Bacterial defense mechanisms involving reverse transcriptase

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Outline

- Bacterial defense mechanisms against bacteriophages
- Role of reverse transcriptase in bacterial defense mechanisms
- Recent discoveries and applications of the defense systems
- Conclusion

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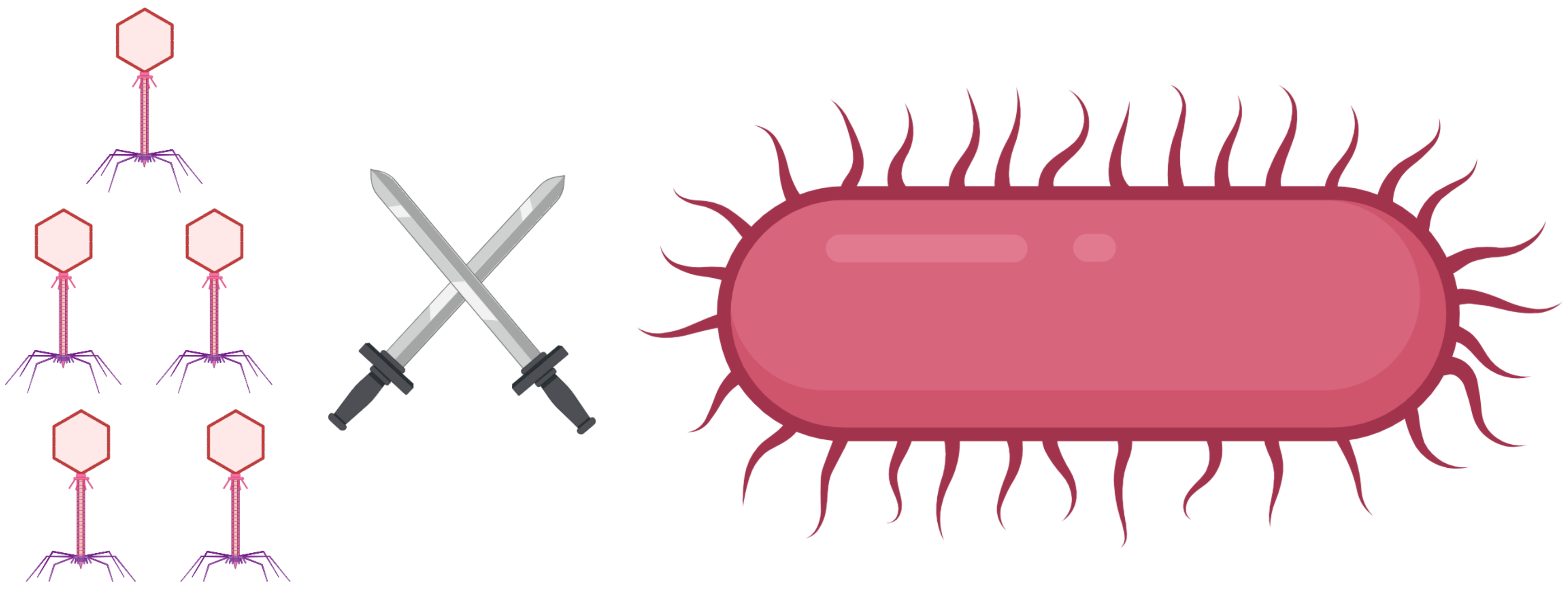
The perpetual war



Hampton *et al.*, Nature, 2020; Image created in https://BioRender.com

Bacterial defense mechanisms against bacteriophages

Degrading foreign DNA & RNA

Inhibiting synthesis of DNA & RNA

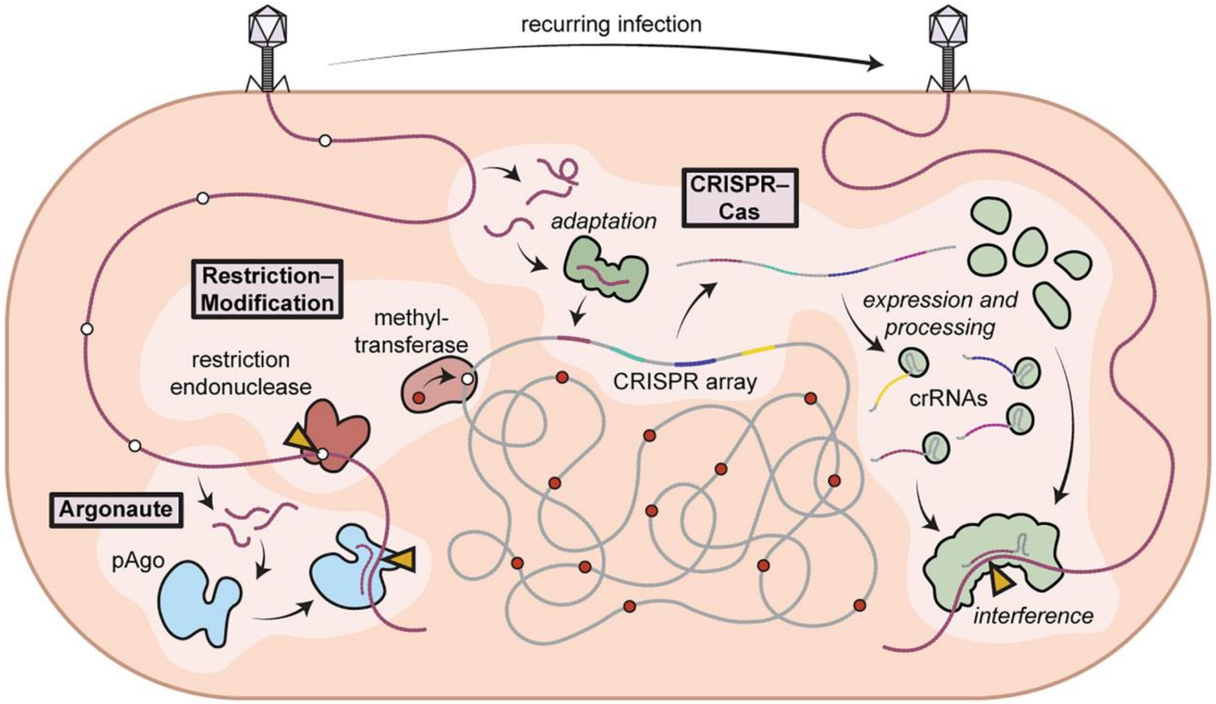
Sacrificing individuals to protect population

