CRISPR-Cas in nature and the lab

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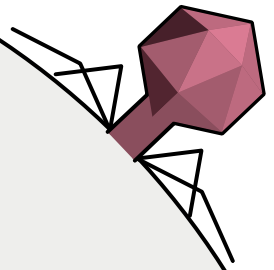
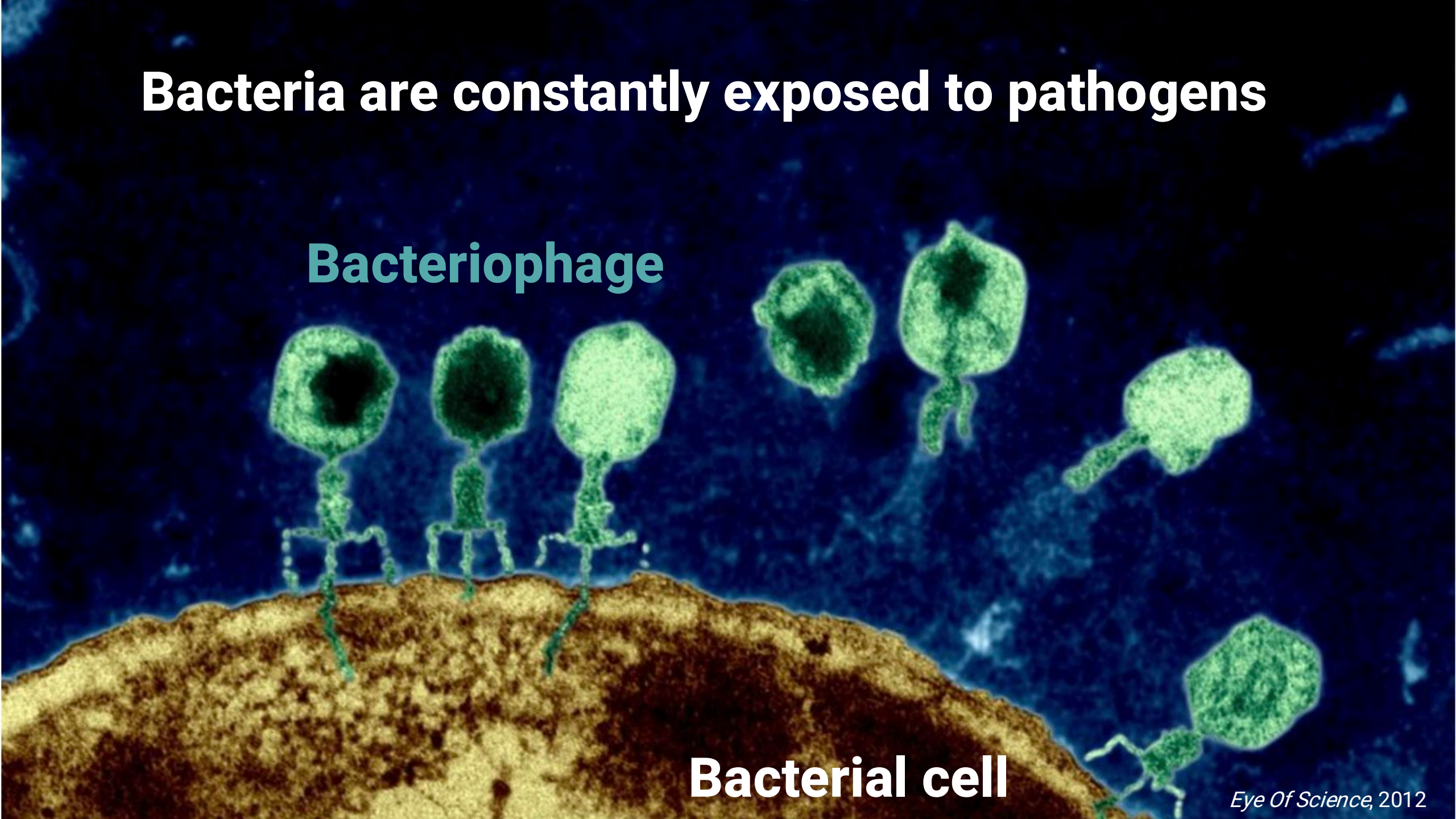
Molecular Biology Program



1. CRISPR-Cas intro
2. Type III CRISPR-Cas10

Bacteria are constantly exposed to pathogens



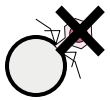
Eye Of Science, 2012

Bacteria have evolved diverse immune strategies

For many years, it was thought that bacteria encode only **innate** immune mechanisms.

Immunity

Individual

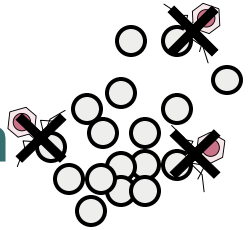


Infected cell survives

Invader is destroyed before the cell is harmed

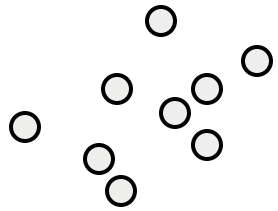
Restriction

Population



Non-infected cells survive

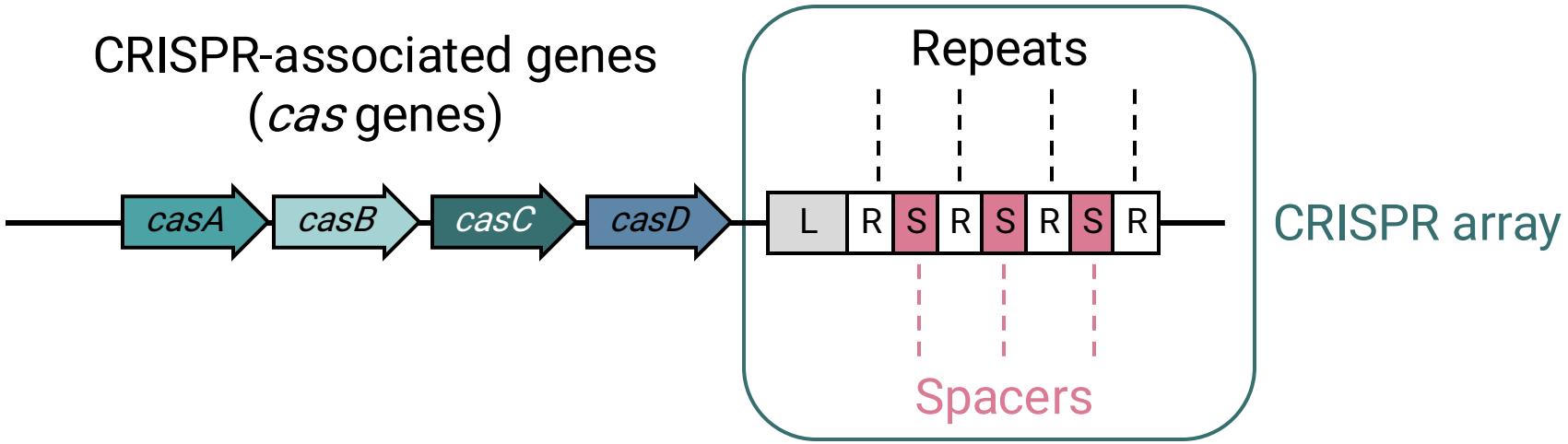
Cell is sacrificed to sequester the infection



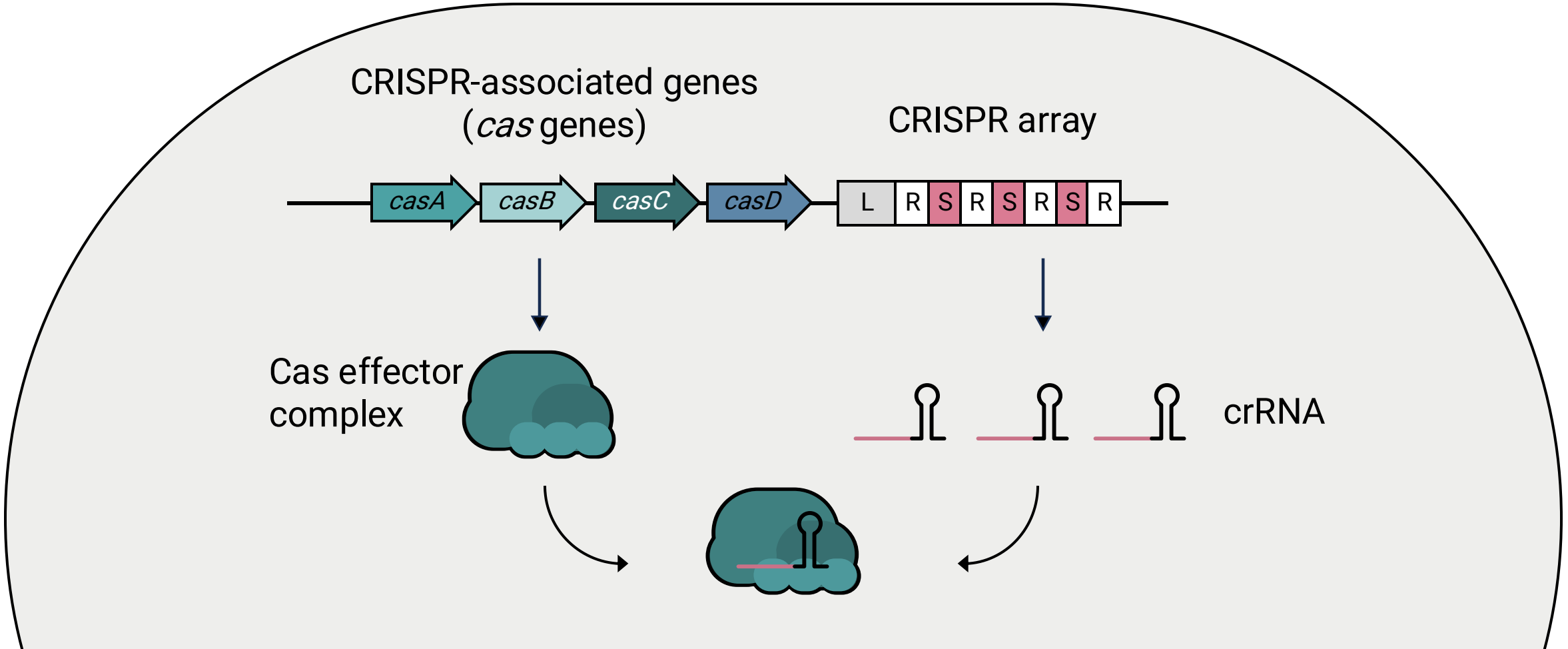
Abortive infection

CRISPR-Cas:
The prokaryotic adaptative immune system

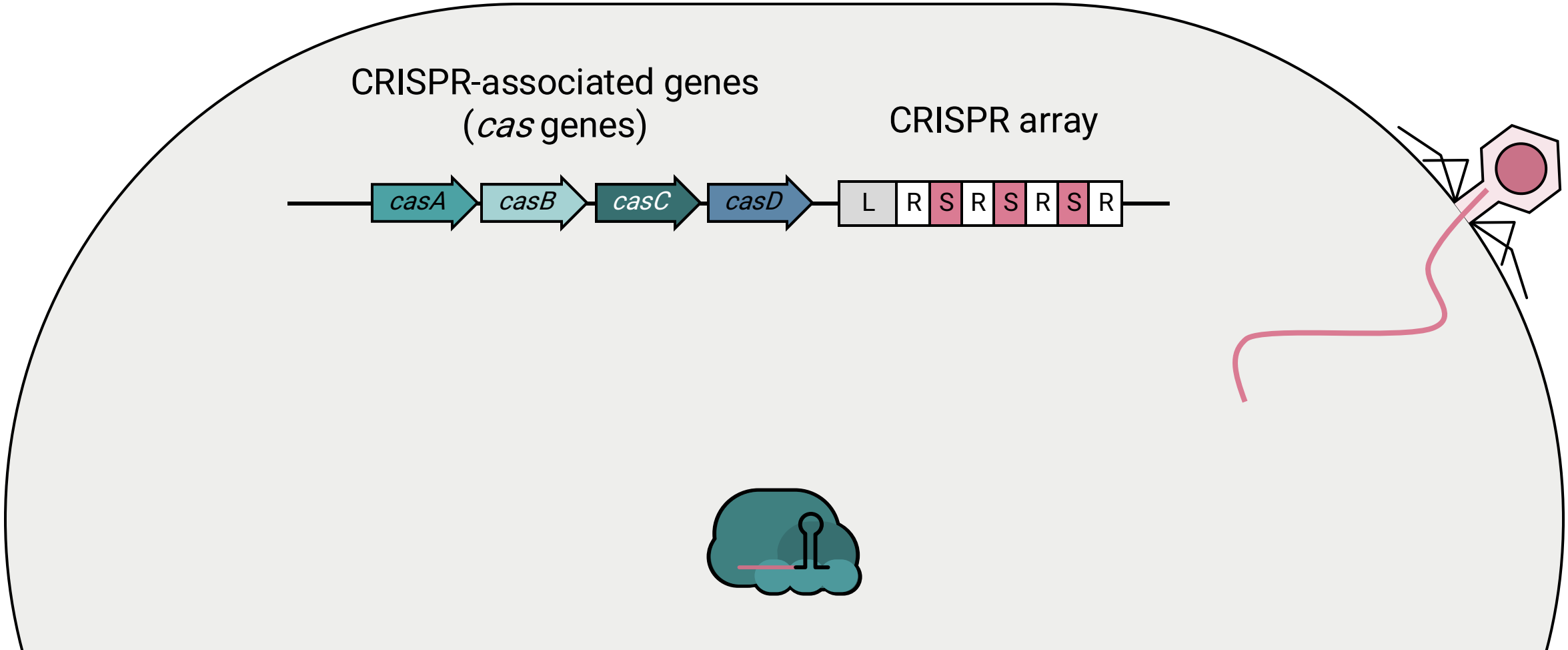
Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats



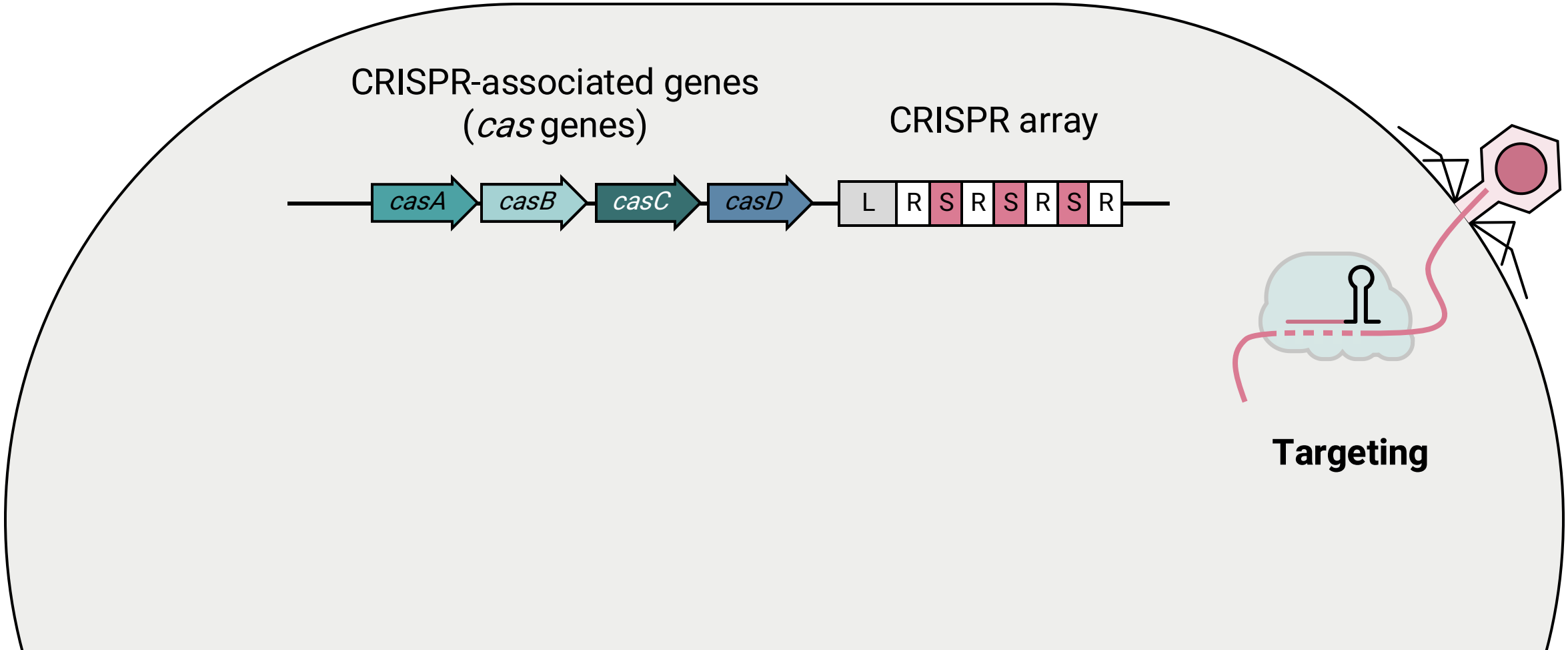
CRISPR-Cas:
The prokaryotic adaptative immune system



CRISPR-Cas:
The prokaryotic adaptative immune system



CRISPR-Cas:
The prokaryotic adaptative immune system



How it started…

1987 – “direct-repeats” are described in *E. coli*

TGAAAATGGGAGGGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC (1,451)
(1,452) CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC (1,512)
(1,513) CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG (1,573)
(1,574) CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC (1,634)
(1,635) CGGTTTATCCCCGCTGGCGCGGGGAACTCG (1,664)

consensus: CGGTTTATCCCCGCT$^{GG}_{AA}$CGCGGGGAACTC

FIG. 5. Comparison of direct-repeat sequences consisting of 61 base pairs in the 3′-end flanking region of *iap*. The 29 highly conserved nucleotides, which contain a dyad symmetry of 14 base pairs (underlined), are shown at the bottom. Homologous nucleotides found in at least two DNA segments are shown in boldface type. The second translational termination codon is boxed. The nucleotide numbers are in parentheses.

Ishino et al., *Journal of Bacteriology* 1987

1993 – similar repetitive sequences described in the archaeon *H. mediterranei*

A

GTTACAGACGAACCCTAGTTGGGTTGAAGCCTCGCCATCGCCGCGAACTCGGTCCTCCTC
GGGGTGGTTACAGACGAACCCTAGTTGGGTTGAAGCAAGCCTTGAGAGTGTCTGTTGGTA
TGATGAATGTTGTTACAGACGAACCCTAGTTGGGTTGAAGCAAGTAGACCGCGCTCAGTT
ACGACAGCTGCTCGAGTTACAGACGAACCCTAGTTGGGTTGAAGC

Mojica et al., *Molecular Microbiology* 1993

In following years, Mojica and others published several comparative genomics works, showing these repeat sequences are present in several archaeal and bacterial species.

2002 – First use of the acronym “CRISPR”

“…To appreciate their characteristic structure, we will refer to this family as the **c**lustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats (CRISPR).”

CRISPR-Associated (Cas) genes are described.

Hypothesized function:
“The *cas3* gene showed motifs characteristic for helicases… the *cas4* gene showed motifs of the RecB family of exonucleases, suggesting that these genes are involved in DNA metabolism or gene expression.”

