA-interacting proprierties** identified in other retrons and with an **putative sensory role for the retron msDNA**

Role of U56 as a phage-encoded Retron-Eco1 inhibitor

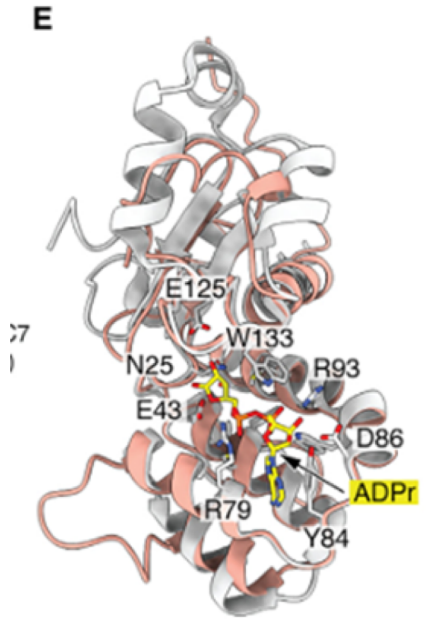




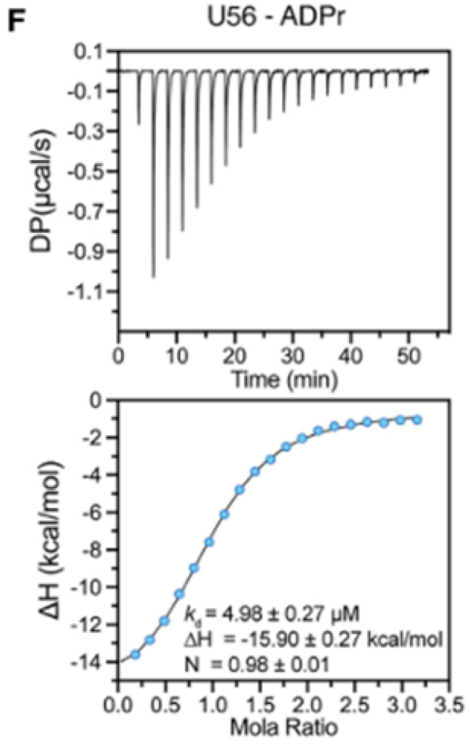
Independent expression of the three genes in cells containg the Retron-Eco1 and subjected to phage infection shows that **only U56 abolish the defense phenotype**

U56 sequence analysis revealed that this protein is a member of the **DarG1/DarG1-like NADAR family** which **play a role in NAD+ metabolism** → Could U56 bind ADPr?