Mayo-Muñoz *et al.*, Cell Reports, 2023

Degrading foreign DNA & RNA

- Methylation of host DNA sequences
  - Non-methylated foreign DNA sequences degraded by restriction endonucleases
  - Restriction-modification

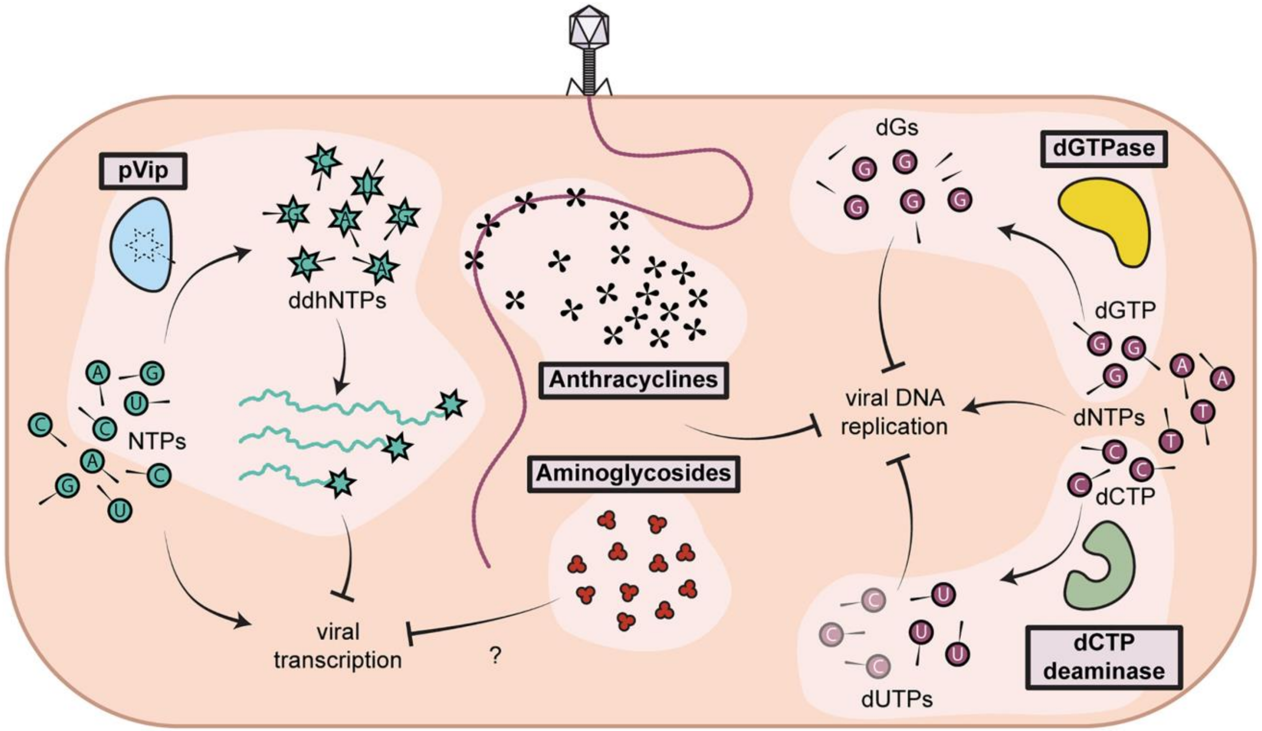
- Recognizing specific foreign sequences for cleaving
  - Argonaute proteins (pAgos)
  - Clustered regularly interspaced short palindromic repeats (CRISPRs) & CRISPR-associated (Cas) proteins



Mayo-Muñoz *et al.*, Cell Reports, 2023

Inhibiting synthesis of DNA & RNA

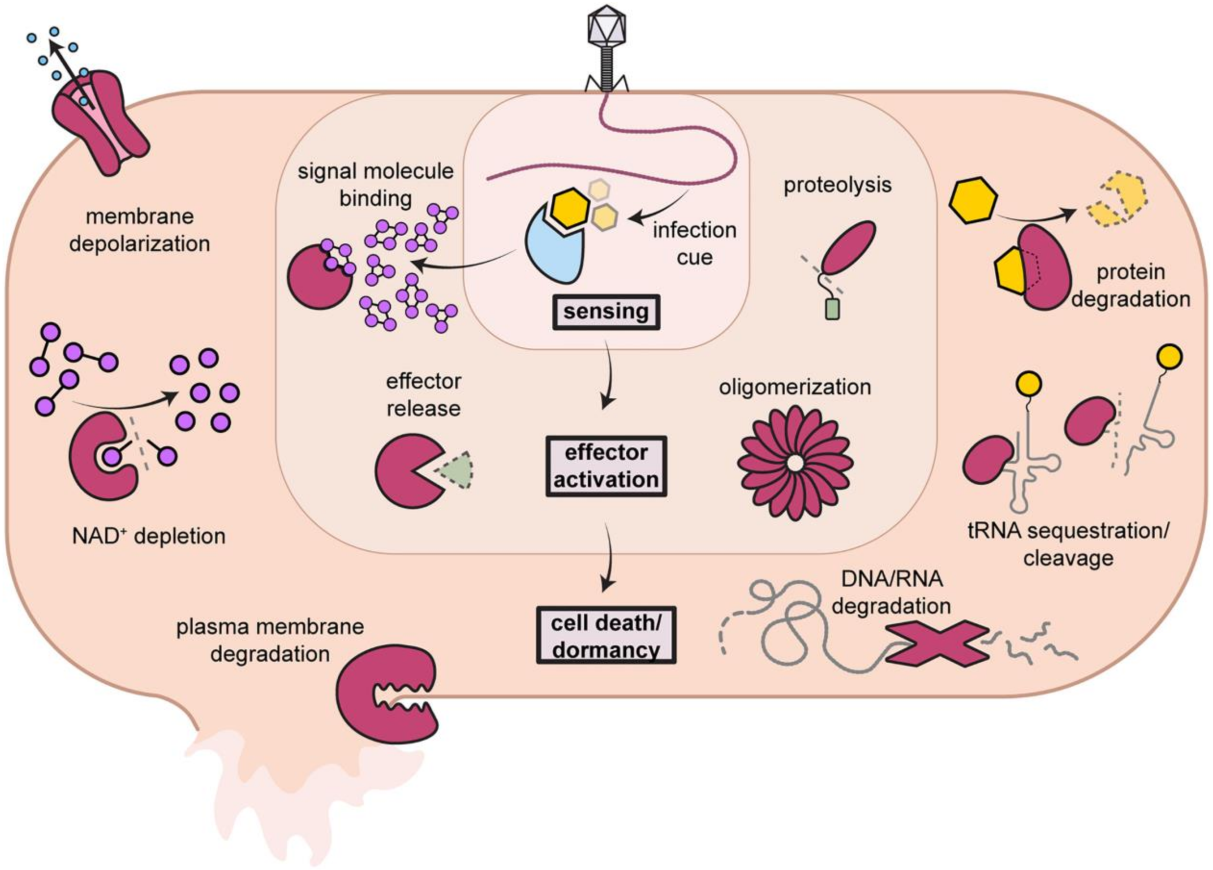
- Blocking viral replication & transcription
  - Examples: anthracyclines, 3’-deoxy-3’ ,4’ -didehydro ribonucleotides (ddhNTPs)
- Depleting the nucleotide materials
  - Examples: dGTPase, dCTP deaminase