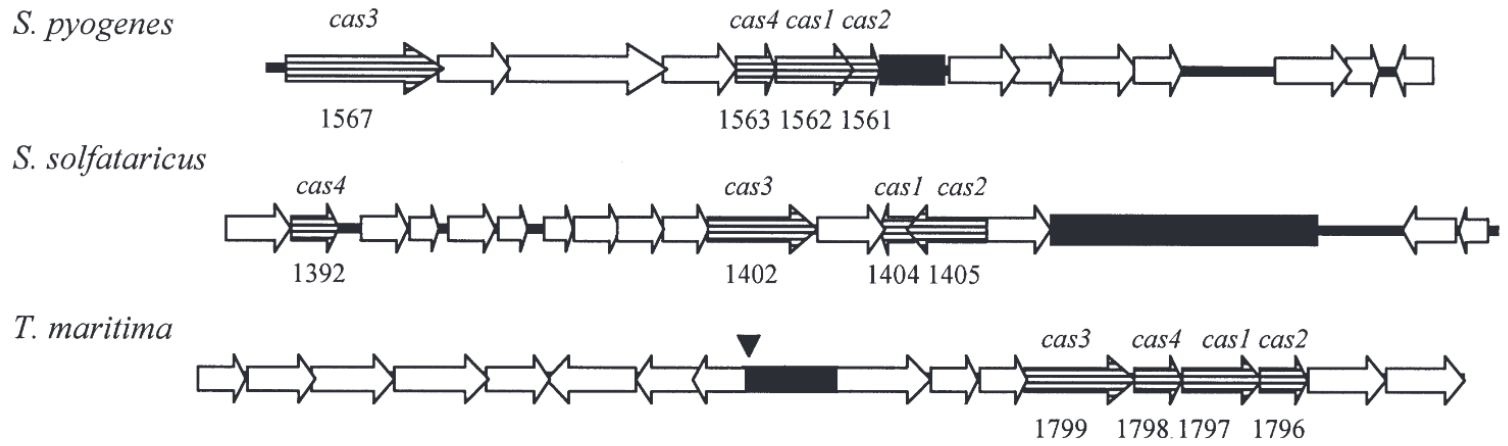


Fig. 1. Genetic organization of the CRISPR locus and the cas genes of the published genomes that contain all four cas genes. The CRISPR loci are depicted as black boxes. The position of the leader is indicated by a black triangle. The putative genes cas1, cas 2, cas3 and cas4 are indicated by dashed arrows. Other putative open reading frames (ORFs) are depicted as white arrows. The numbers below the ORFs indicate the gene numbers as assigned by the individual genome projects. The cas2 genes of P. horikoshii and A. aeolicus have no gene number because these were not annotated by these genome projects.

Jansen et al. *Molecular Microbiology* 2002

2005 – turning point: spacers steal the show

Table 2. Distribution of CRISPR-spacer homologs

Strain	No. of spacers analyzed	No. of spacers with homologs in		
		Phages[a]	Plasmids	NF[b]
Chlorobium tepidum TLS	62		1	
Clostridium tetani Massachusetts E88	62	1		6
Corynebacterium efficiens YS-314T	22		1	2
Escherichia coli ECOR42	14		1	
Escherichia coli ECOR44	10	1		
Escherichia coli ECOR47	17	1		
Escherichia coli ECOR49	11		1	
Listeria innocua Clip11262	9	3		
Listeria monocytogenes EGD-e	4	1		
Methanothermobacter thermoautotrophicum ΔH	169	9		
Mycoplasma gallisepticum R	71			1
Neisseria meningitidis Z2491 (serogroup A)	16			4
Photorhabdus luminescens laumondii TT01	65	7		3
Porphyromonas gingivalis W83	44			4
Pyrobaculum aerophilum IM2	129			1
Salmonella typhimurium LT2 SGSC1412	57	1		
Shigella sonnei 53G	3			1
Streptococcus agalactiae NEM316	13	1		1
Streptococcus agalactiae 2603V/R	25	1	1	3
Streptococcus pyogenes MI GAS SF370	9	8		
Sulfolobus solfataricus P2	424	6	3	
Sulfolobus tokodaii 7	471	2	2	
Thermoanaerobacter tengcongensis MB4T	306			5
Yersinia pestis CO-92 (Biovar Orientalis)	16	4		
Yersinia pestis KIM5P12 (Biovar Mediaevalis)	10	1		

[a]Prophages are included.
[b]Number of spacers with homology to chromosomal sequences not directly related to foreign DNA (prophages are excluded).

Mojica et al., *Journal of Molecular Evolution* 2005

2005 – turning point: spacers steal the show

Table 6. Features of the sequences most similar to CRISPR spacers from *E. coli*

Strain	Gene	Element	Activity	Alignment[a]
ECOR42	*traI*	Plasmid F	Helicase	gtttcccgtgcgtcgtatgaggcagaaaagag ||||||||||||||||||||||||||||| gtttcccgtgcgtcgtatgaggcagaaaagag
ECOR44	Unannotated	Phage P1	Unknown	ctgttggcaagccaggatctgaacaataccgt ||||||||| ||||||||||||||||| ctgttggcaggccaggatctgaacaataccgt
ECOR47	*darB*	Phage P1	Methylase	gctggtggcgcgggcaaacggaacaatcccgc ||||||||||||||| ||||||||||||||| gctggtggcgcgggcgaacggaacaatcccgc
ECOR49	*resD*	Plasmid F	Resolvase	atcgacttatgccccatcaggctctgcaatac || ||||||||||||||||||| |||| || atggacttatgtcccatcaggctttgcagaac

[a]CRISPR-spacer sequence (top line) and best-match homologous sequence (bottom line).

Mojica et al., *Journal of Molecular Evolution* 2005

2005-2006 – CRISPR suggested to function in phage defense

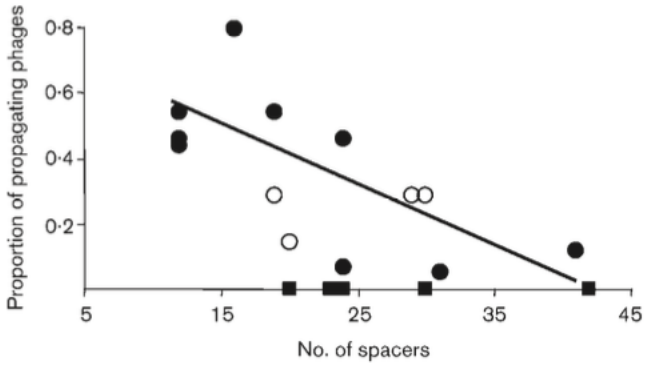


Fig. 6. Correlation of *S. thermophilus* phage resistance and the number of spacers in a CRISPR locus. Filled symbols correspond to data obtained from strains tested with the panel of 59 phages. The line of best-fit refers to strains that were not fully phage resistant (●), and for which $y=-0{\cdot}02x+0{\cdot}77$ and $R^2=0{\cdot}51$. Fully phage-resistant strains (■), were not taken into account for the correlation shown. ○, Strains tested with the panel of seven phages.

Bolotin et al., *Microbiology* 2005

"… it seems *likely* that the inserts are transcribed and silence the cognate phage or plasmid genes via the formation of a duplex between the prokaryotic small interfering RNA (psiRNA) and the target mRNA, followed by cleavage of the duplex or translation repression."

RAMPs = Repeat-associated mysterious proteins

Makarova et al., *Biology Direct* 2006

2007 – First experimental proof of spacer acquisition and phage defense

CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes

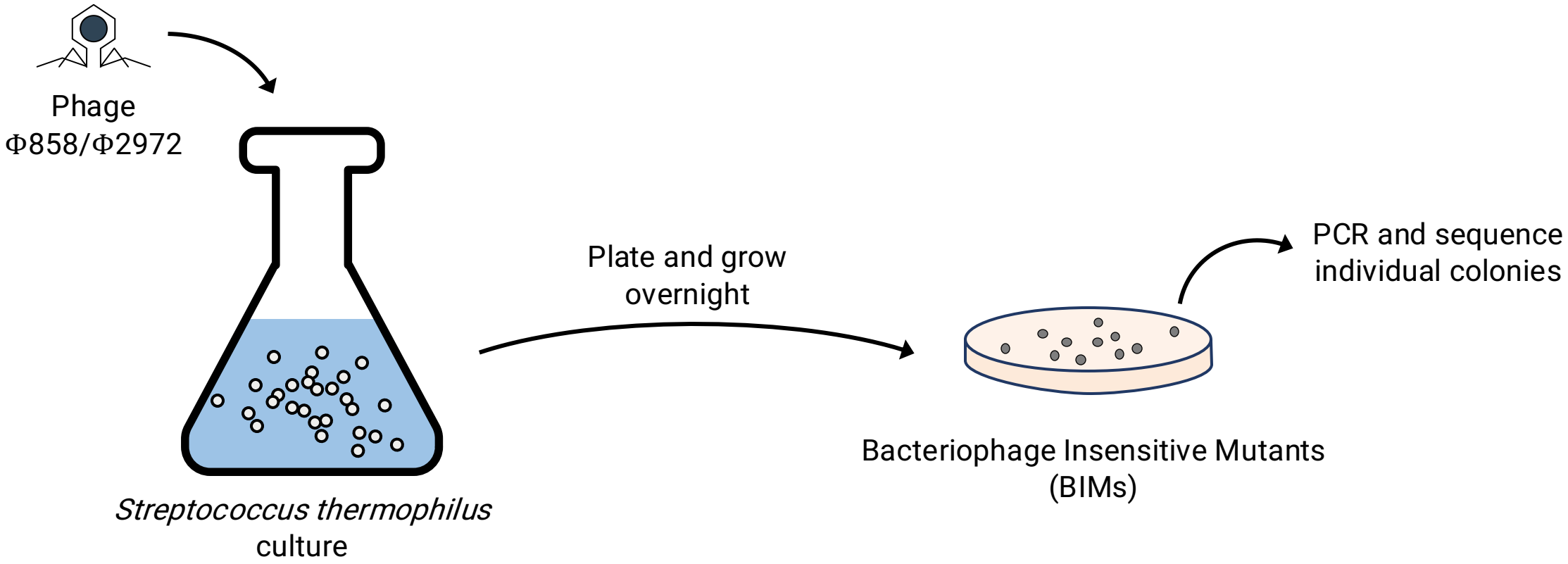
Rodolphe Barrangou,[1] Christophe Fremaux,[2] Hélène Deveau,[3] Melissa Richards,[1] Patrick Boyaval,[2] Sylvain Moineau,[3] Dennis A. Romero,[1] Philippe Horvath[2]*

[1]Danisco USA Inc., 3329 Agriculture Drive, Madison, WI 53716, USA. [2]Danisco France SAS, Boîte Postale 10, F-86220 Dangé-Saint-Romain, France. [3]Département de Biochimie et de Microbiologie, Faculté des Sciences et de Génie, Groupe de Recherche en Ecologie Buccale, Faculté de Médecine Dentaire, Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval, G1K 7P4 Québec, Canada.

*To whom correspondence should be addressed. E-mail: philippe.horvath@danisco.com