U56 mechanism of action as a Retron-Eco1 inhibitor involves ADPr binding

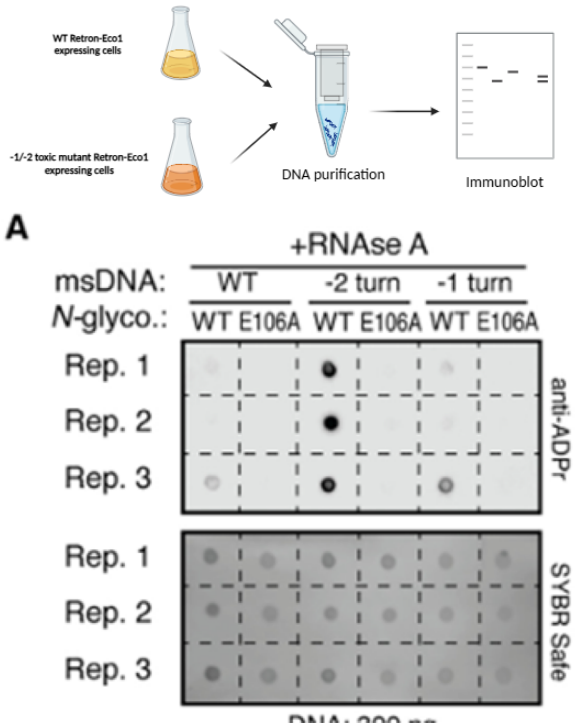


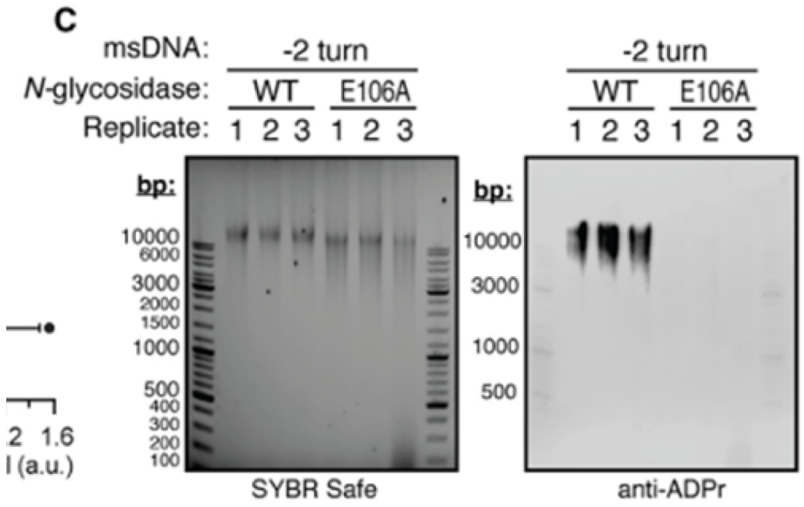
An AlphaFold-generated U56 model was compared to PnNADAR protein showing that **the folding and the catalytic residues are conserved**

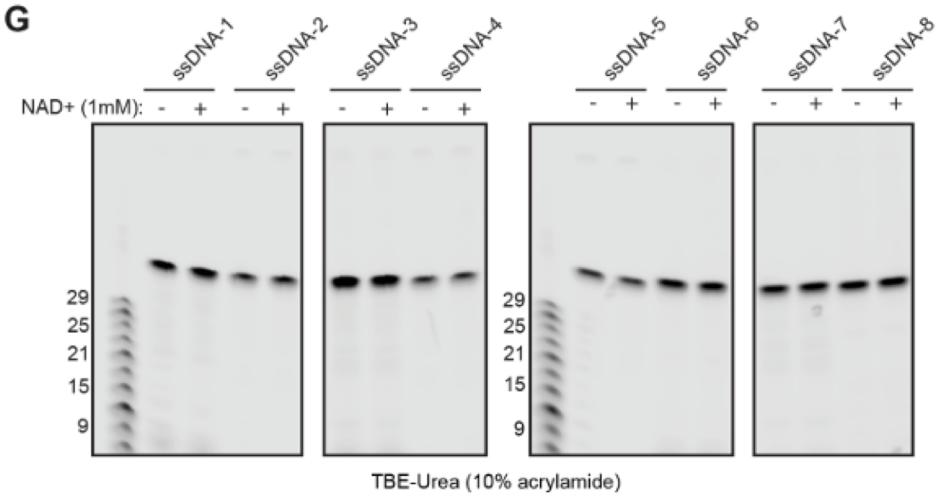


A functional test through Isotermal Calorimetry **confirmed that U56 binds ADPr**