Mayo-Muñoz *et al.*, Cell Reports, 2023

Sacrificing individuals to protect population

- Stopping virus propagation by inducing cell suicide
- Sensing of phage activity activates effector
- Different pathways to cell death:
  - Depleting nicotinamide adenine dinucleotide (NAD+)
  - Breaking down cell membrane
  - Indiscriminately degrading DNA/RNA



Mayo-Muñoz *et al.*, Cell Reports, 2023

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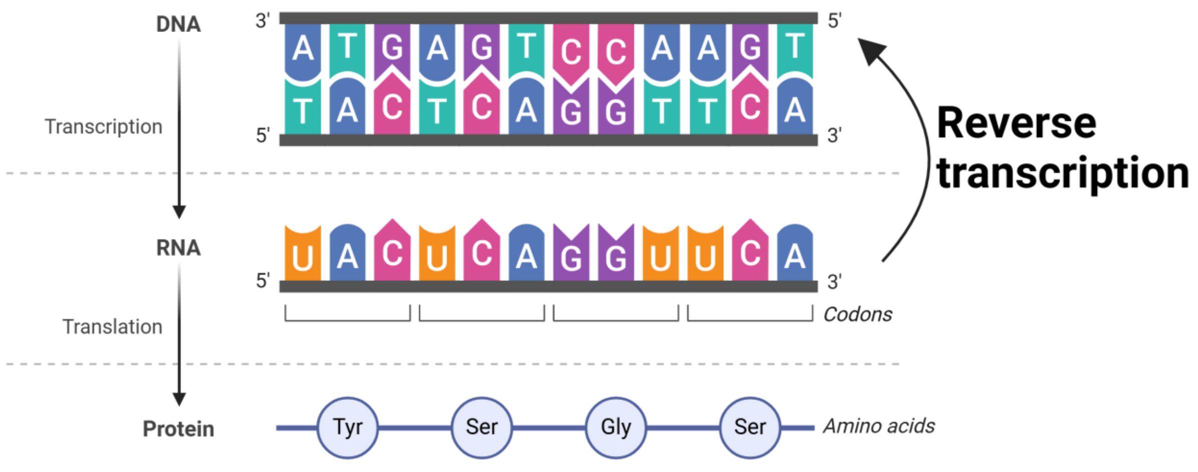
Reverse transcriptases (RTs)

- Enzymes that synthesize complementary DNA (cDNA) from RNA template



- Prokaryotic RTs have variety of functions:
  - Genomic diversification
  - Adaptation to environment
  - Aiding in bacterial defense

- Likely originate from mobile genetic elements
  - Incorporated in bacteria genome during evolution

- “Mercenaries”, role in the war against bacteriophages

Gonzalez-Delgado *et al.*, FEMS Microbiology Reviews, 2021; Image created in https://BioRender.com