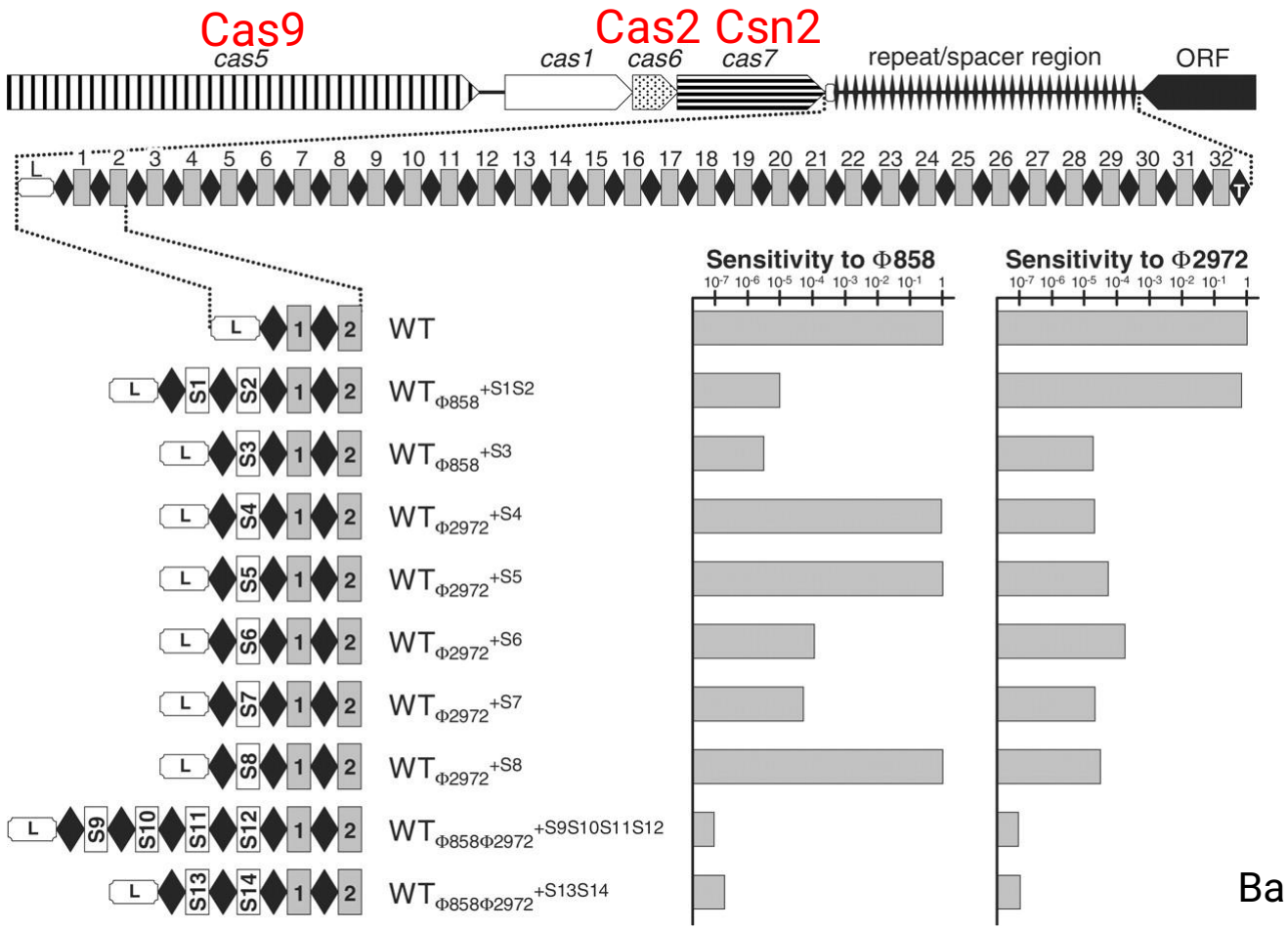
Barrangou et al., *Science* 2007

2007 – First experimental proof of spacer acquisition and phage defense



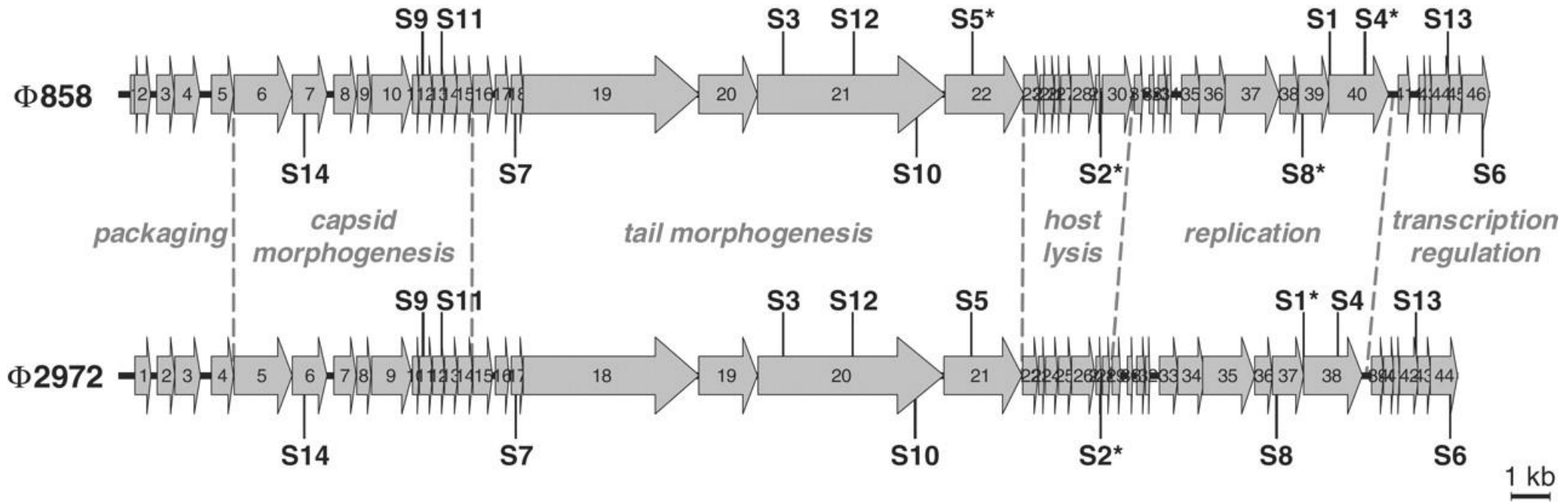
Barrangou et al., *Science* 2007

2007 – First experimental proof of spacer acquisition and phage defense



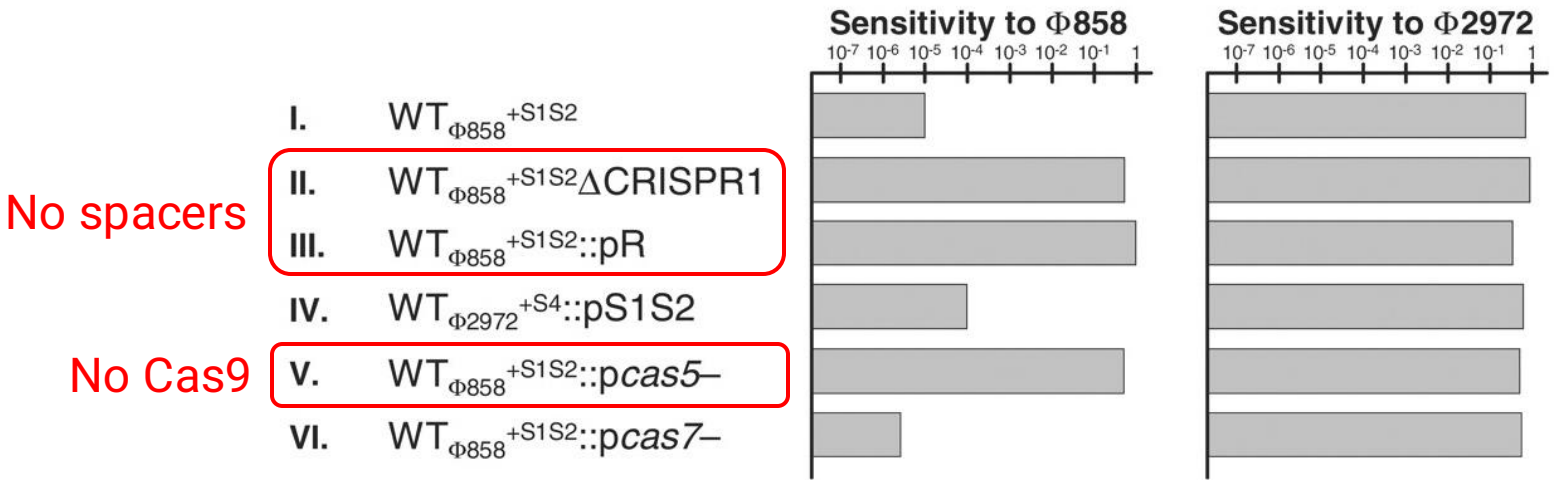
Barrangou et al., *Science* 2007

2007 – First experimental proof of spacer acquisition and phage defense



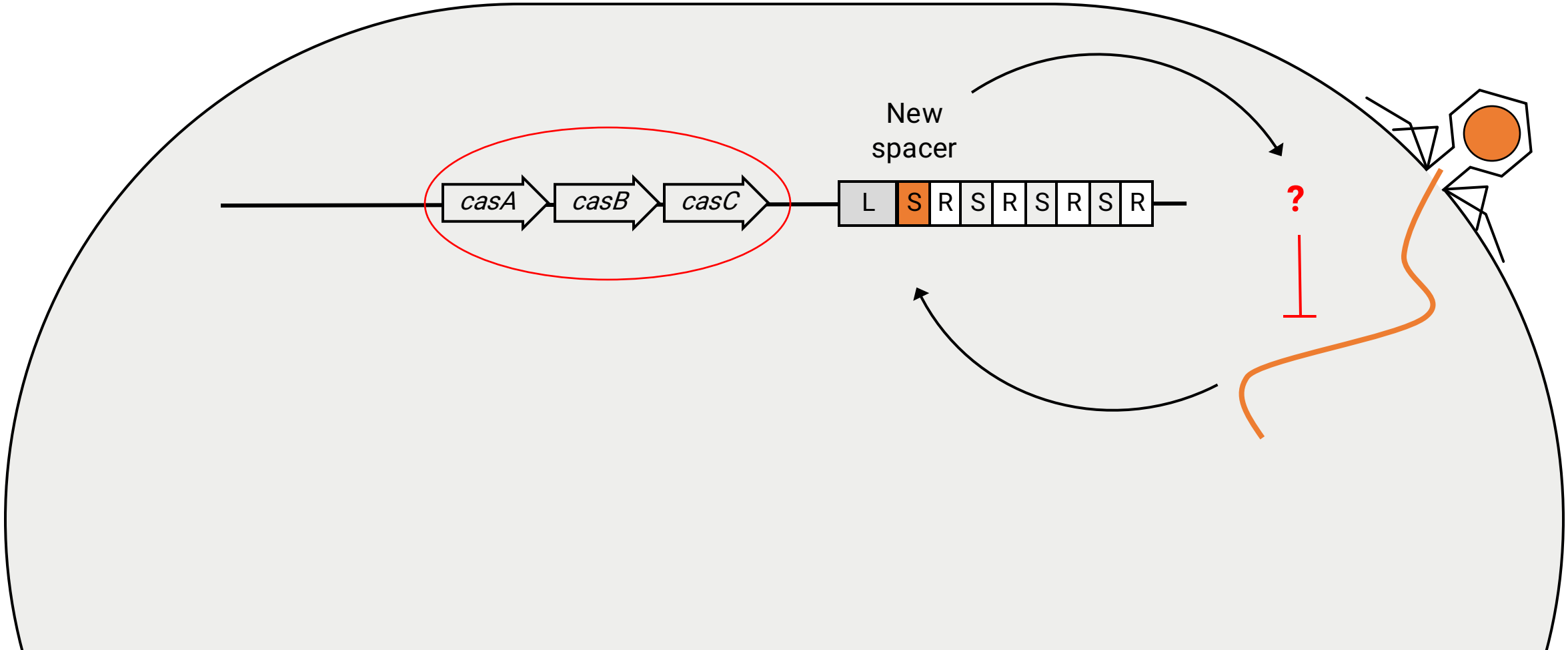
Barrangou et al., *Science* 2007

2007 – First experimental proof of spacer acquisition and phage defense



Barrangou et al., *Science* 2007

Spacer acquisition - immunological memory formation within a single cell

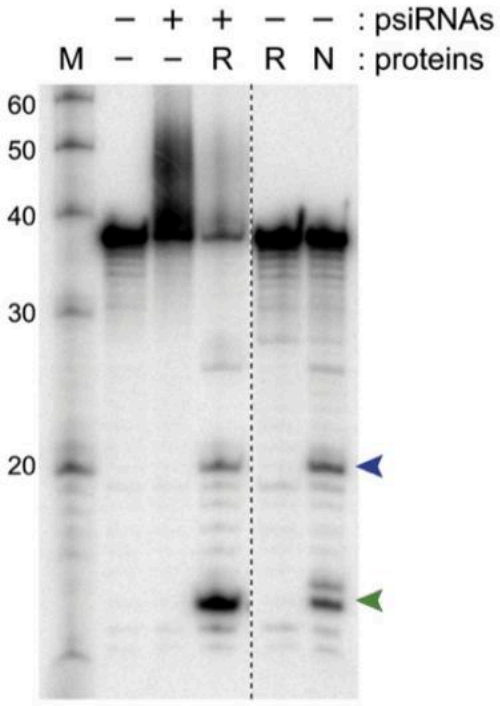


What took them so long?

The CRISPR-array was described in 1987. Why was its function discovered only in 2007?

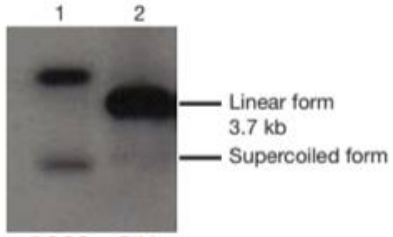
2009-2010 – What is the target of CRISPR-Cas?

RNA?



Halle et al., *Cell* 2009

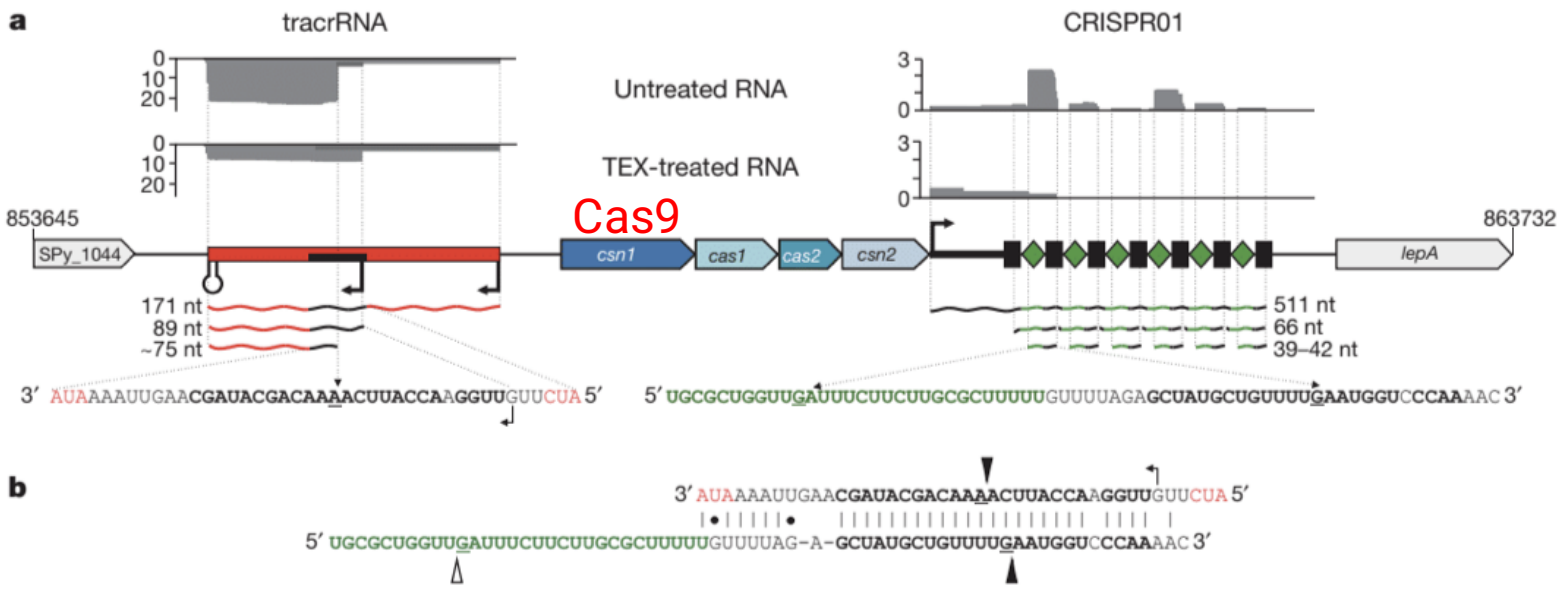
DNA?



Garneau et al., *Nature* 2010

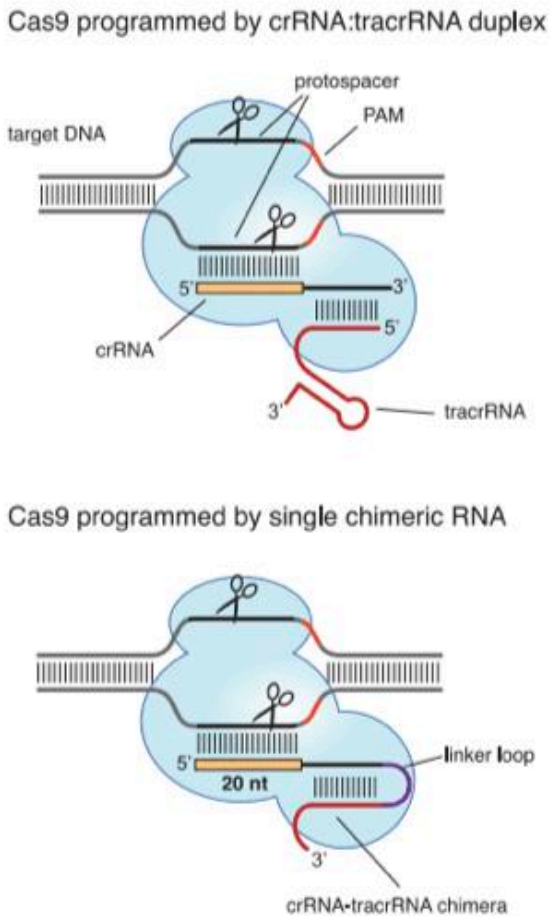
BOTH!
Depending on the
CRISPR-Cas type.

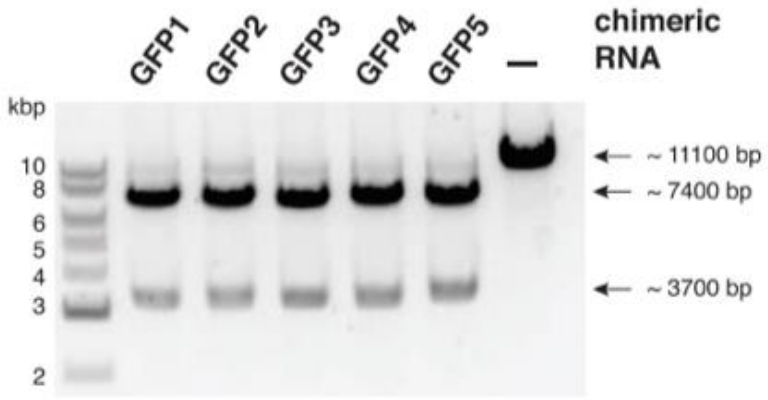
2011-2012 – CRISPR-Cas as a programable tool 🤯



Deltcheva et al., *Nature* 2011

2011-2012 – CRISPR-Cas as a programable tool 🤯



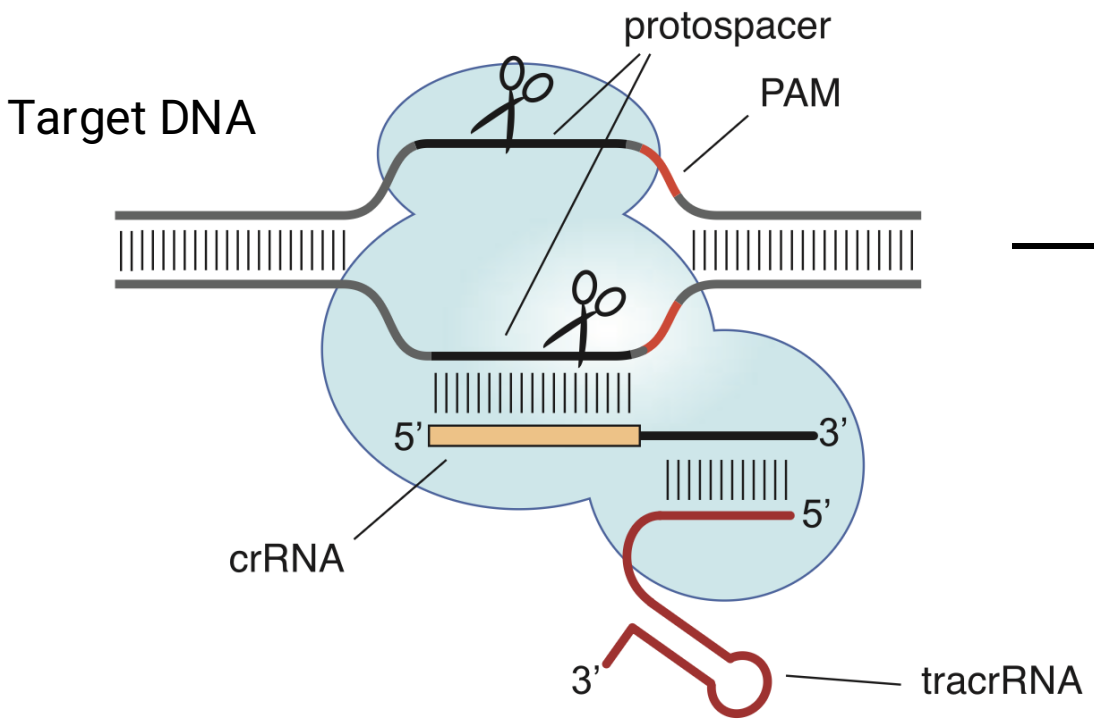


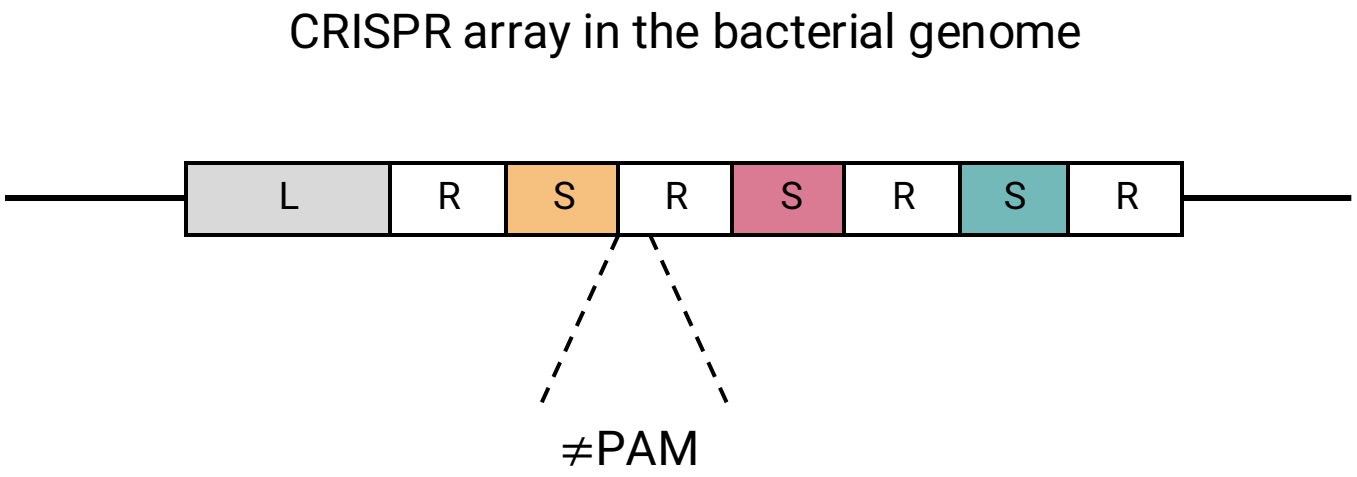
Jinek et al., *Science* 2012

How do bacteria balance immunity with the risk of self-targeting?