Possible toxic mechanism of Retron-Eco1: DNA ADP-ribosylation





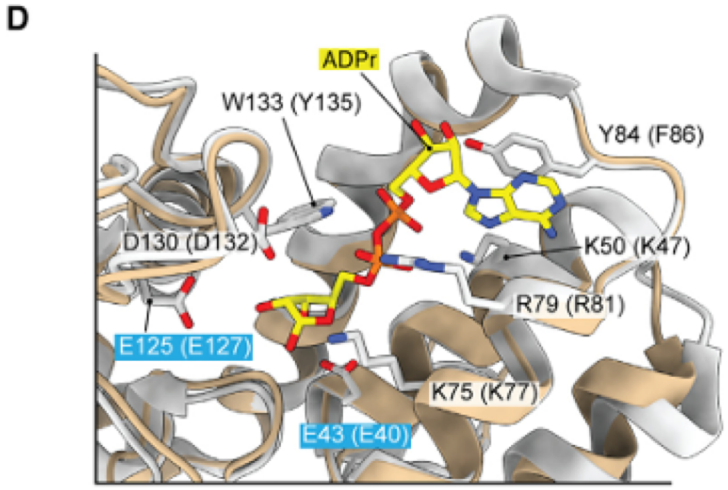


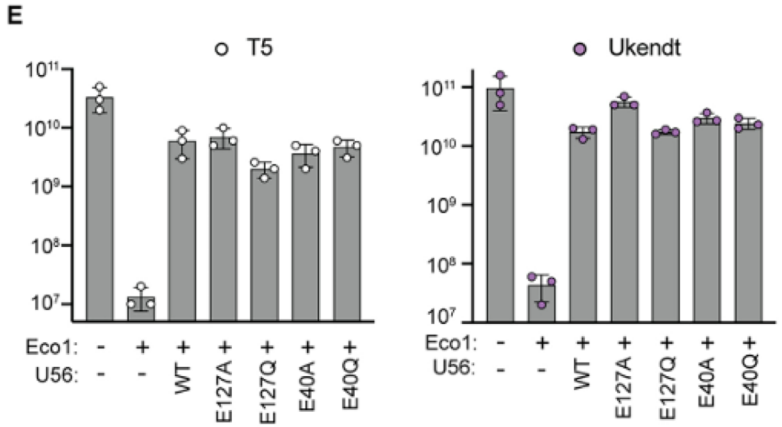
In *in vivo* condition, toxic mutants -1 and -2 show **higher level of DNA ADP-ribosylation** compared to WT Retron-Eco1.

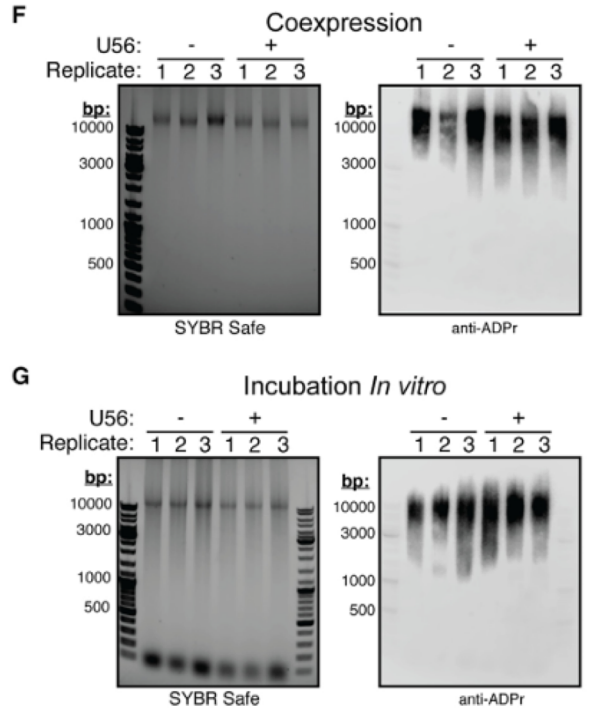
10.000??

Electrophoretic run **in denaturating condition** of eight different ssDNAs after incubation with the Retron-Eco1 toxic mutant -1.
No shift were obeserved → **Retron-Eco1 does not ADP-ribosylate DNA under these conditions**

U56 hydrolytic activity isn't involved in the inhibition of Retron-Eco1 immunity







U56 is very similar to DarG1 and other NADAR. Can U56 remove ADPr from the DNA, like they do?

Expression of a catalytic inactive U56 didn't impair the ability of U56 to neutralize Retron-Eco1 defense
→ **the possible hydrolytic activity is dispensable for the inhibition**

Neither the expression of U56 *in vivo* or the treatment of ADP-ribosylated DNA with *U56 in vitro* reversed the DNA-ADP-ribosylation.