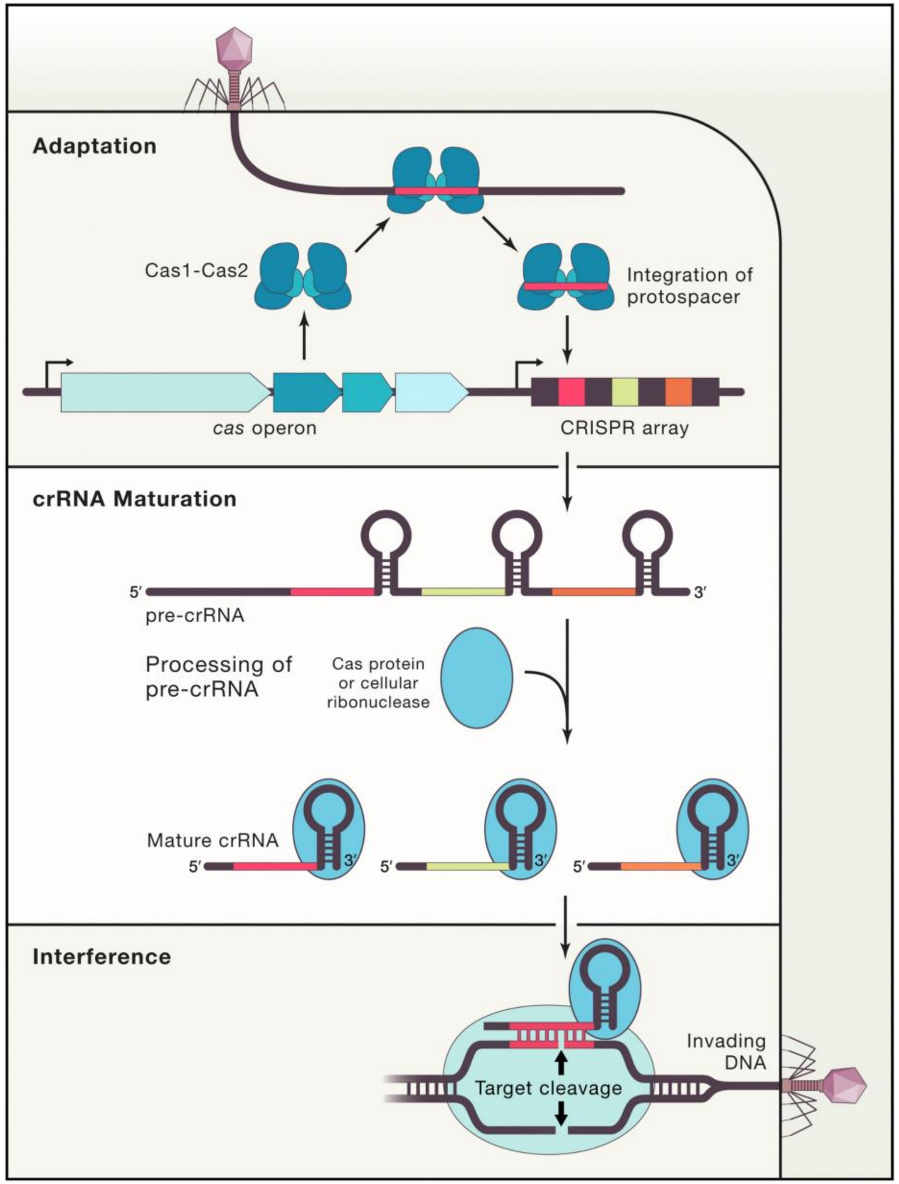
Example 1: CRISPR-Cas system

- The adaptive immune system in bacteria

- **Adaptation**: Short RNA/DNA sequences (spacers) are integrated to the CRISPR array by Cas1-Cas2
  - CRISPR array = memory

- **Expression**: Spacers are expressed in form of CRISPR RNA (crRNA), which is processed further

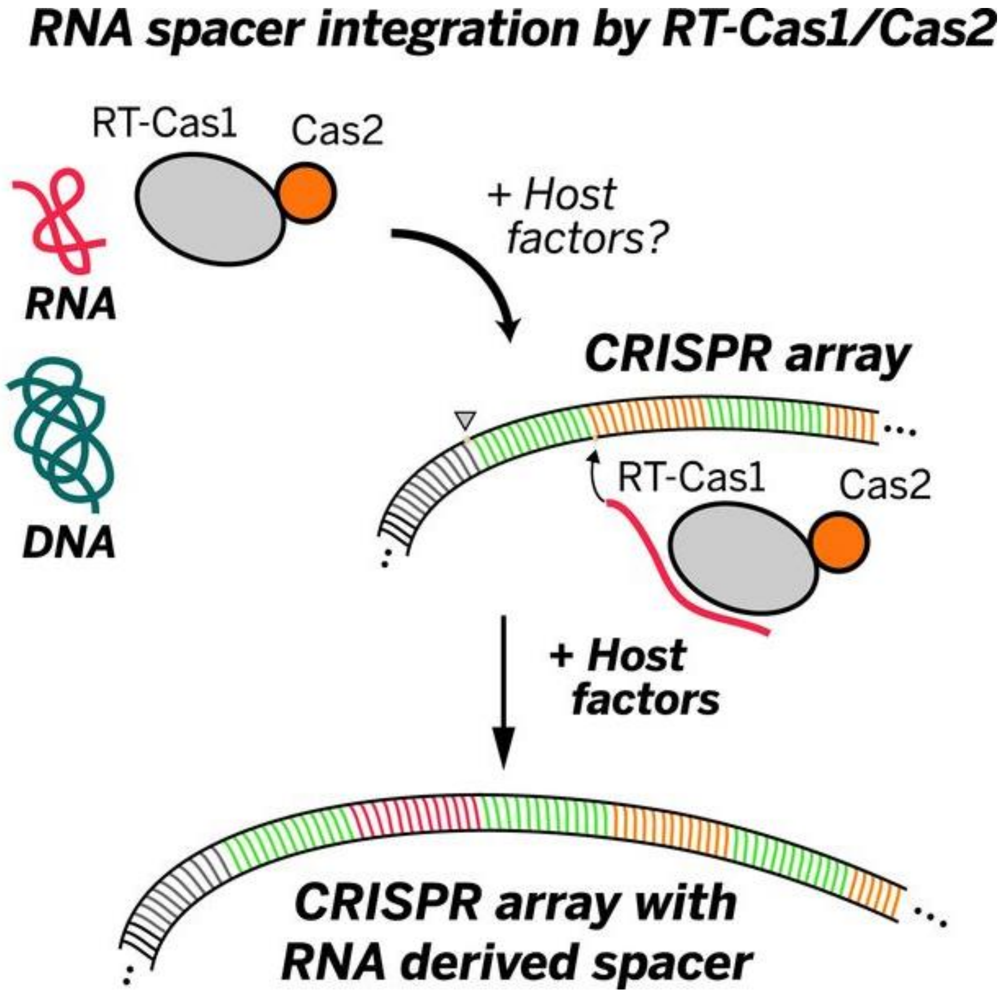
- **Interference**: Mature crRNA forms complex with Cas proteins & guides the Cas proteins to bind and destroy foreign DNA



Hille *et al.*, Cell, 2018

Role of RT in CRISPR-Cas system

- Type III CRISPR-Cas has RT-Cas1 fusion protein
- Can target both RNA & DNA
    - Processing RNA requires RT
- RT-Cas1 is suggested to be involved in adaptation
- But RNA phages are poorly understood
- Only one known natural example of acquiring spacers from RNA phage
    - a CRISPR spacer in *Candidatus Accumulibacter* is similar to RNA-dependent RNA polymerase (Wolf *et al.*, 2020)