Self vs. non-self

DNA-targeting systems: PAM



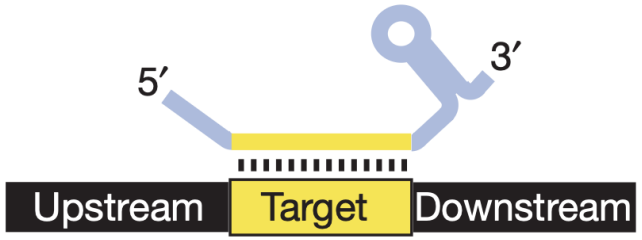


Jinek et al., *Science* 2012

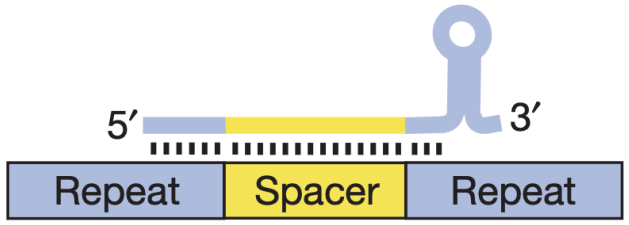
Self vs. non-self

RNA-targeting systems: anti-tag

Type III



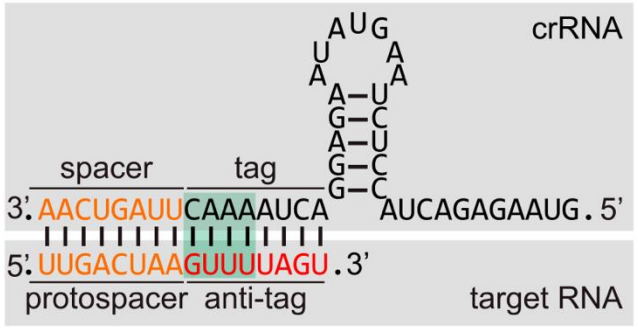
Targeting of non-self DNA

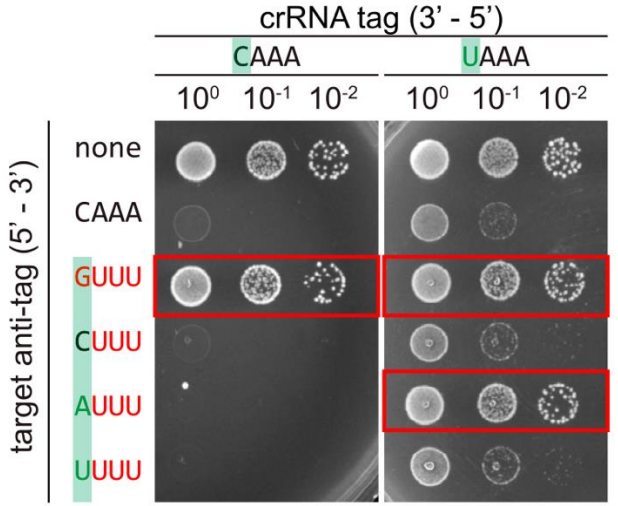


Protection of self DNA

Marraffini et al., *Nature* 2010

Type VI





Meeske & Marraffini et al., *Mol Cell* 2019

Phages fight back

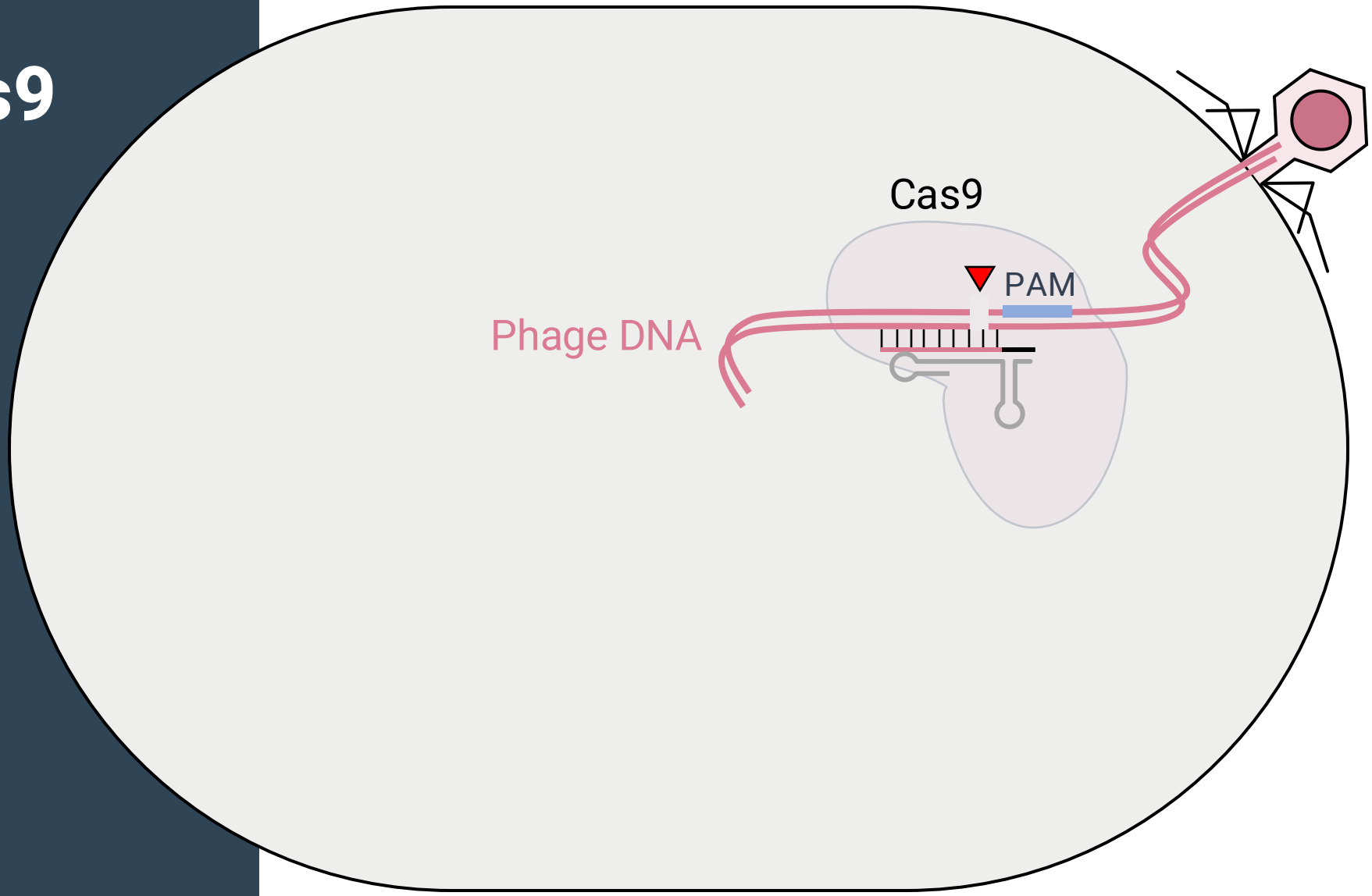
Phage-encoded CRISPRs Seed et al., *Nature* 2013

Anti-CRISPRs Bondy-Denomy et al., *Nature* 2013

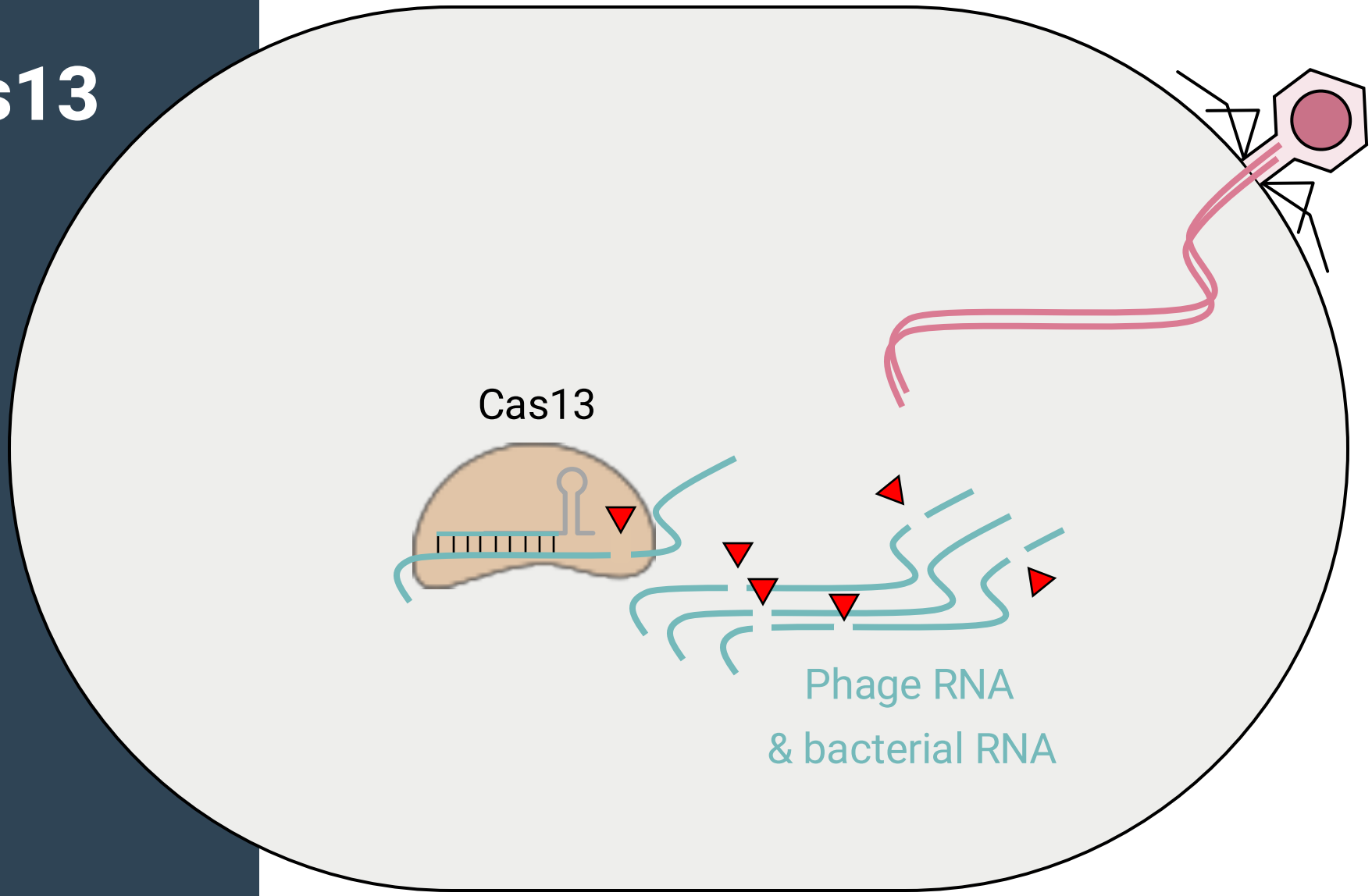
Pawluk et al., *Cell* 2016

1. CRISPR-Cas intro
2. **Type III CRISPR-Cas10**

Type II-A CRISPR-Cas9



Type VI-A CRISPR-Cas13



Molecular mechanisms of CRISPR-Cas: A rich source for biomedical discoveries

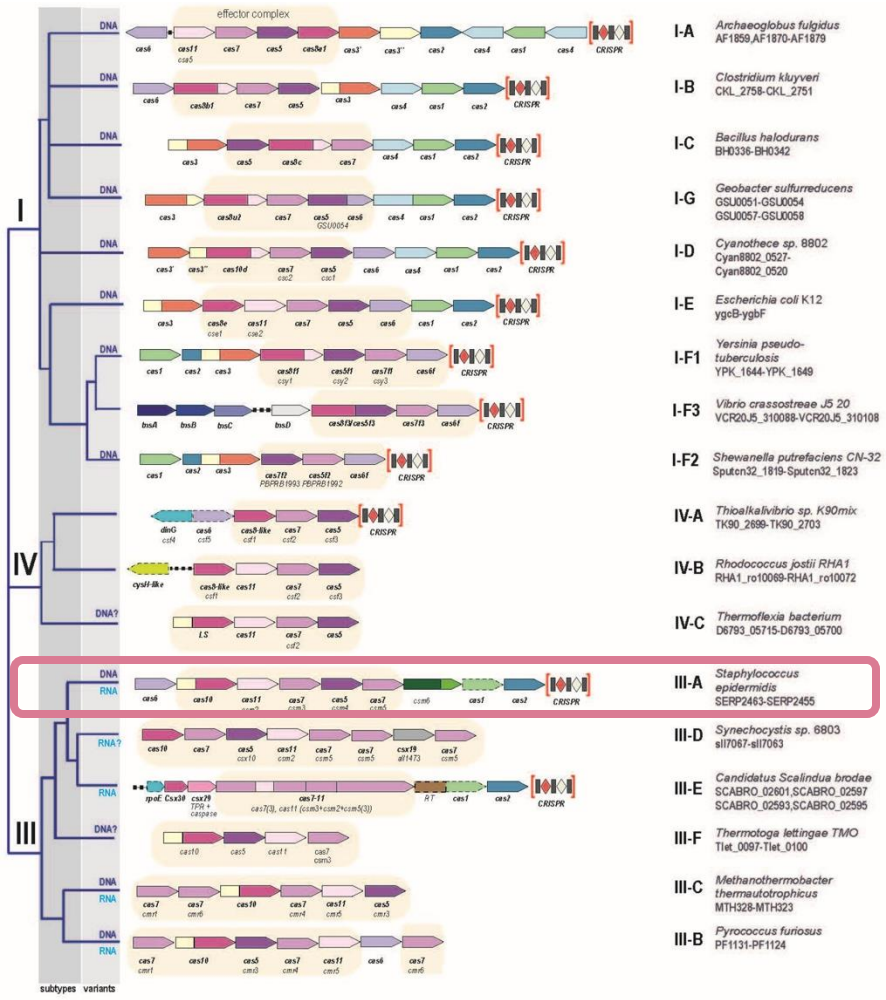
Class 1

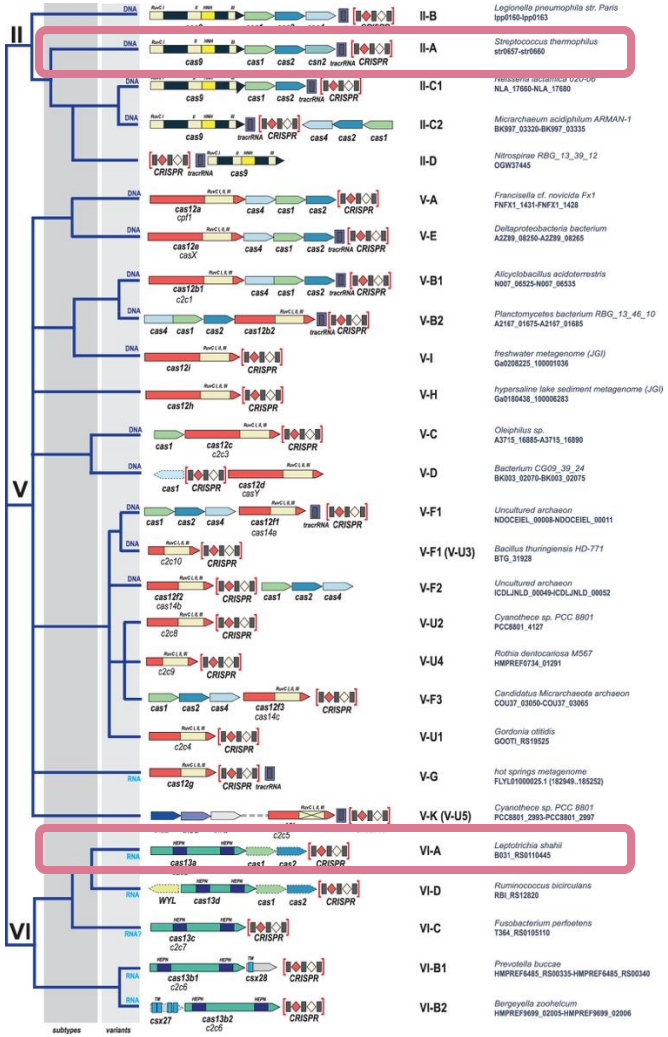
Class 2

Cas10 (type III)

Cas9 (type II)

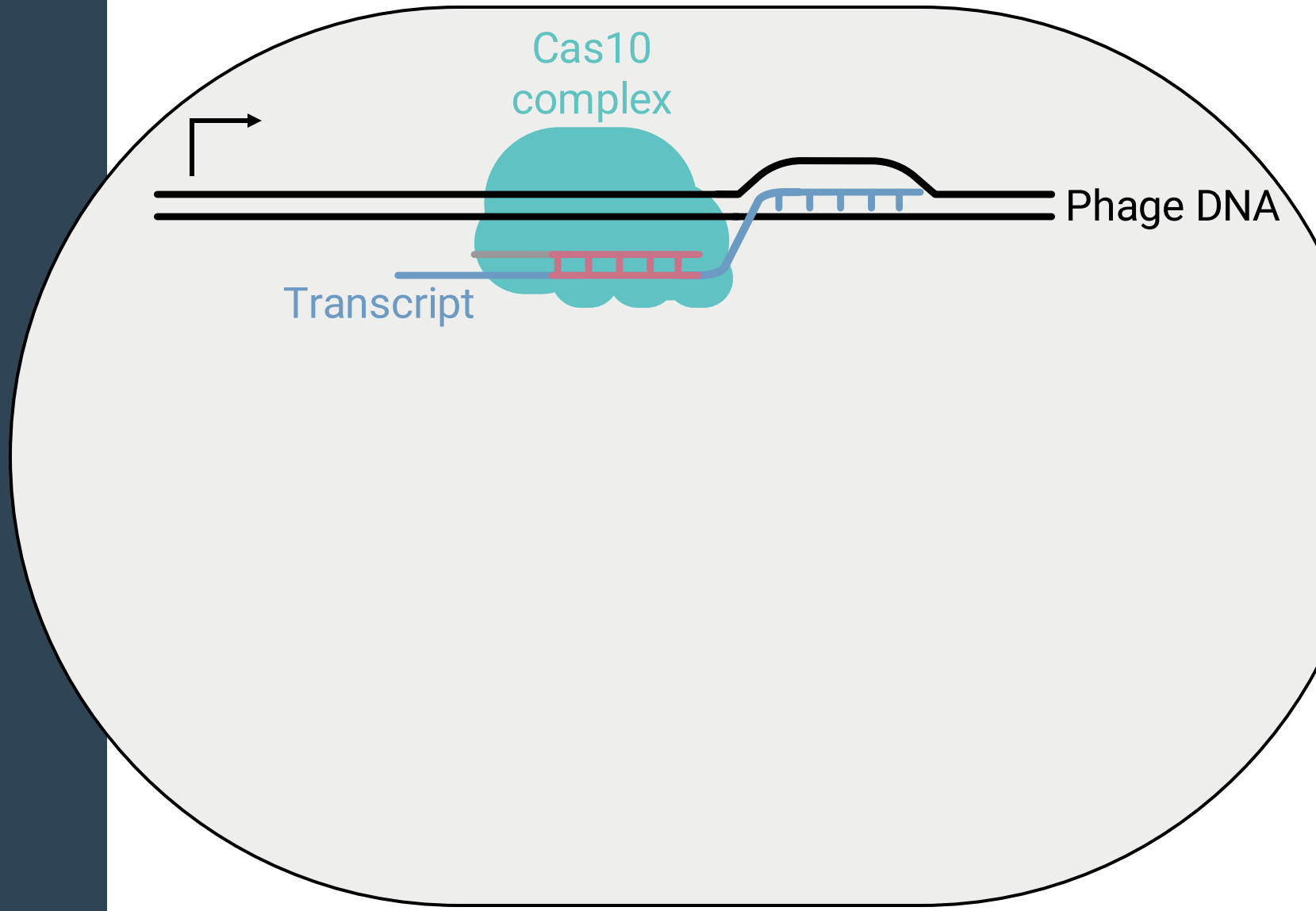
Cas13 (type VI)