Key points – Retron-Eco1 escape mechanisms in phages

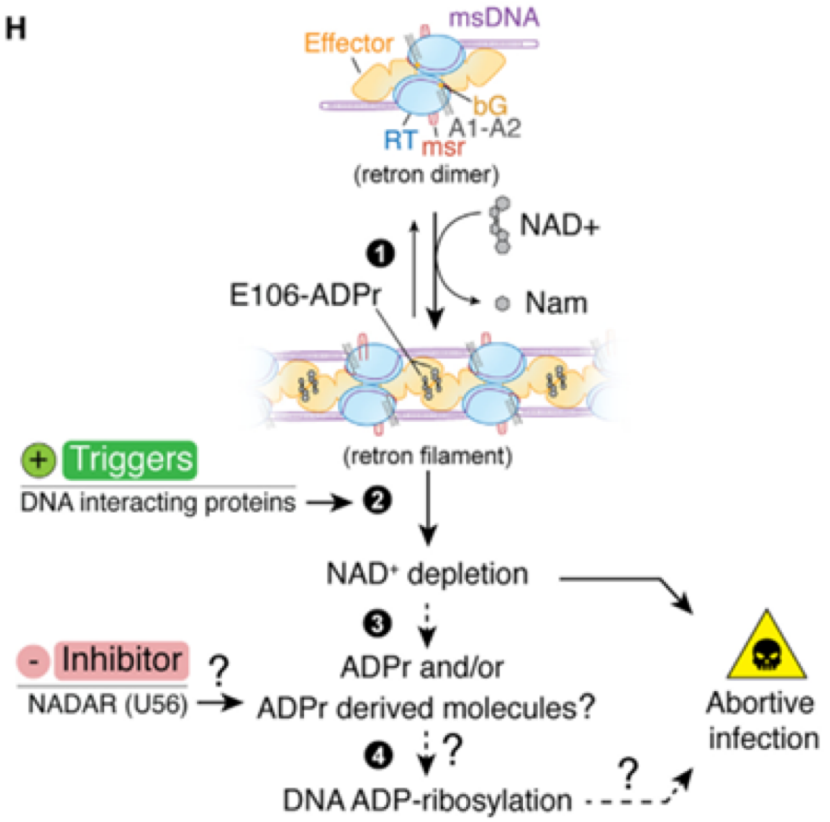
1) **Phages can acquire mutations to escape the Retron-Eco1 defense system**.
2) U69 expression enhances the toxic effect of Retron-Eco1 probably interacting with the msDNA.
3) **U56 amplification in phages abolishes Retron-Eco1 immunity**.
4) U56 is an homolog of the DarG1/NADAR family that can bind and hydrolyze ADPr however its **catalytic activity is not involved in the immune escape mechanism**.