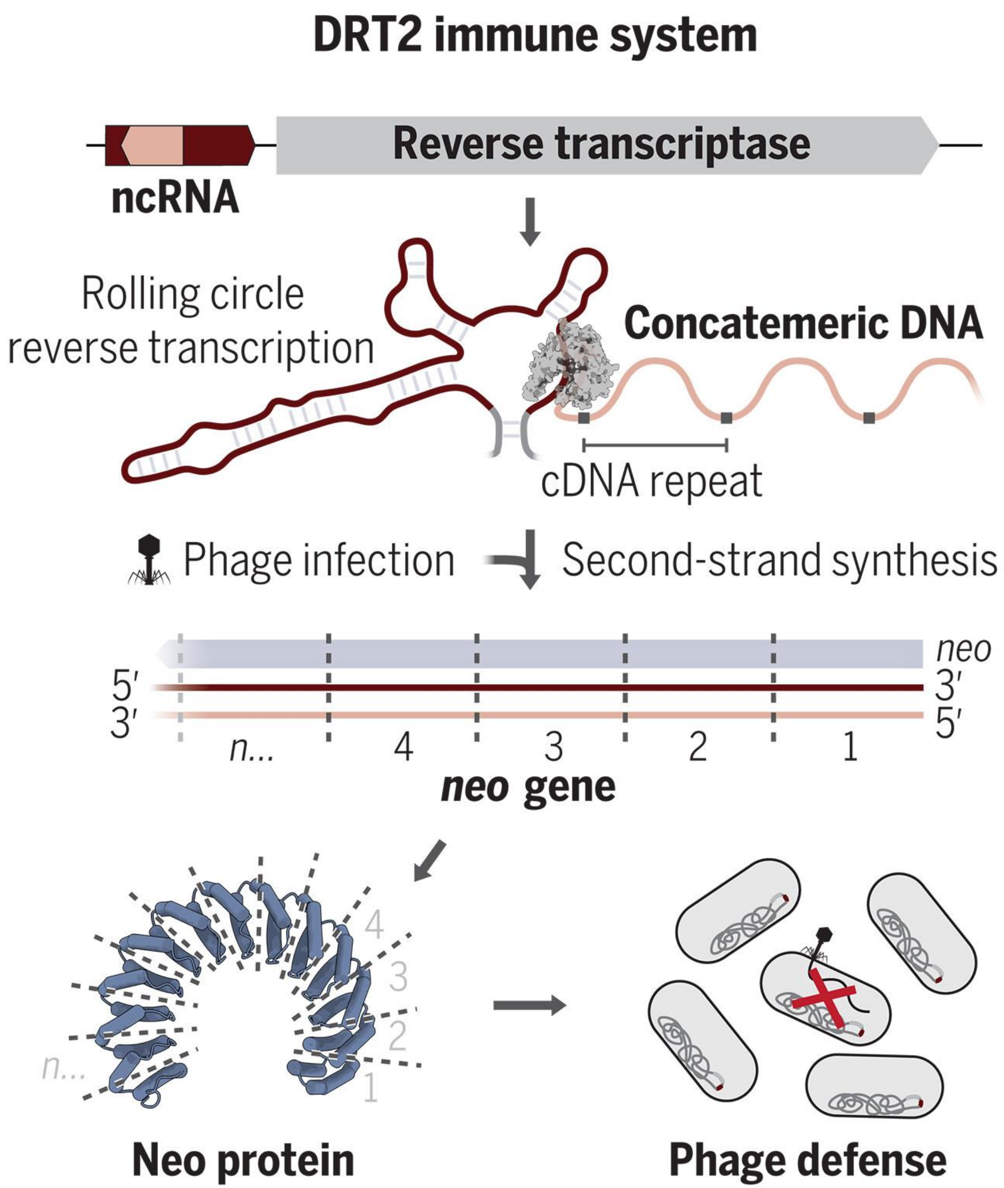


Silas *et al.*, Science, 2016; Wolf *et al.*, Nature Microbiology, 2020

Example 2: Defense-associated Reverse Transcriptase (DRT2) system

- Mechanism recently investigated independently by two teams
- Only consists of non-coding RNA (ncRNA) & RT domain
- Induce growth arrest
- Population-level immunity by sacrificing individual cells

Tang *et al.*, Science, 2024

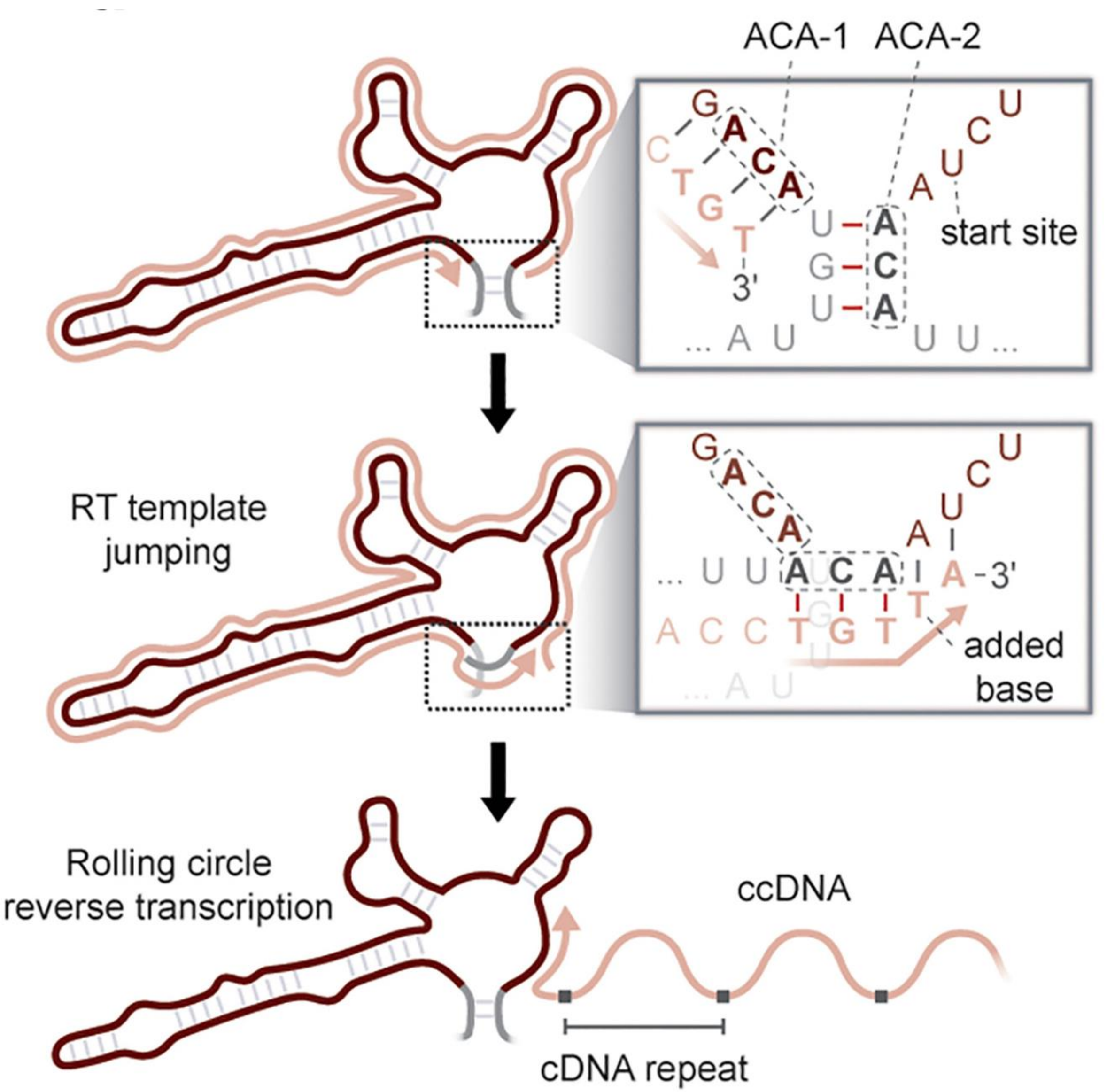


Generation of concatemeric DNA (ccDNA)