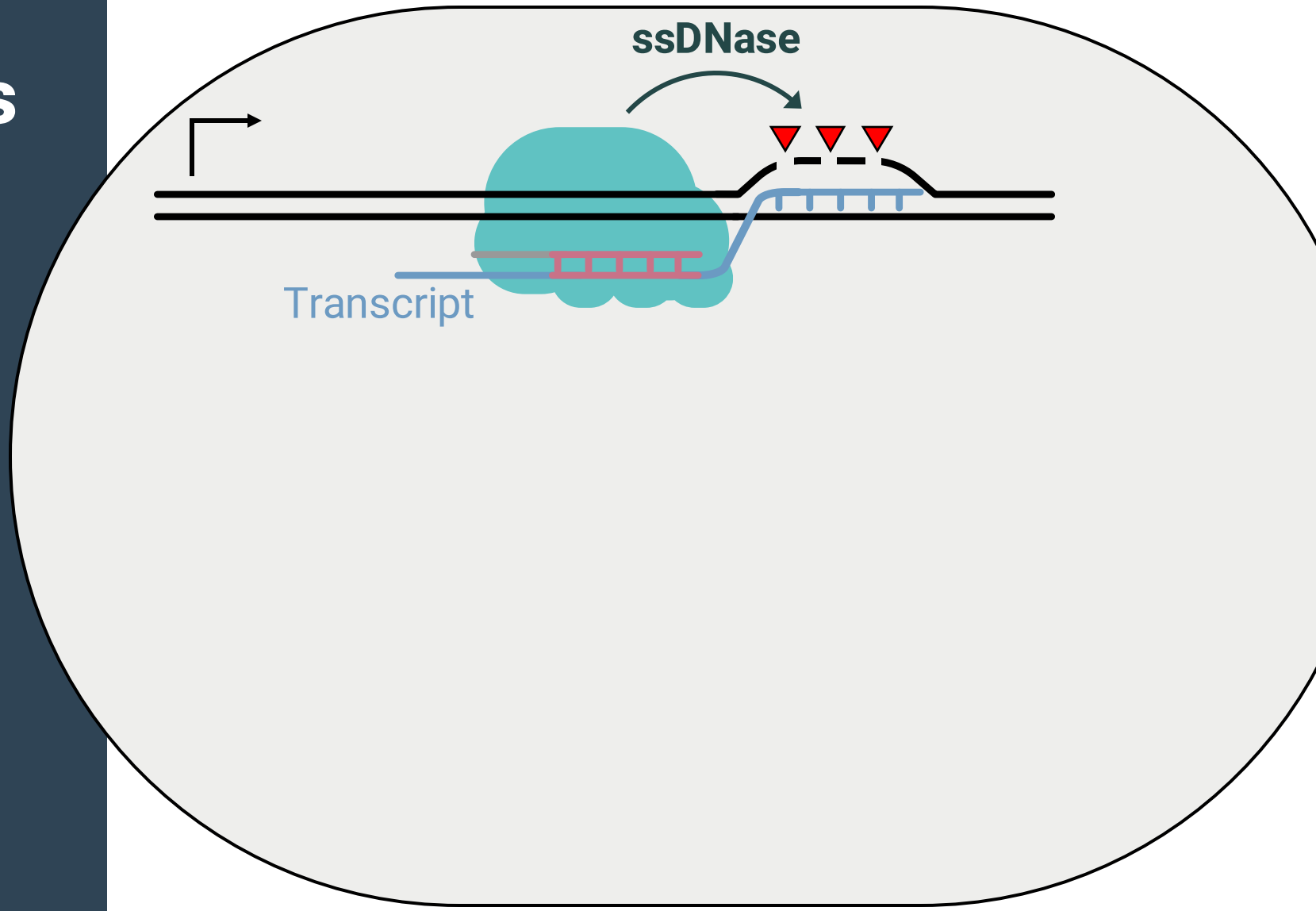


Koonin et al, *Curr Opin Microbiol* 2017

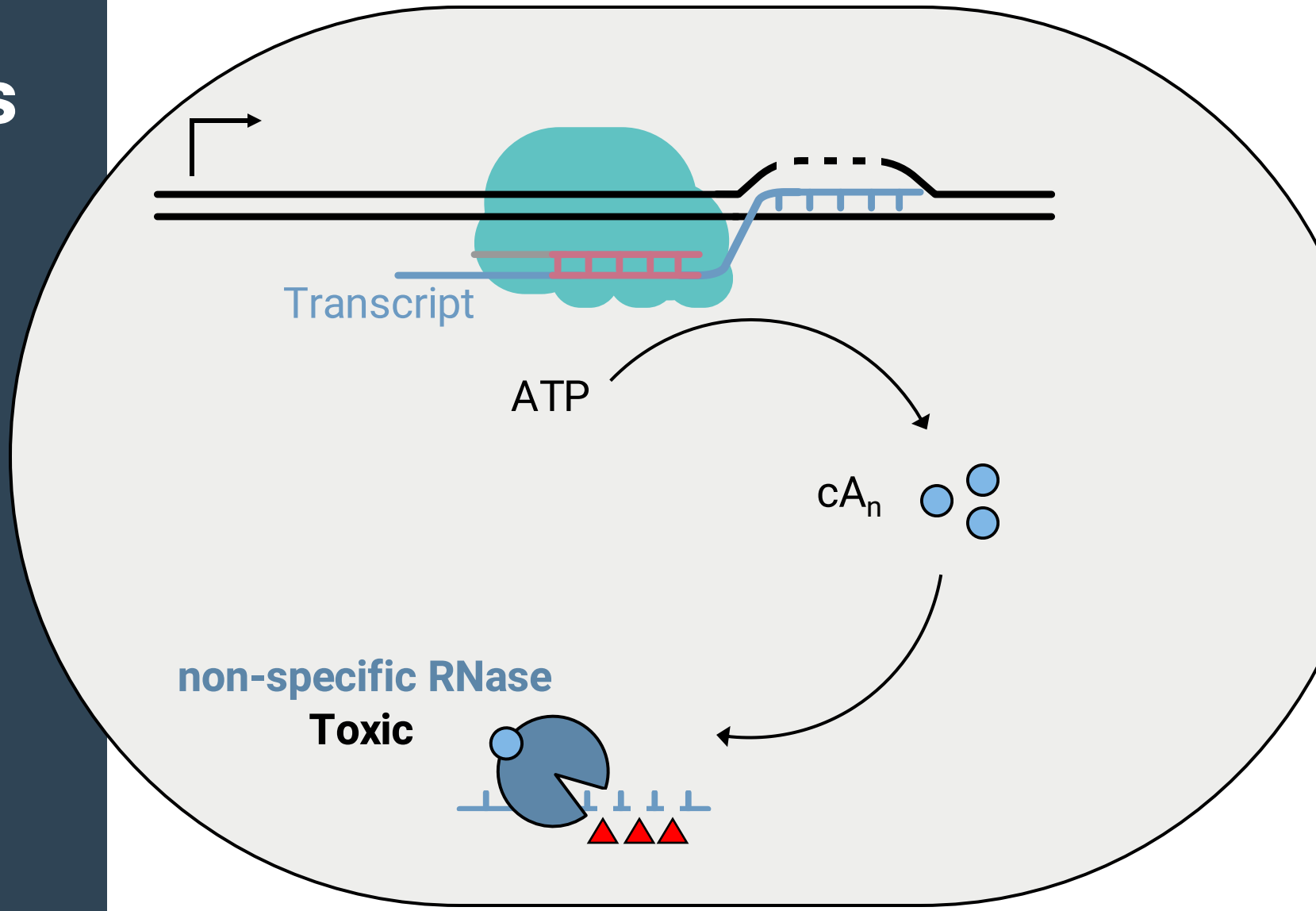
Type III-A CRISPR-Cas: the Cas10 complex



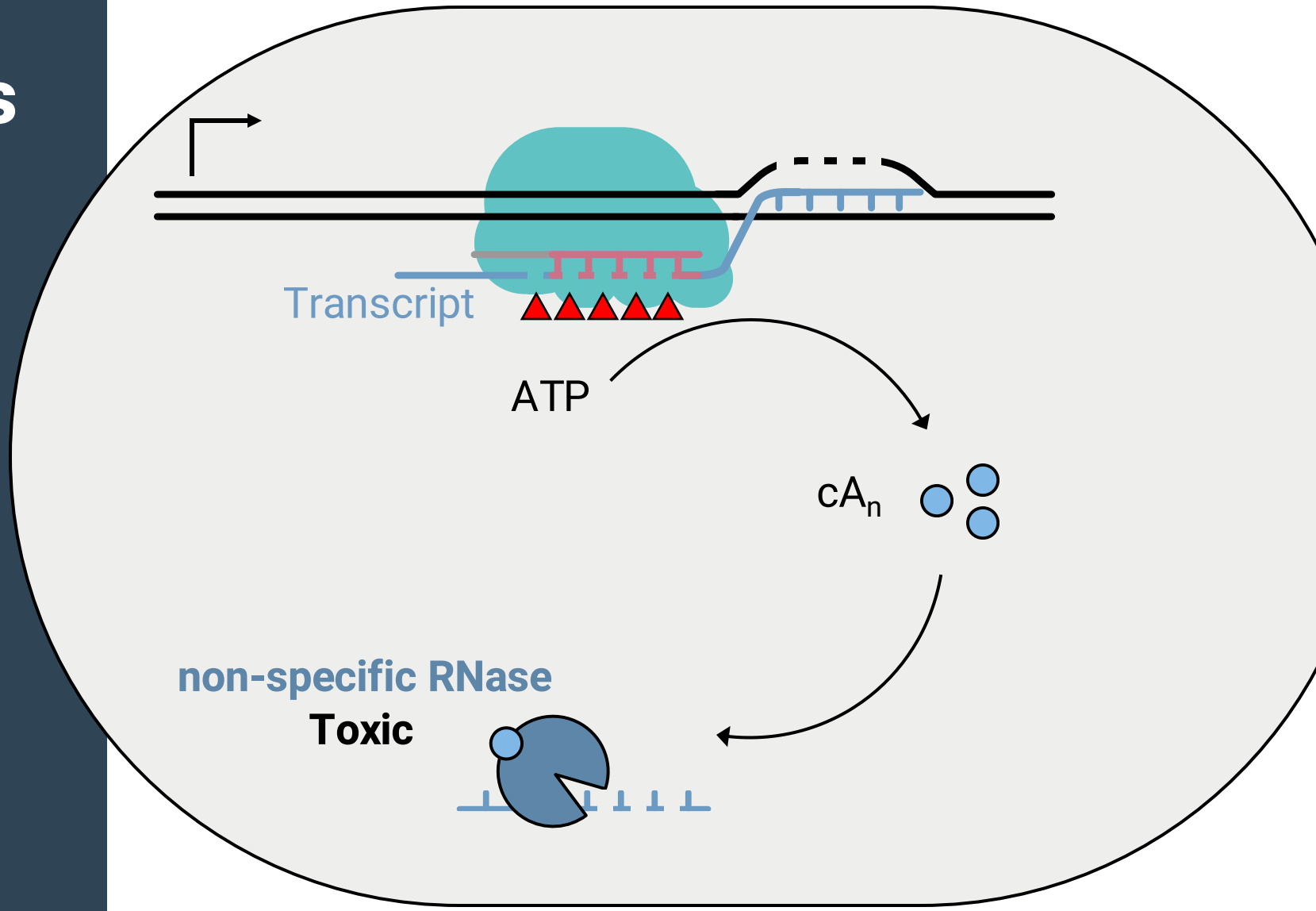
Transcript binding activates the Cas10 complex



Transcript binding activates the Cas10 complex

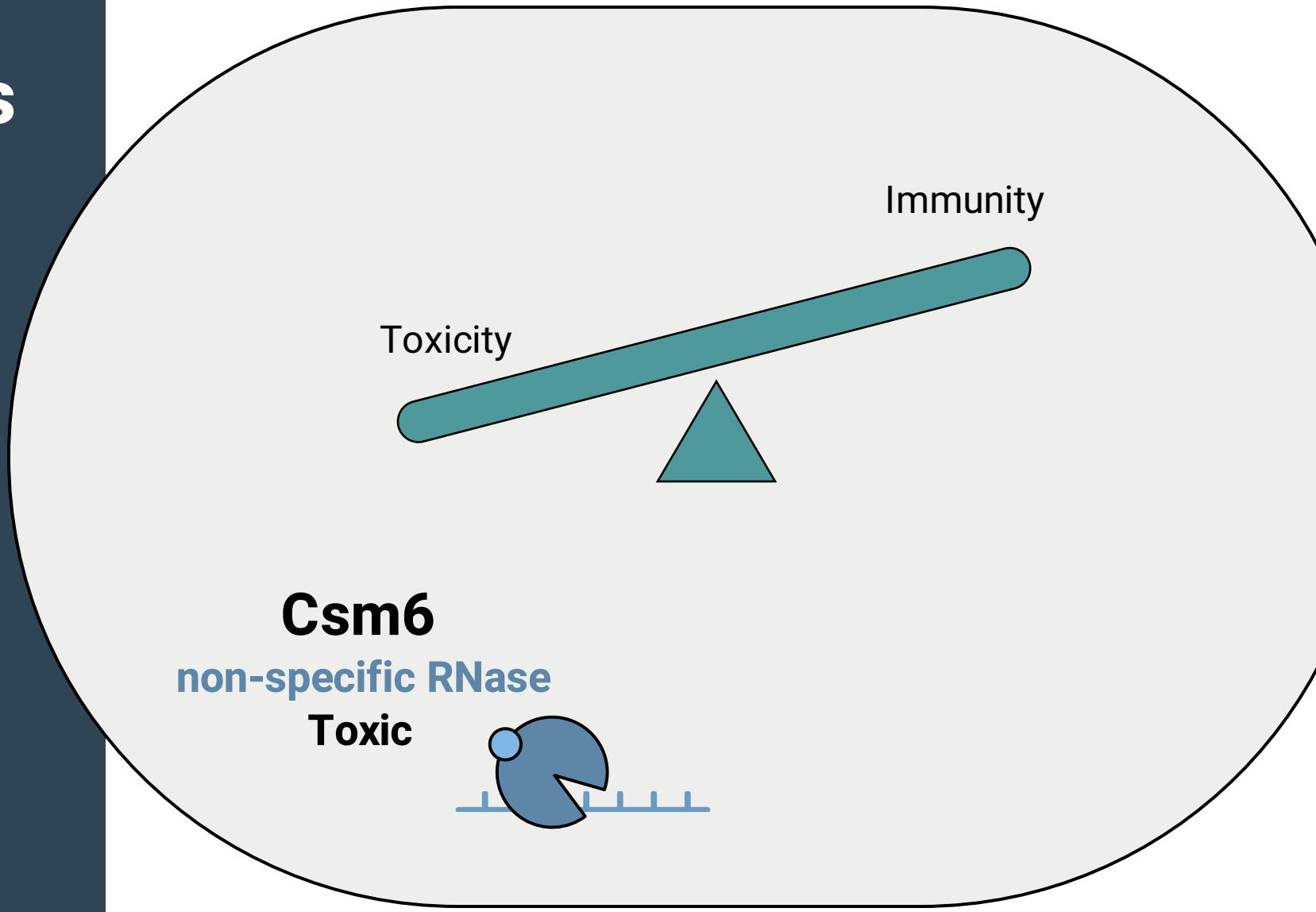


Transcript binding activates the Cas10 complex

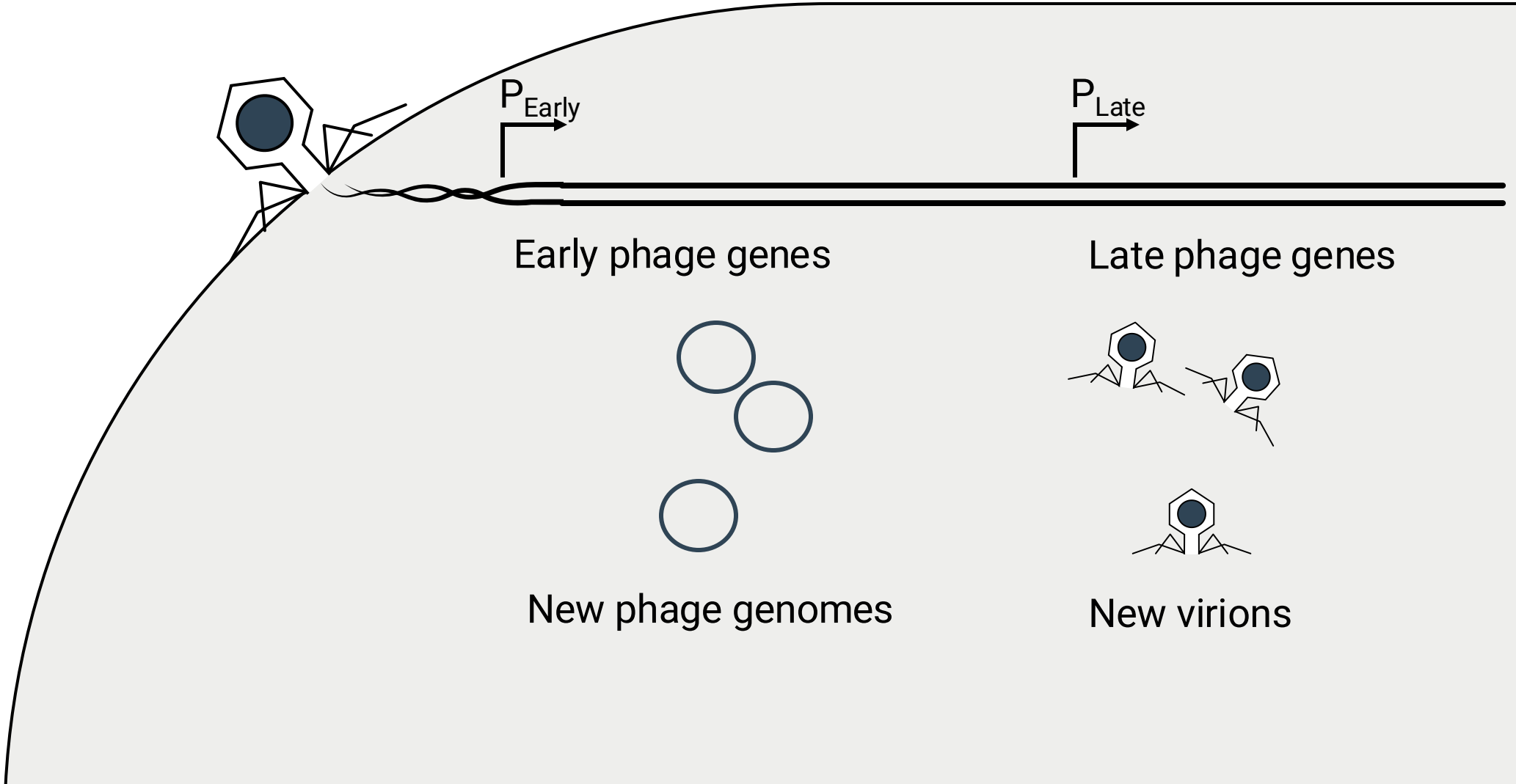


Transcript binding activates the Cas10 complex

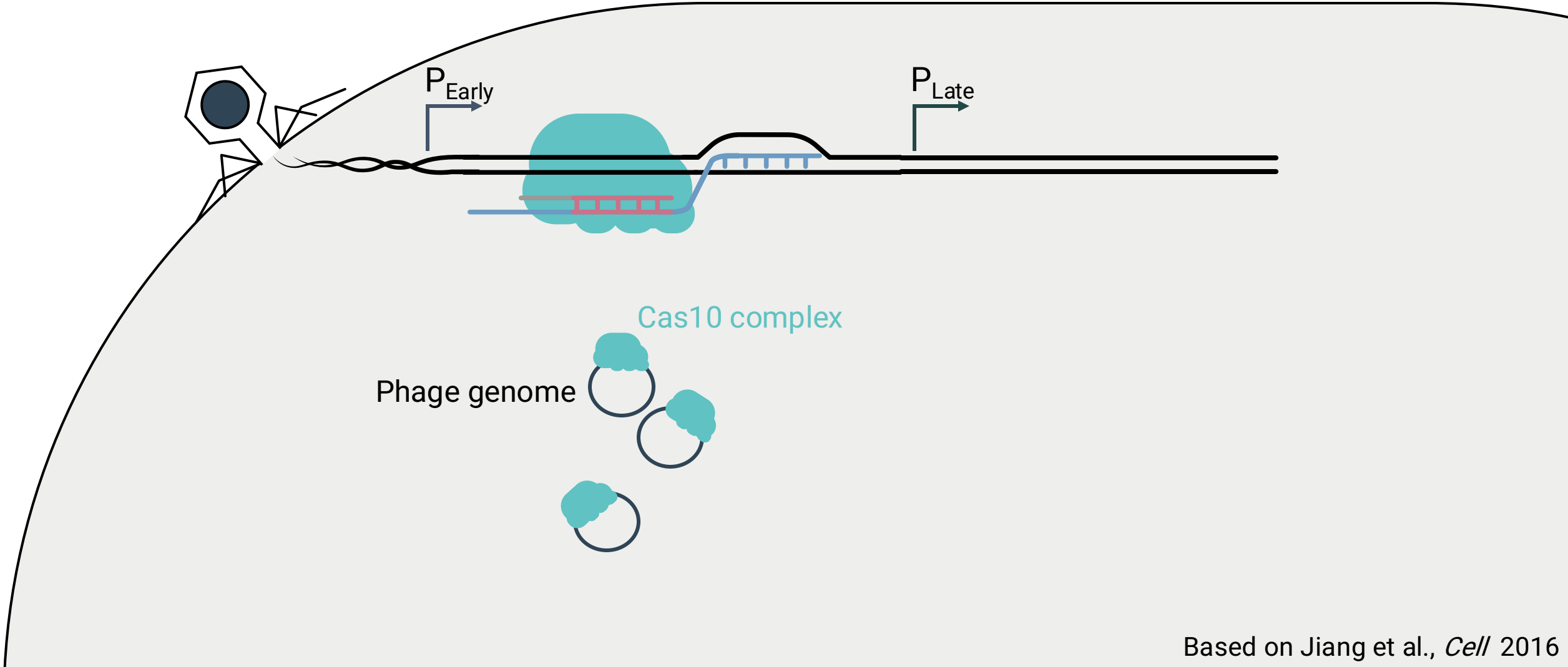
How are spacers maintained in the population despite **inhibiting bacterial growth?**