Key points – Retron-Eco1 possible DNA ADP-ribosylation activity

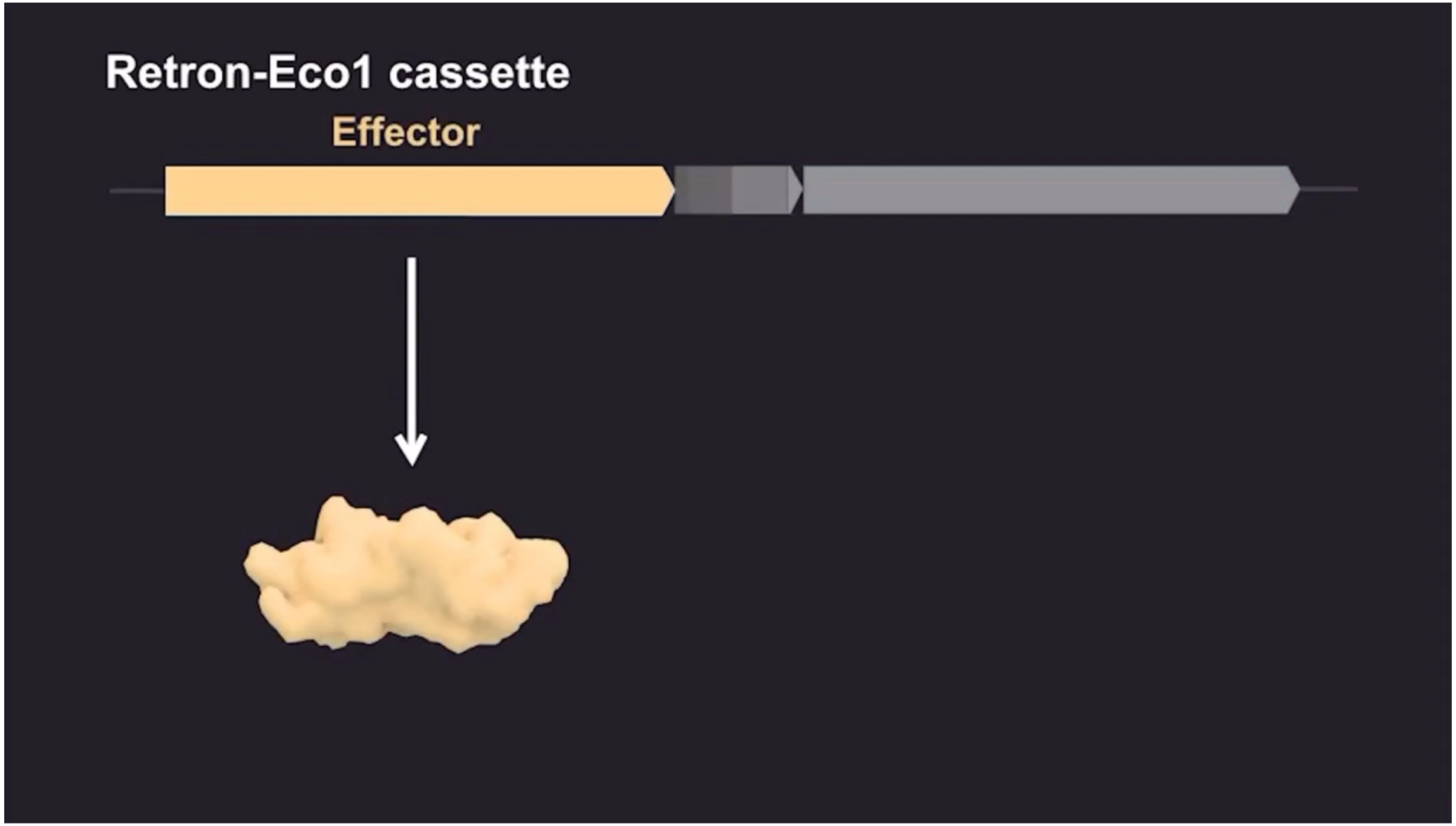
1) The DNA ADP-ribosylation could be another way the Retron uses to induce Abi in bacteria.
2) **DNA ADP-ribosylation is observed in vivo but not in vitro** suggesting that **a cellular intermediate may be required**.

Conclusions

1) Retron-Eco1 is an antiphage system that relies on the formation of **filamentous structures**, which are assembly of Eco1 dimers
2) Filament assembly occurs in absence of phage infection, constituting a **prepared immune state** that enables a rapid response to infections
3) **Effectors dimerize at interface of the N-glyco domains and are encapsulated by the msDNA**, keeping them in a **low activity state**
4) During infection, **DNA-interacting proteins are thought to trigger Retron-Eco1 activation destabilizing the msDNA** an therefore the filament formation, leading to effector release and toxicity
5) Toxicity is achieved by **NAD+ depletion** and possibly **DNA ADP-ribosylation** mediated by an intermediate factor, which lead to **bacterial growth arrest and death (Abi),** preventing the spreading of the phages
6) **Phages develop resistance** by encoding inhibitors of Retron-Eco1 and downregulating the expression of proteins implicated in triggering Retron-Eco1 activation



Conclusions – Video recap



Future perspectives

Standing questions

- How is Retron-Eco1 activated during phage infection?
- What are the molecular mechanisms by which Retron-Eco1 exerts toxicity?
- How do phage-encoded inhibitors, like U56, inhibit Retron-Eco1 activity?

Why is it relevant to study bacterial antiphage systems?

- With the emerging of antimicrobial resistance**, phage therapy** is emerging as a promising alternative. Understanding the molecular basis of bacteria-phage interactions is crucial for designing effective phage therapies
- The **parallels between Retron-Eco1 activation and eukaryotic innate immune systems**, such as the oligomerization of immune components, could lead to insights into evolutionary adaptations of immune systems in both prokaryotes and eukaryotes

Thank you for your attention!

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