- Once the RT arrives at the end of template region, it jumps back to the start
  - Rolling circle reverse transcription of ncRNA

- Results in repeats in synthesized DNA - concatemeric DNA (ccDNA)

- Promoter elements being aligned together allows transcription of ccDNA

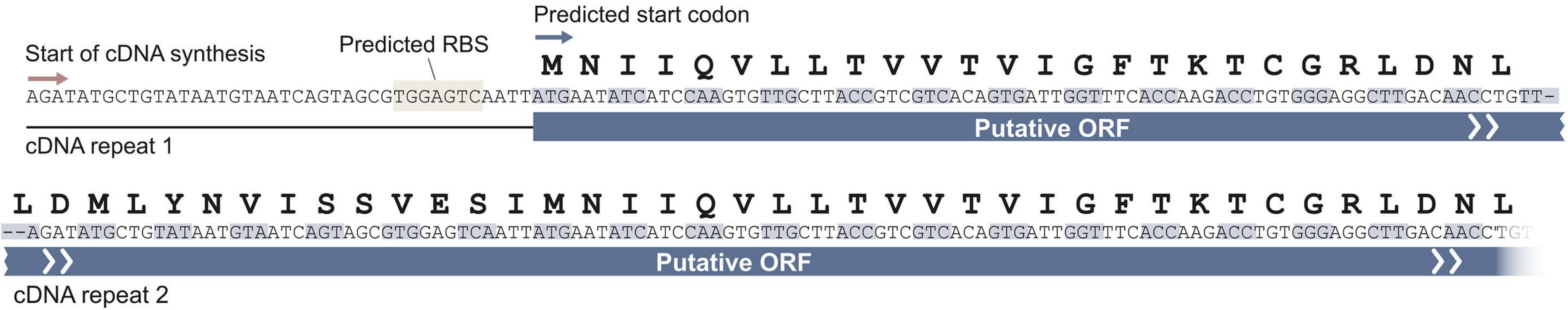


Tang *et al.*, Science, 2024

Transcription & translation of ccDNA

- ccDNA has no stop codon
  - Hence has nearly endless open reading frame (ORF)
  - Nearly endless ORF (Neo) protein

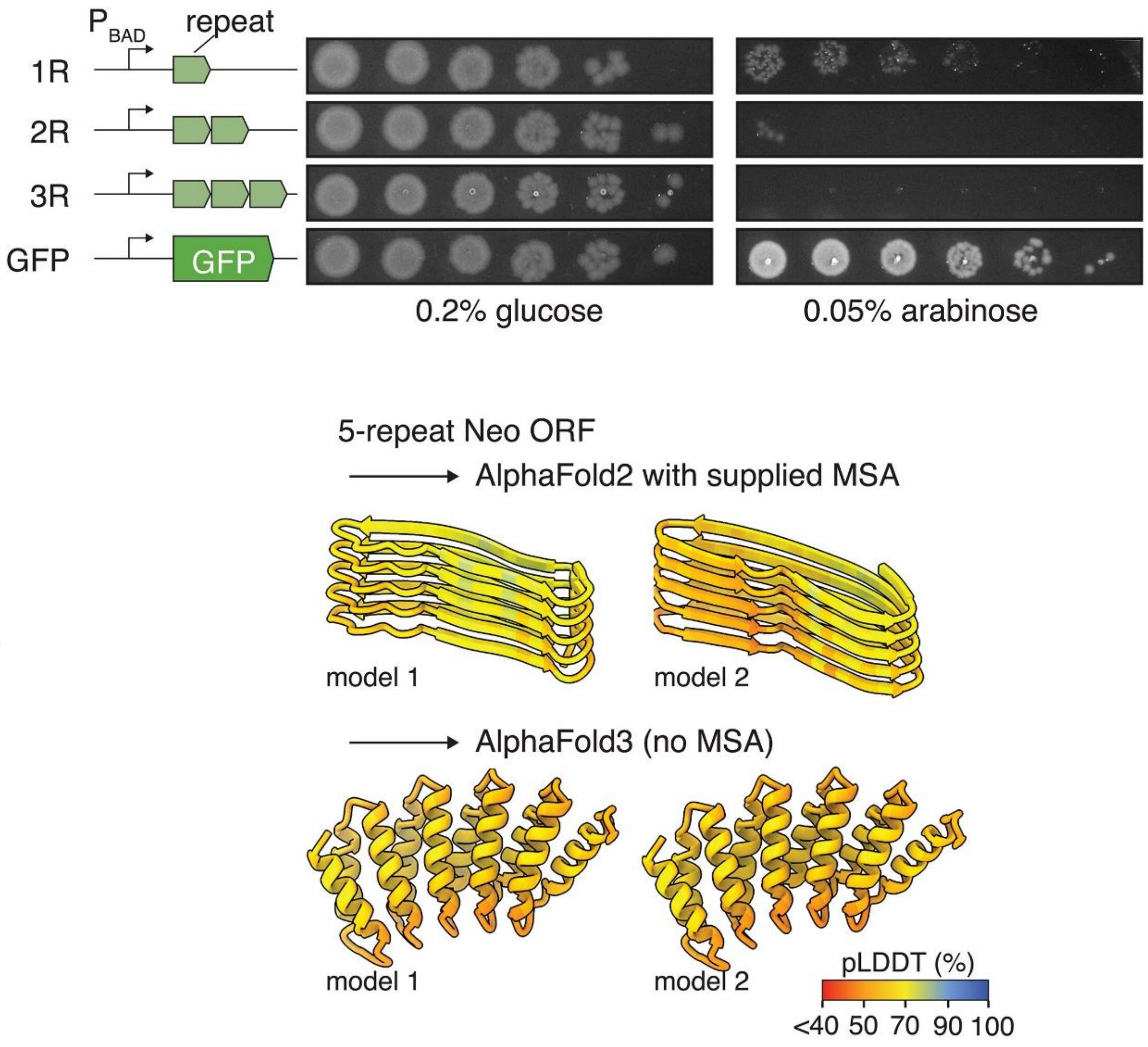
- Lead to variable lengths of repeats



Tang *et al.*, Science, 2024

DRT2 product: Neo protein

- Neo transcripts may include hundreds of repeats
- But strains encoding >3 repeats are difficult to isolate due to cell toxicity of Neo
- Predicted Neo protein structure does not bear resemblance to any annotated proteins
- Unknown mechanism of inducing growth arrest