Csm6 activation during phage infection

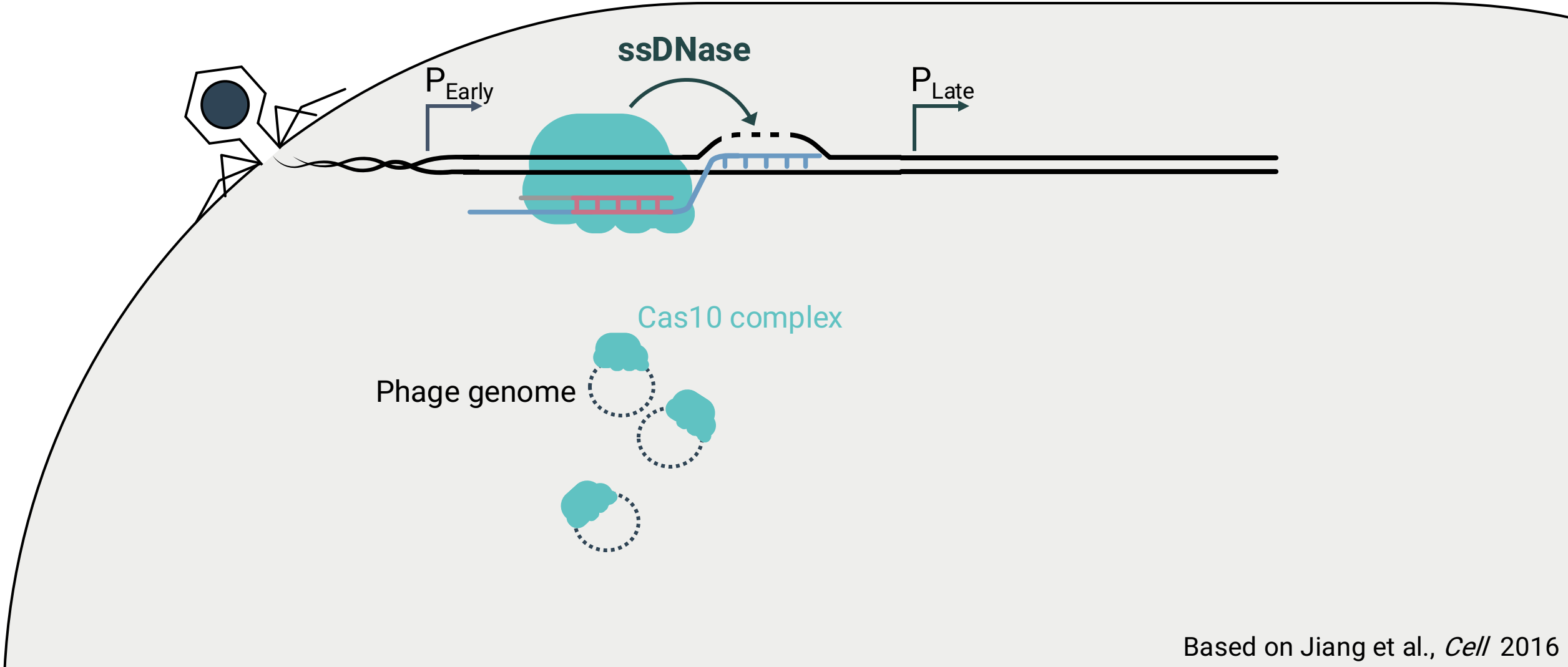


Csm6 activation during phage infection



Based on Jiang et al., *Cell* 2016

Csm6 activation during phage infection



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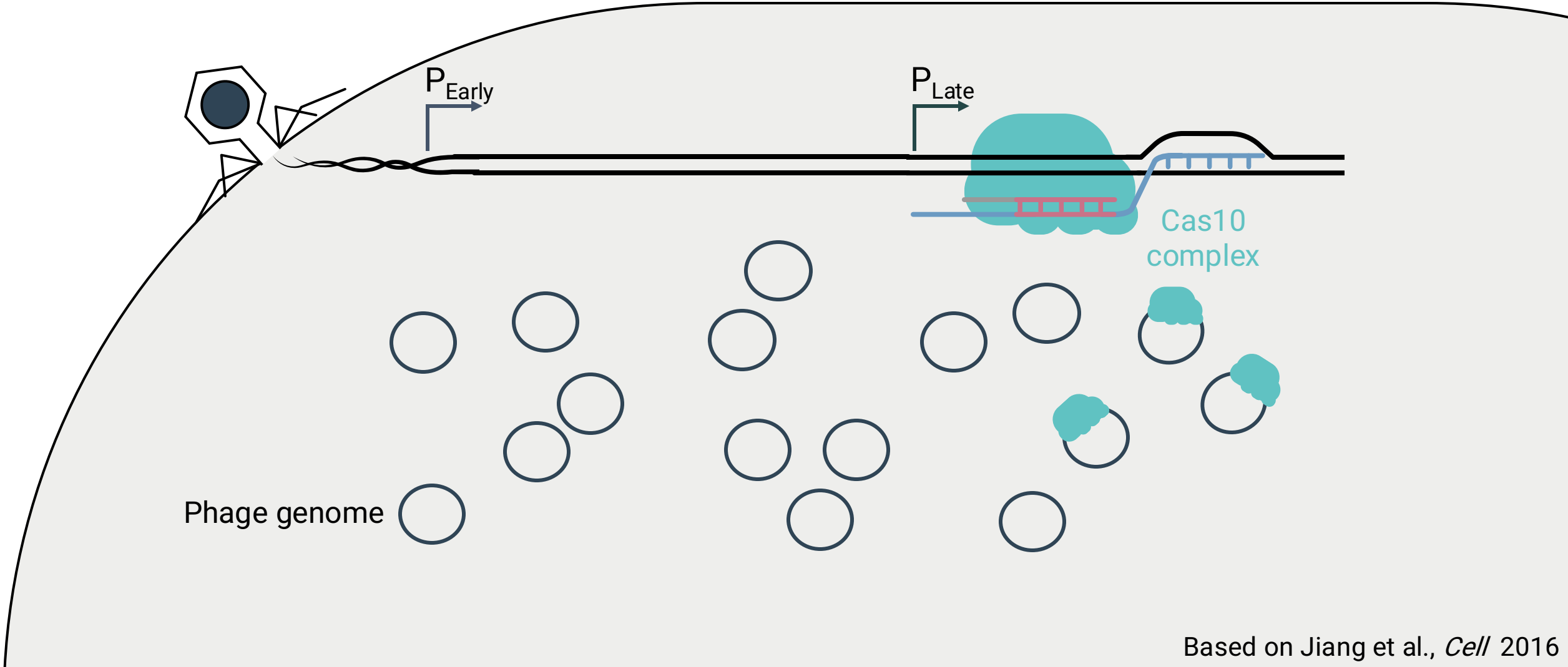

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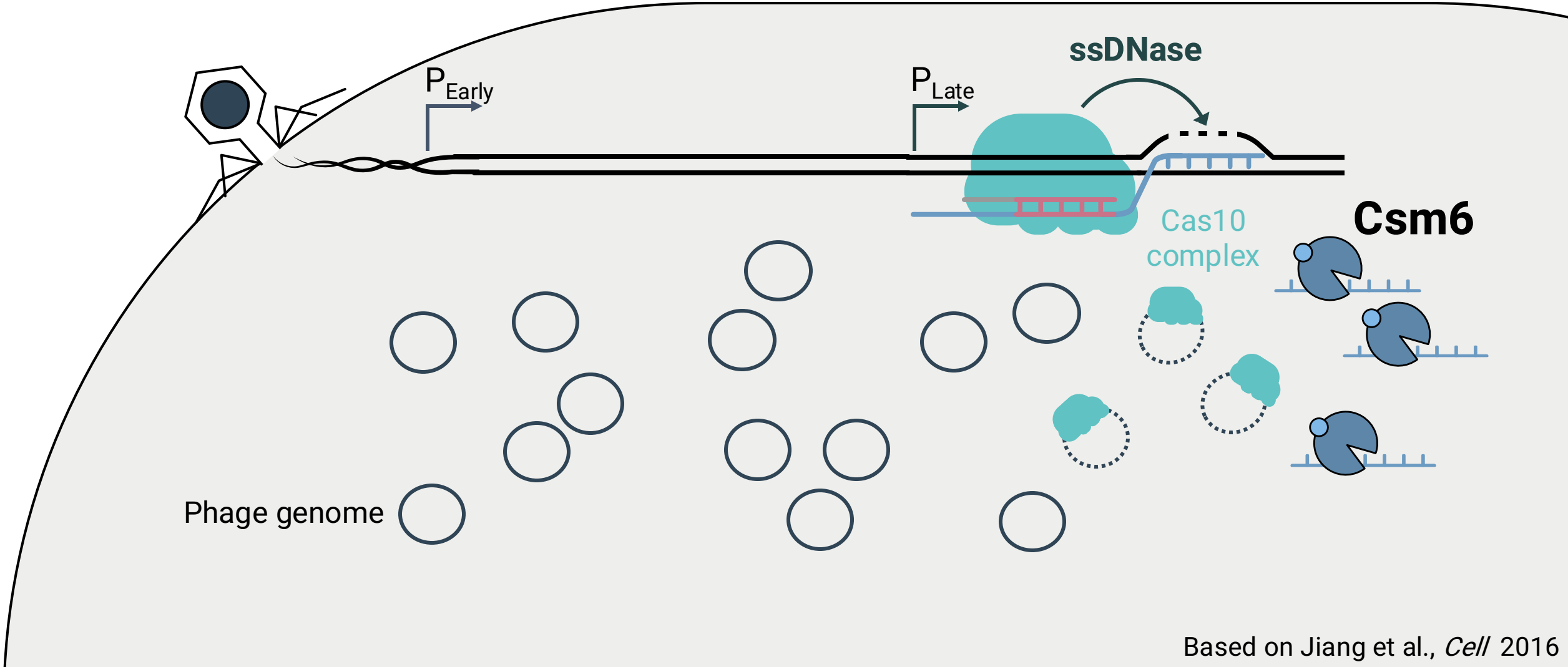
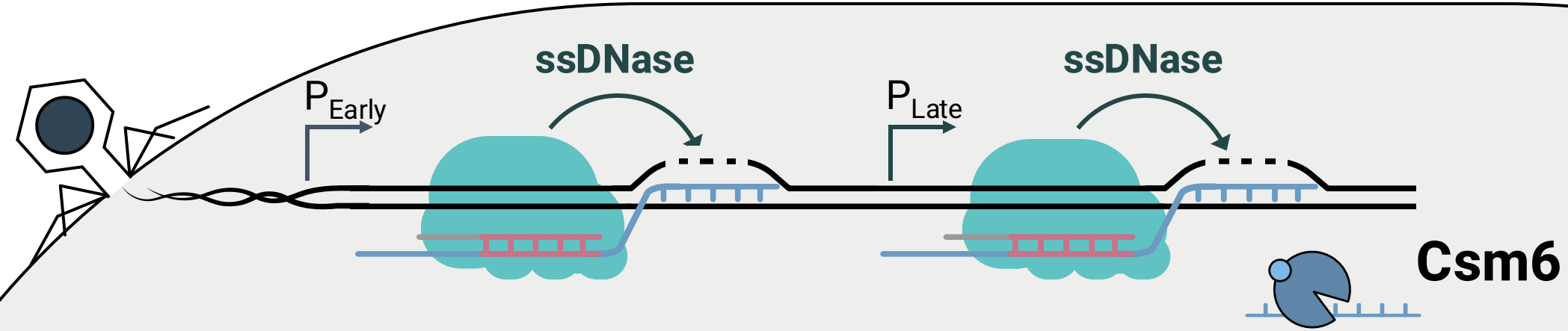
Csm6 activation during phage infection



Based on Jiang et al., *Cell* 2016

Type III CRISPR-Cas can confer either individual or population immunity



Individual

"Early" spacer ∝ Csm6-independent

DNase activity is sufficient for immunity

The **infected** cell survives

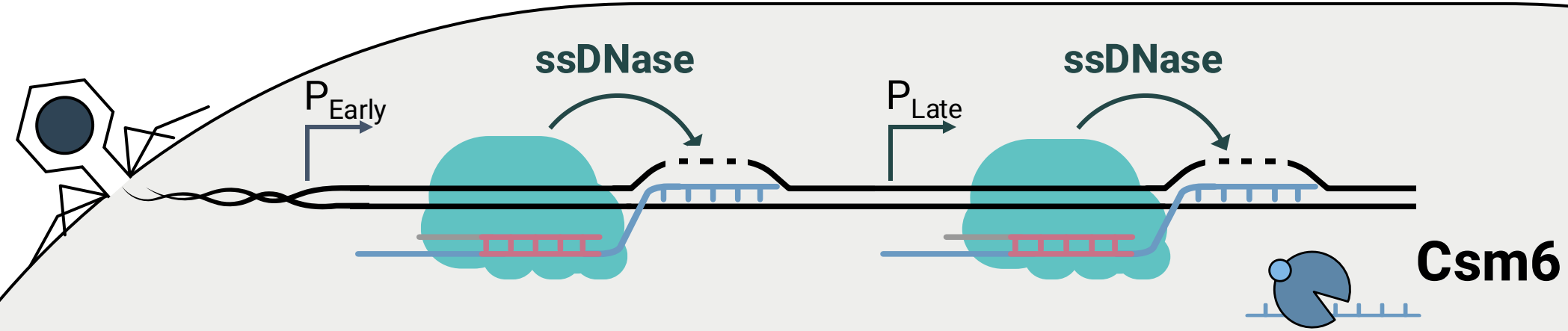
Population

"Late" spacer ∝ Csm6-dependent

Growth inhibition of the infected cell

Non infected cells survive

Type III CRISPR-Cas can confer either individual or population immunity



Individual

"Early" spacer ∝ Csm6-independent

DNase activity is sufficient for immunity

The **infected** cell survives

Population

"Late" spacer ∝ Csm6-dependent

Growth inhibition of the infected cell

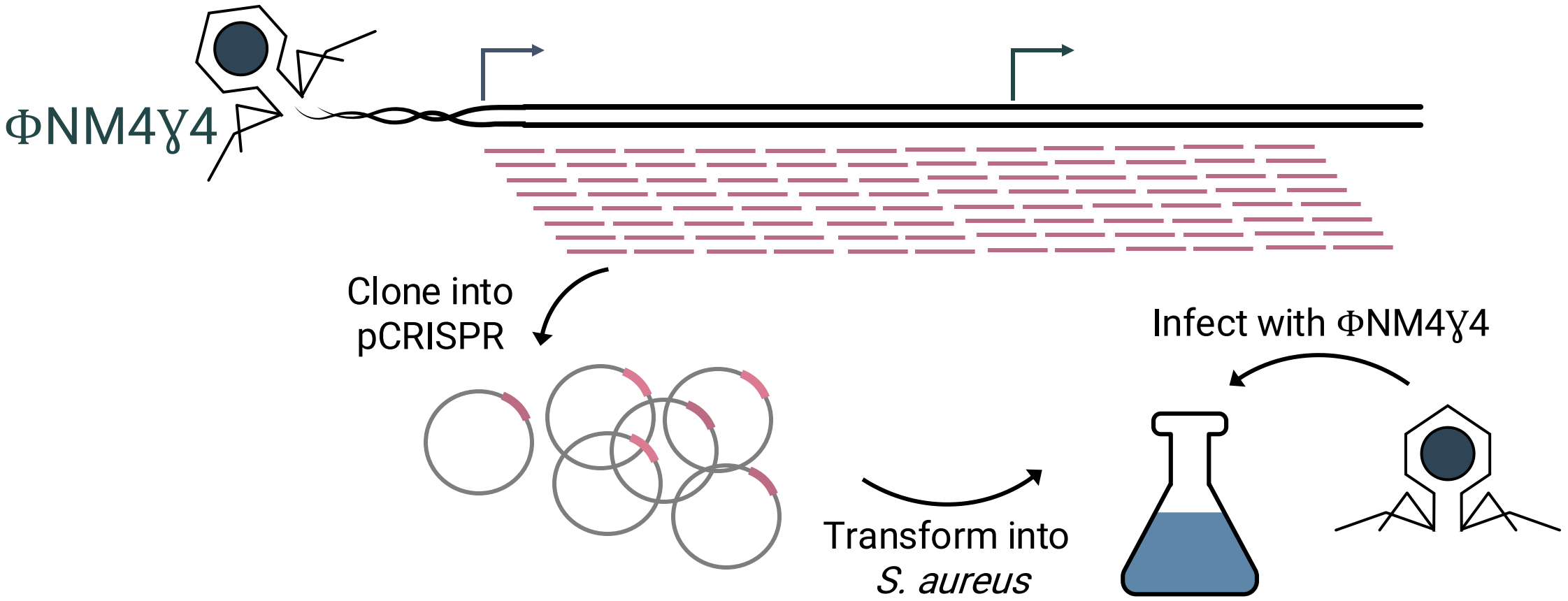
Non infected cells survive ?

Half of annotated spacers in metagenomes are predicted to induce Csm6.

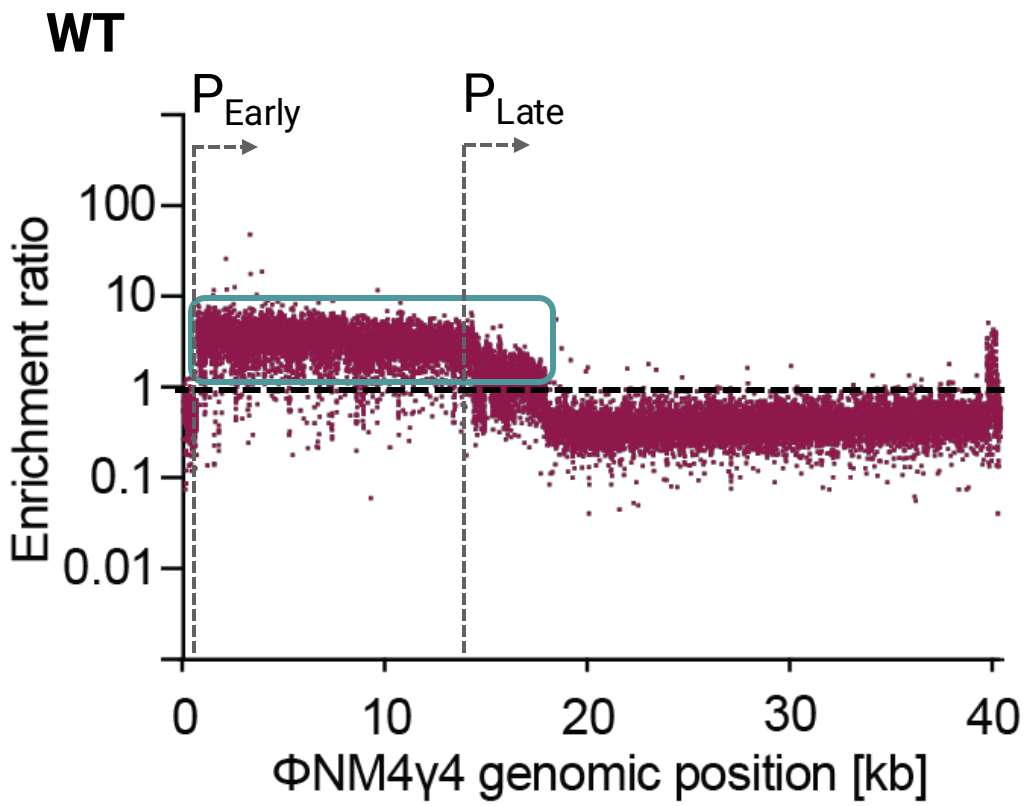
Tiled spacer library across the viral genome to study the type III-A immune response

A synthetic library of 40,338 spacers tiled every 2 nucleotides matching the phage genome

Tiled spacer library across the viral genome to study the type III-A immune response



Tiled spacer library across the viral genome to study the type III-A immune response