Wilkinson *et al.*, Science, 2024

Summary of RT-associated bacterial defense systems

Bacterial defense systems	Mechanisms	Knowledge gaps
CRISPR-Cas (Type-III)	• Acquisition of RNA sequences from phages • Integration of spacers into the CRISPR array • Conversion of RNA to cDNA	• Only one natural example discovered • Lack of knowledge on RNA phages
DRT2	• Generation of ccDNA from ncRNA • ccDNA encodes toxic protein Neo	• How phages trigger the system is unclear • Neo protein not resembling other annotated proteins

Silas *et al.*, Science, 2016; Tang *et al.*, Science, 2024; Wilkinson *et al.*, Science, 2024

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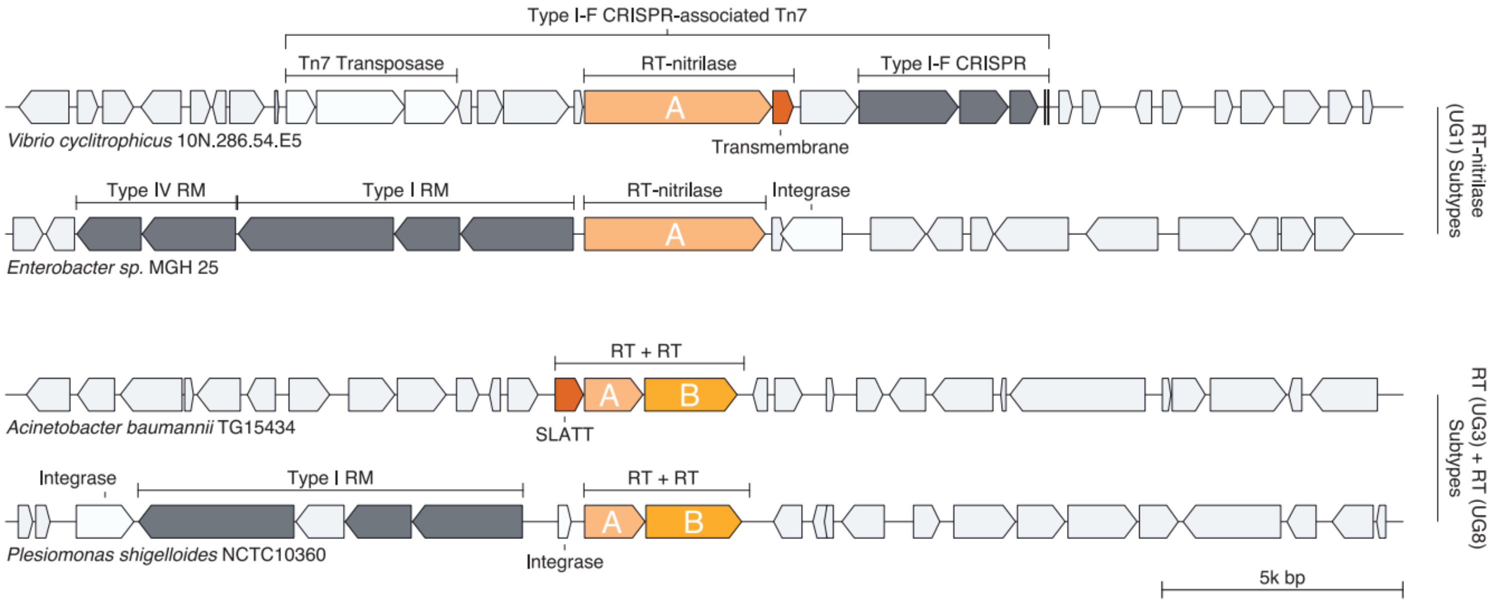
The expanding discovery of novel defense systems

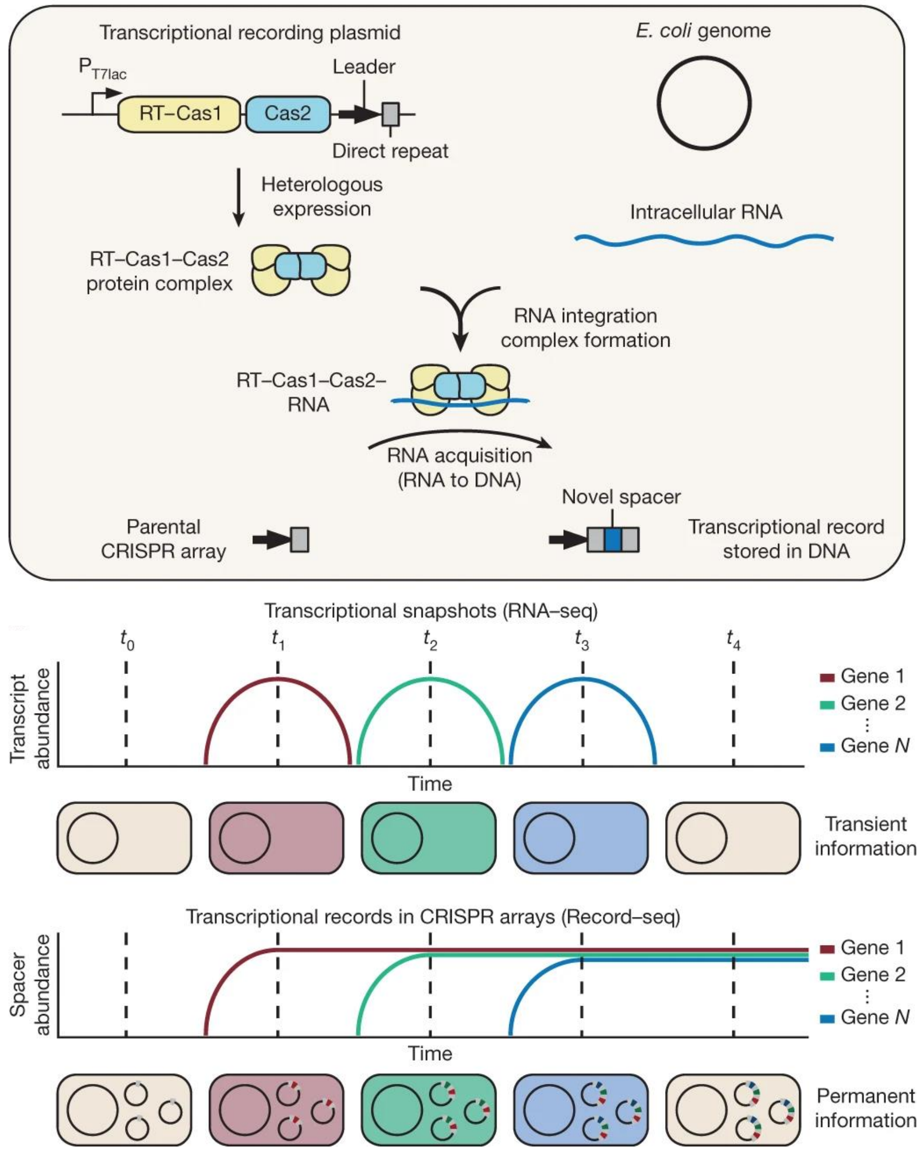
- Main strategy: searching for genes in defense islands
- Predicting possible defense systems by their proximity to other well characterized defense genes
    - Expansion to the types & diversities of known defense systems
- For now, many systems have unknown functions & mechanisms