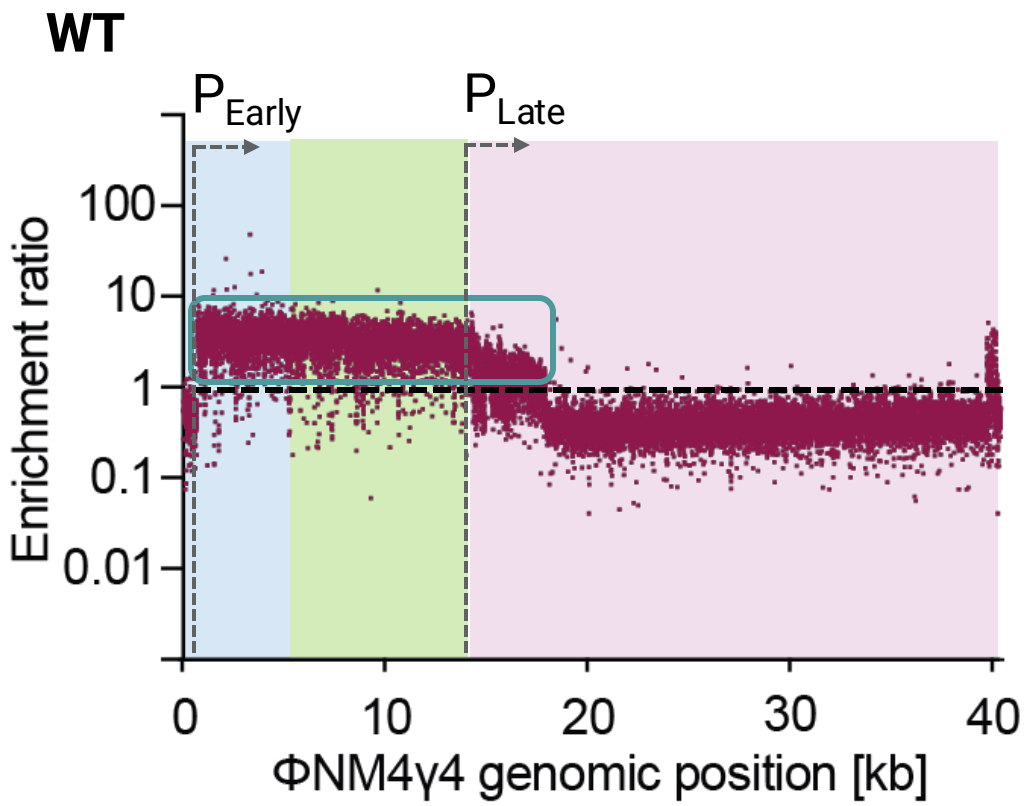


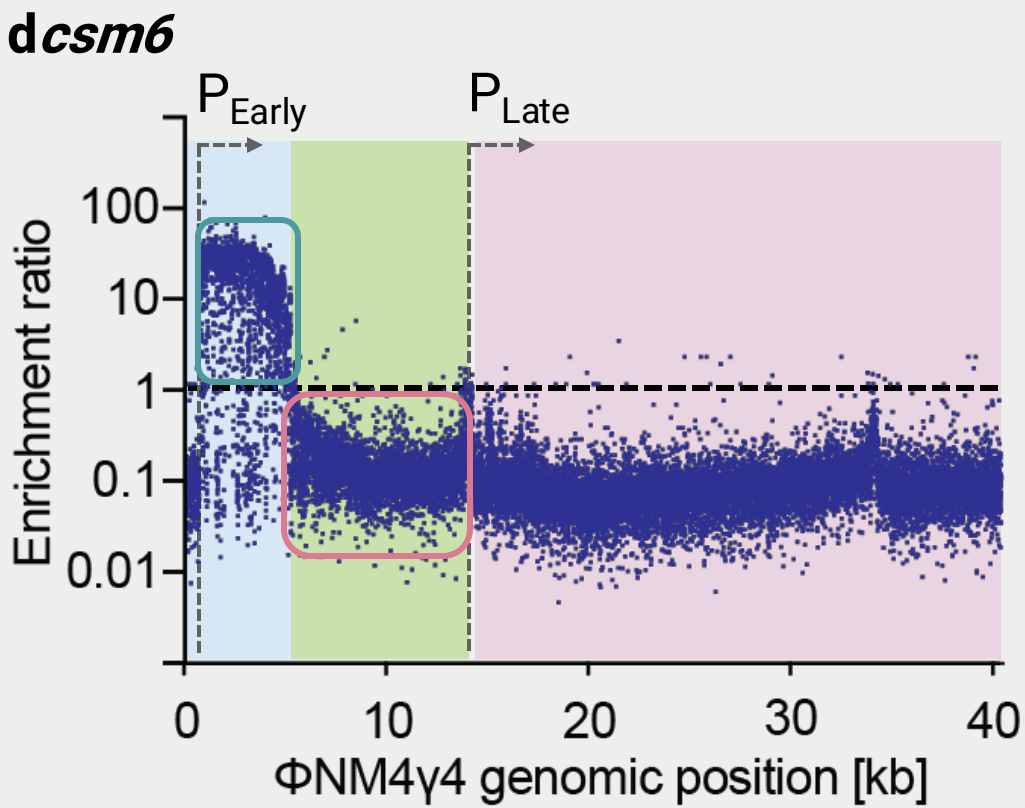
> 1

Depletion due to Csm6-induced growth inhibition

Aviram et al., *Cell Host & Microbe* 2024

Tiled spacer library across the viral genome reveals the Csm6-dependence landscape



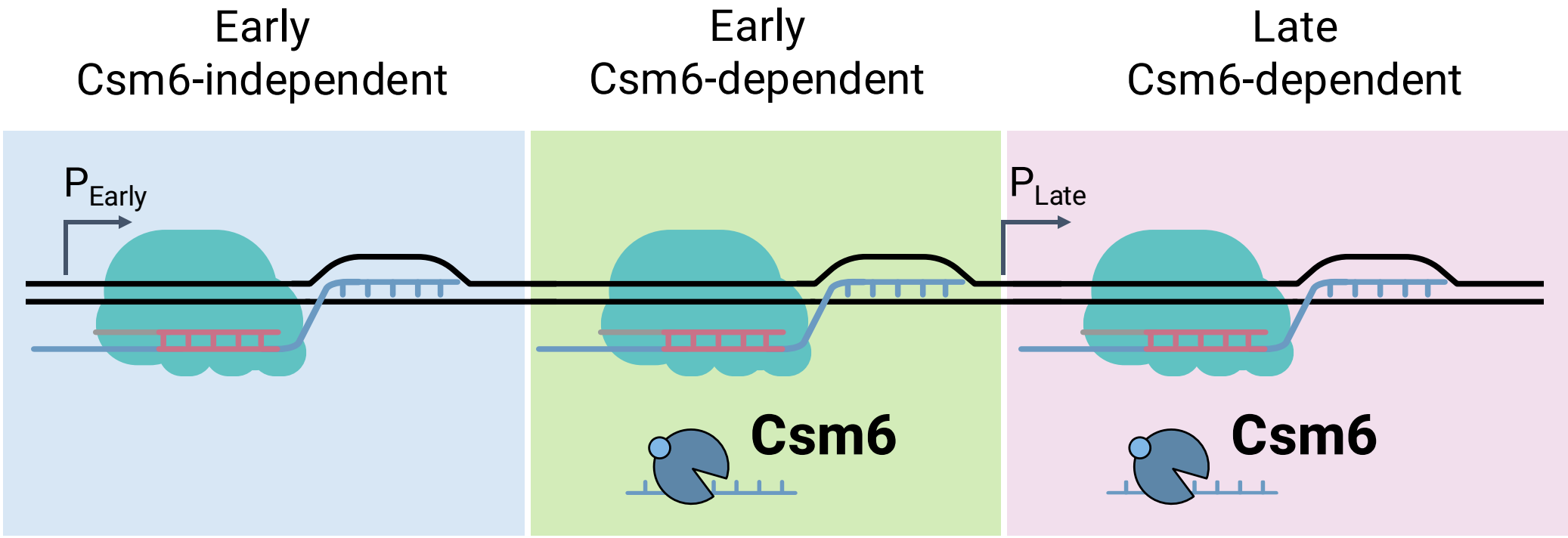


Most spacers targeting **early** genes are **Csm6-dependent.**

Csm6-activation is **not detrimental** to cells.

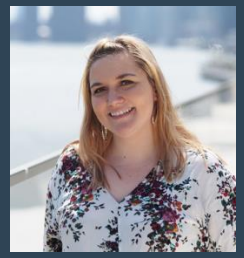
Aviram et al., *Cell Host & Microbe* 2024

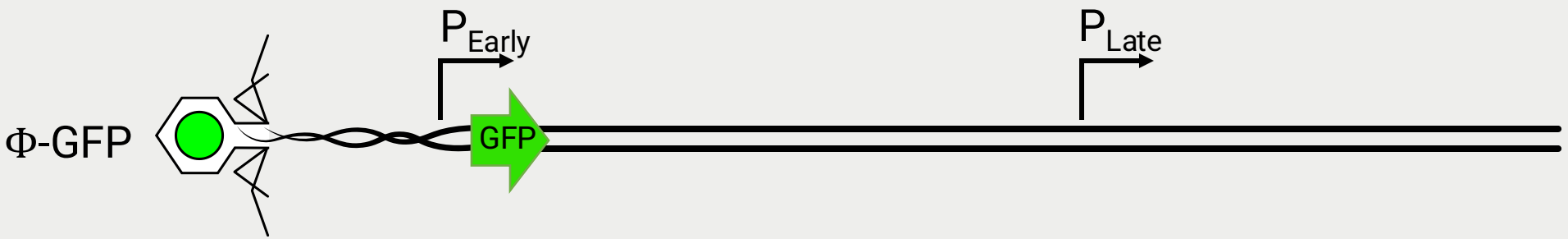
A revised model for Csm6-dependence in type III-A CRISPR-Cas immunity



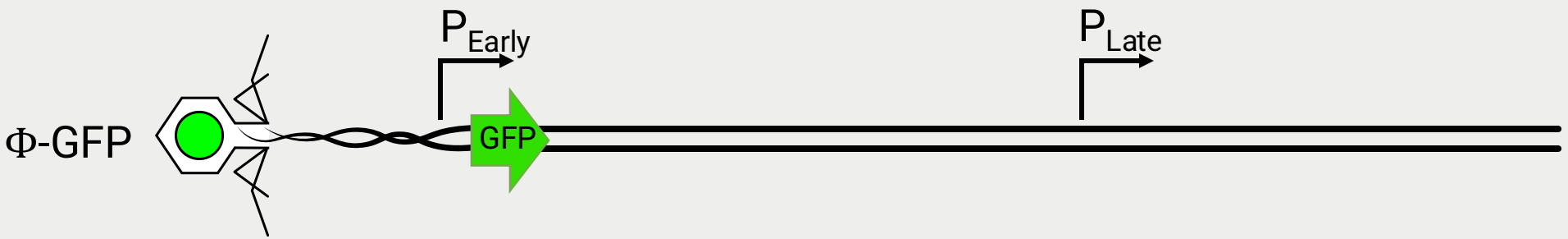
GFP-expressing phage as a tool for tracking infected bacteria

Amanda Shilton

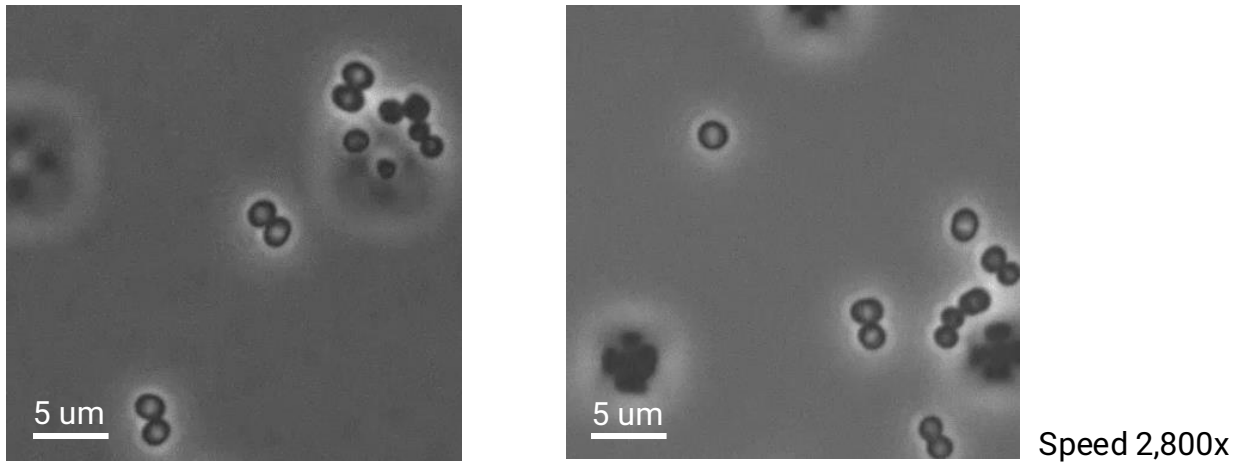




GFP-expressing phage as a tool for tracking infected bacteria



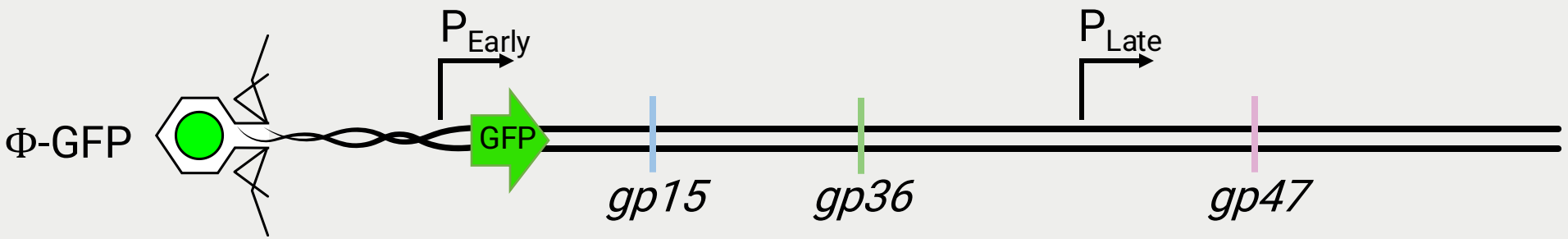
No CRISPR-Cas



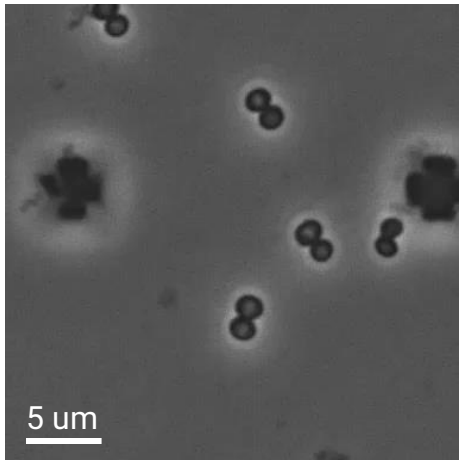
Speed 2,800x

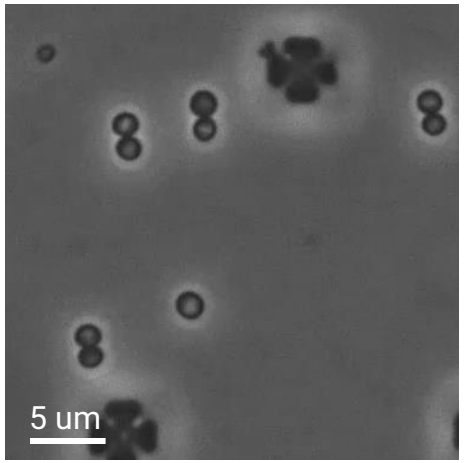
Aviram et al., *Cell Host & Microbe* 2024

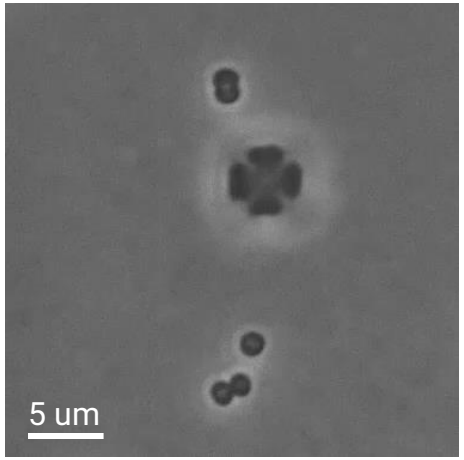
The spacer's target affects the outcome of type III CRISPR-Cas immunity



With CRISPR







Speed 2,800x

Growth arrest?

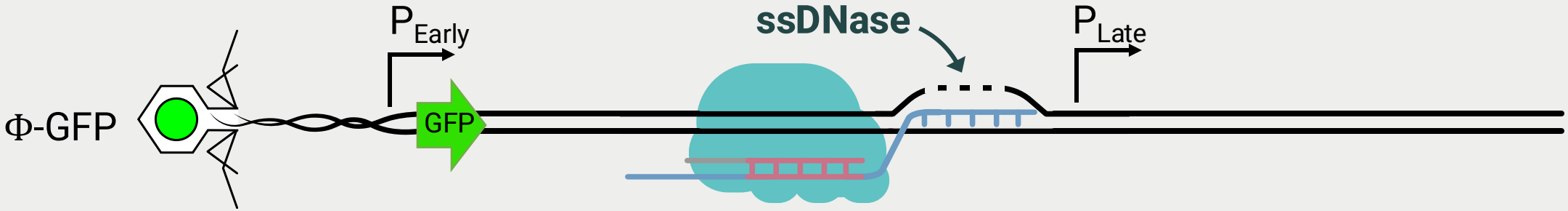
No

Transient

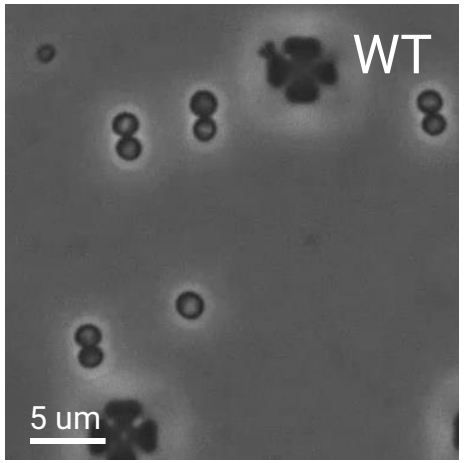
Prolonged

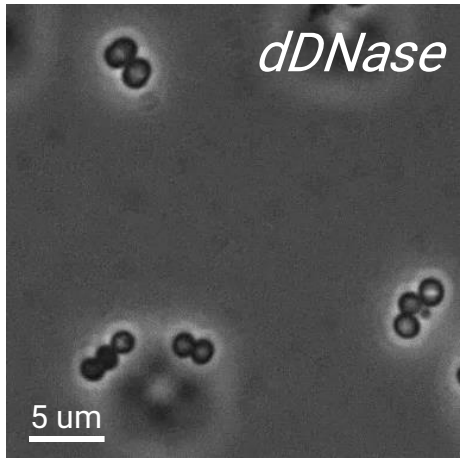
Aviram et al., *Cell Host & Microbe* 2024

The Cas10 ssDNase is essential for resuming growth after Csm6 activation



spc36 *spc36*





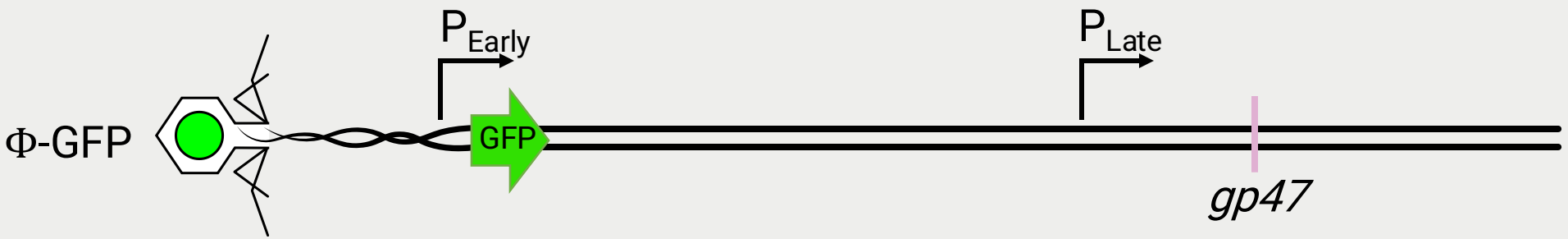
Speed 2,800x

Individual

Population

Aviram et al., *Cell Host & Microbe* 2024

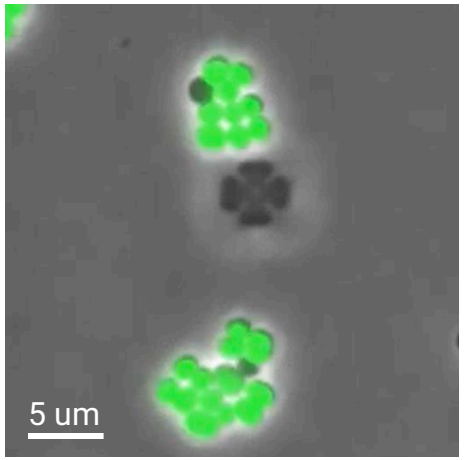
Can the growth arrest conferred by late-targeting spacers be resolved?