Gao *et al.*, Science, 2020

Applications of the defense systems: Record-seq

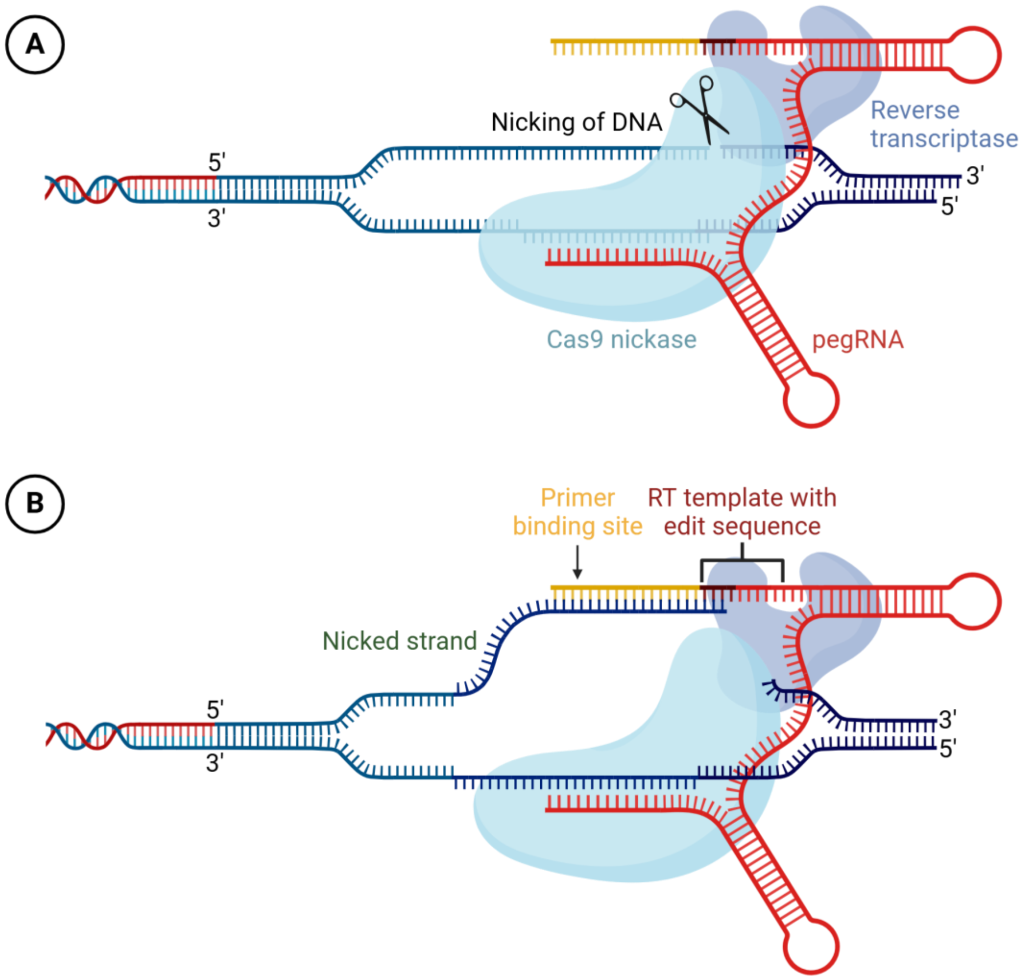
- Records transcription events in bacterial cells
- Transcripts converted to spacers and stored in CRISPR array
- Can record cumulative expression information
- Useful for transcription history or transient transcription events
- Examples: Oxidative stress responses, effect of intestinal environment, exposure to herbicide



Tanna *et al.*, Nature Protocols, 2020; Schmidt *et al.*, Nature, 2018; Schmidt *et al.*, Science, 2022

Applications of the defense systems: Primed editing

- Can correct small mutations such as point mutations
- Mutated Cas9 nicks genome DNA
  - Prime editing guide RNA (pegRNA) serves as primer for RT
  - RT synthesizes cDNA with edited sequence
- Potential of treating pathogenic allele-associated disease
- Successful application in mice models with liver and eye diseases



Anzalone *et al.*, Nature, 2019; Jang *et al.*, Nature Biomedical Engineering, 2022. Image created in https://BioRender.com

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Conclusion

- Diverse bacterial defense mechanisms targeting bacteriophages
  - Different functions and different structures in many defense systems

- Limited knowledge on many bacterial defense systems involving RTs
  - Especially in the context of bacteria in their natural habitats

- The role of reverse transcriptase in complex bacterial defense mechanisms may shed light on novel molecular mechanisms yet to be fully understood

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Thank you for listening!
Any questions?