1. Can cells **recover** from prolonged growth arrest?

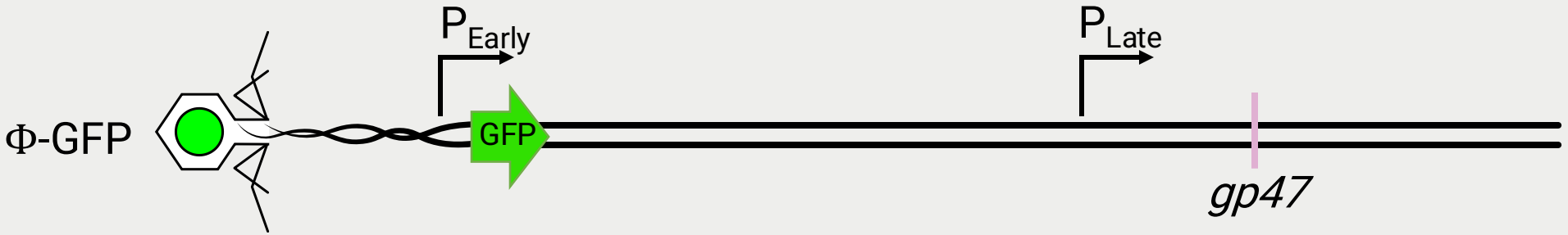
2. If so, does the **Cas10 ssDNase** contribute to this recovery?

spc47

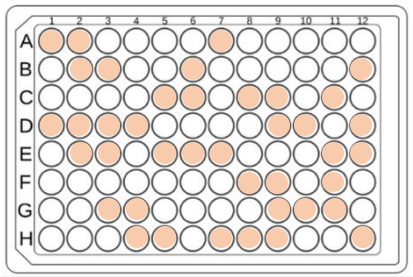


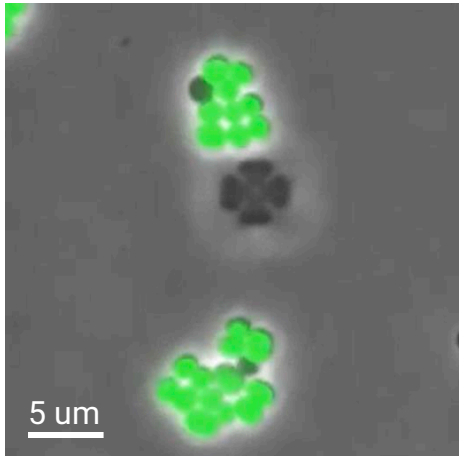
Aviram et al., *Cell Host & Microbe* 2024

Growth arrest conferred by late-targeting spacers can be resolved



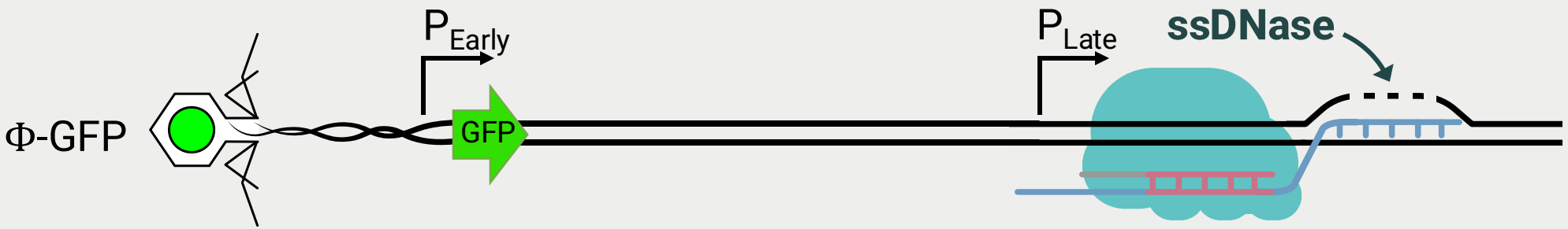
spc47

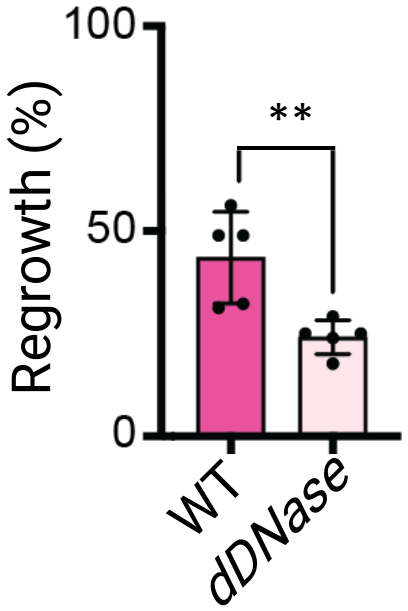


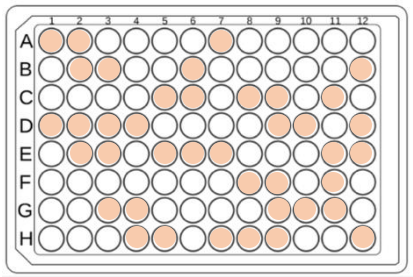


Aviram et al., *Cell Host & Microbe* 2024

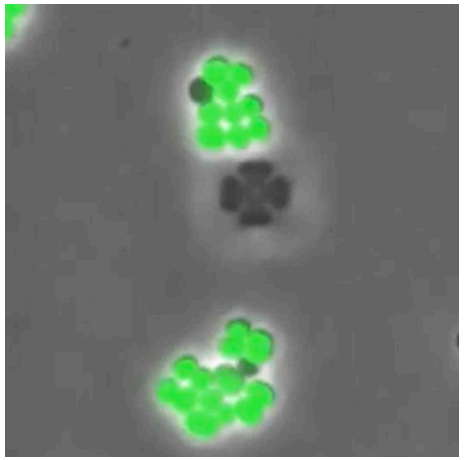
The Cas10 ssDNase alleviates dormancy in a subset of cells





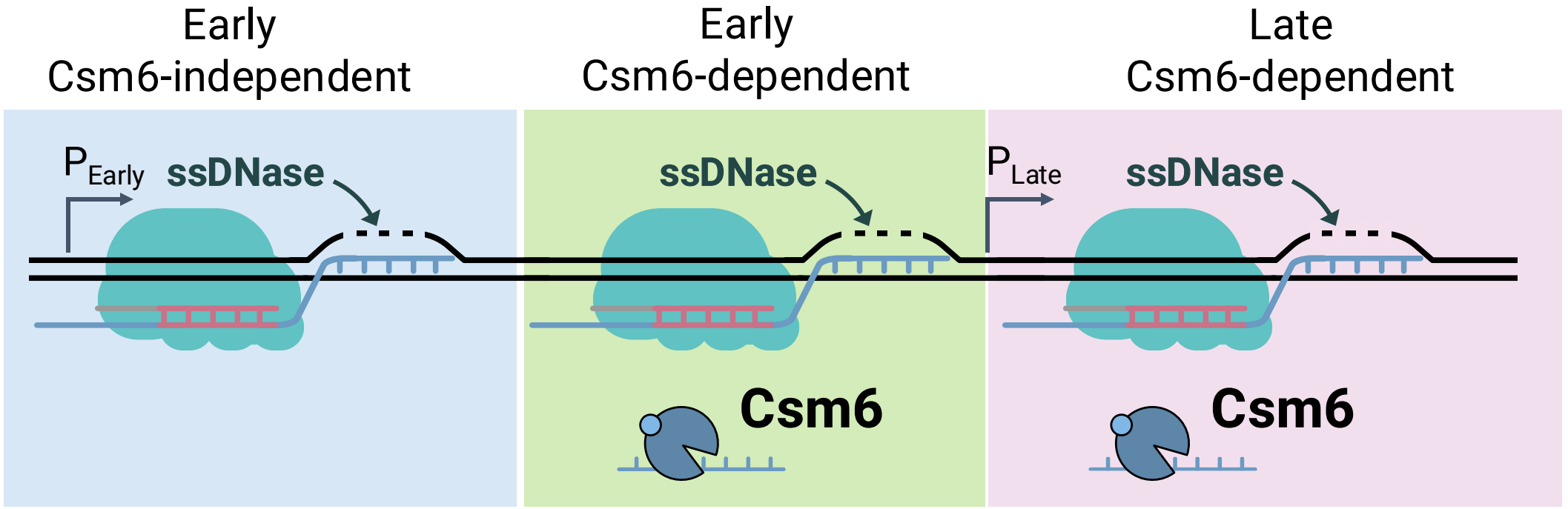


spc47



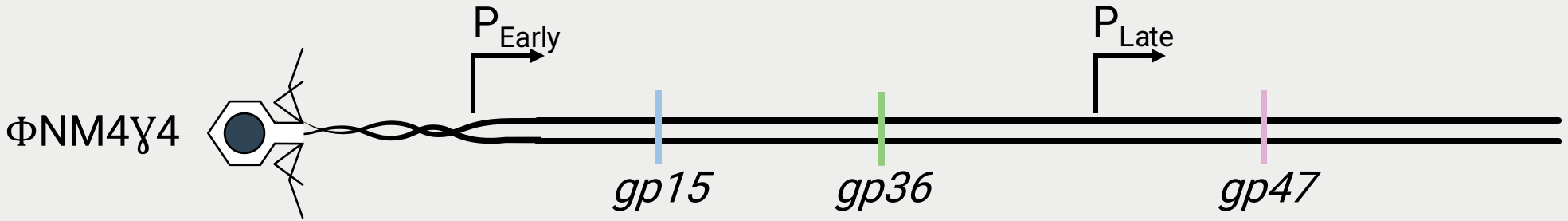
Aviram et al., *Cell Host & Microbe* 2024

How are spacers maintained in the population despite inhibiting bacterial growth?

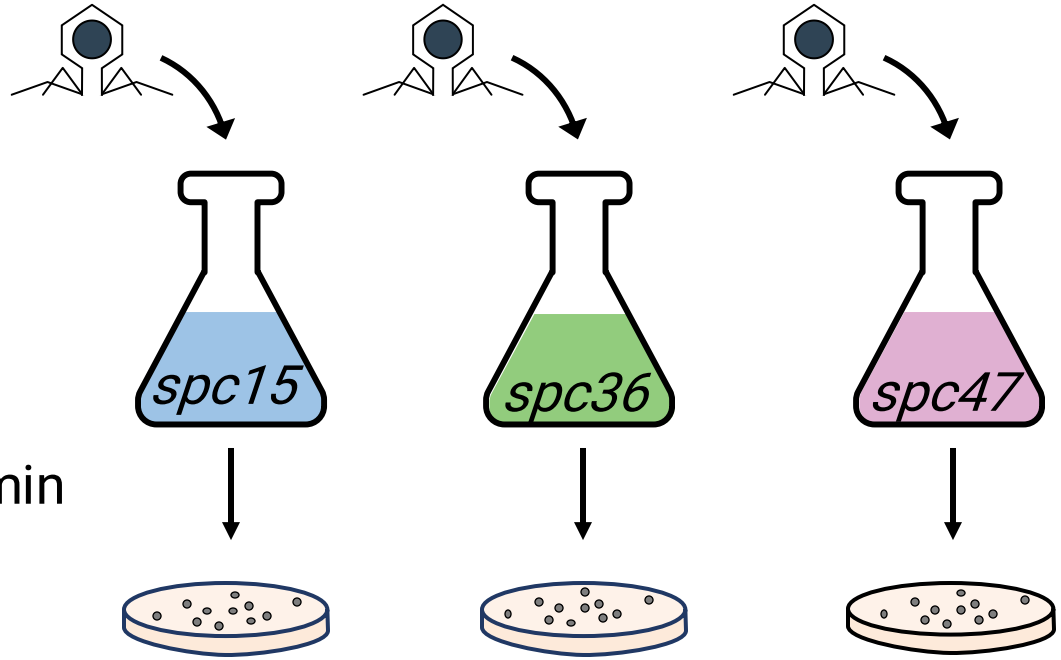


The Cas10 **ssDNase** activity alleviates Csm6-induction, enabling **dormancy-inducing** spacers to be **fixed** in the population.

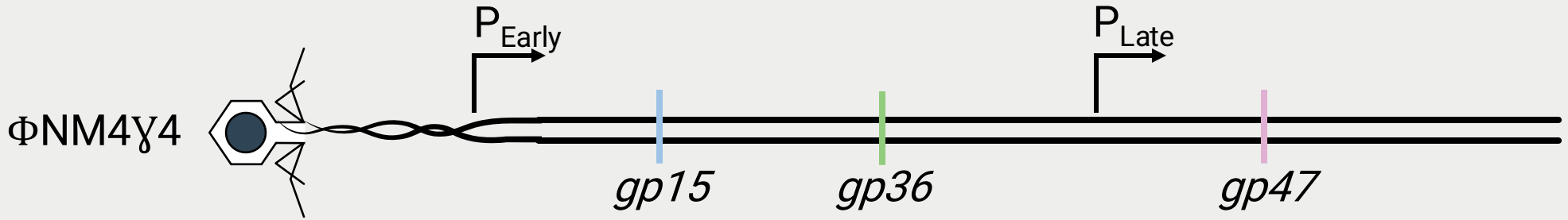
Why was dormancy-based immunity selected for during evolution?

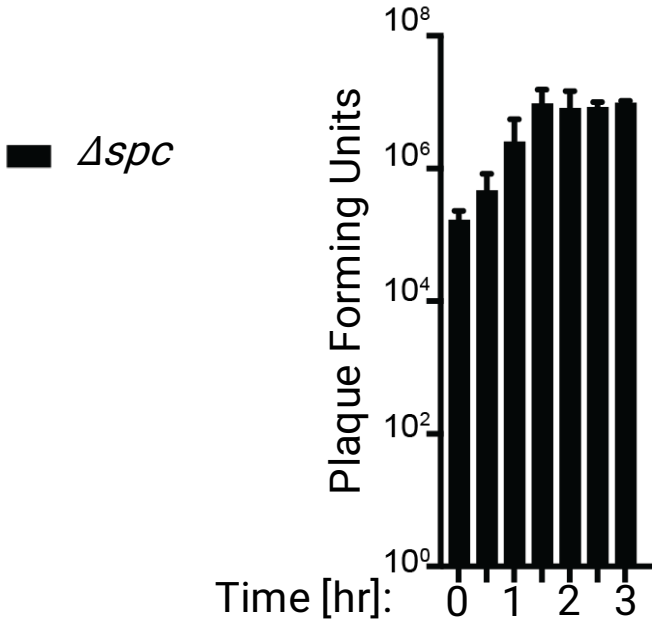


Isolated phage every 30 min



Csm6-induced dormancy inhibits phage-mutants from propagating





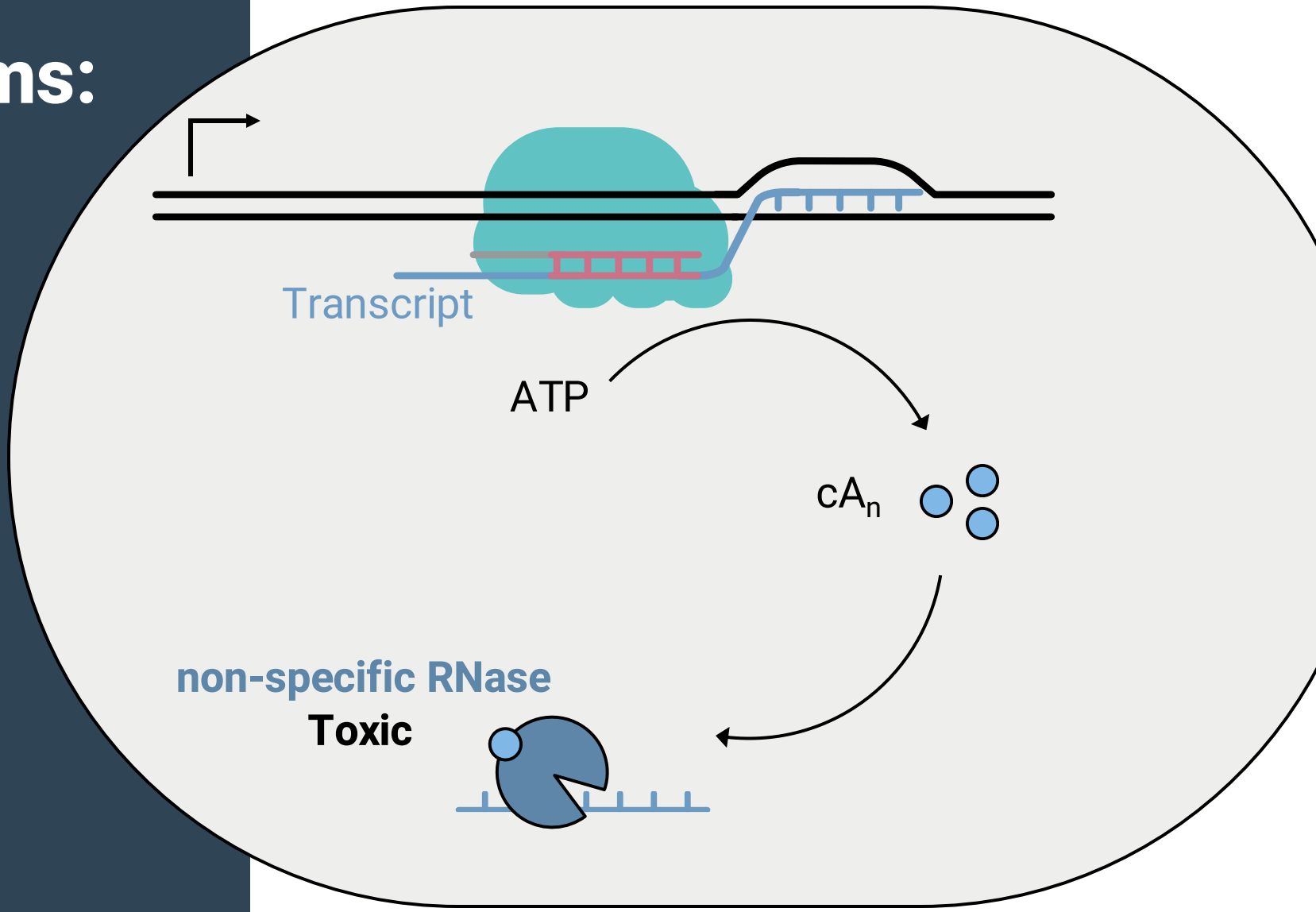
Dormancy confers **selective advantage** by inhibiting escaper-phages.

Dormancy **protects** cells from sequential infections by untargeted phages.

Aviram et al., *Cell Host & Microbe* 2024

Type III-A systems: Open questions

1. How are **functional** spacers selected for acquisition?



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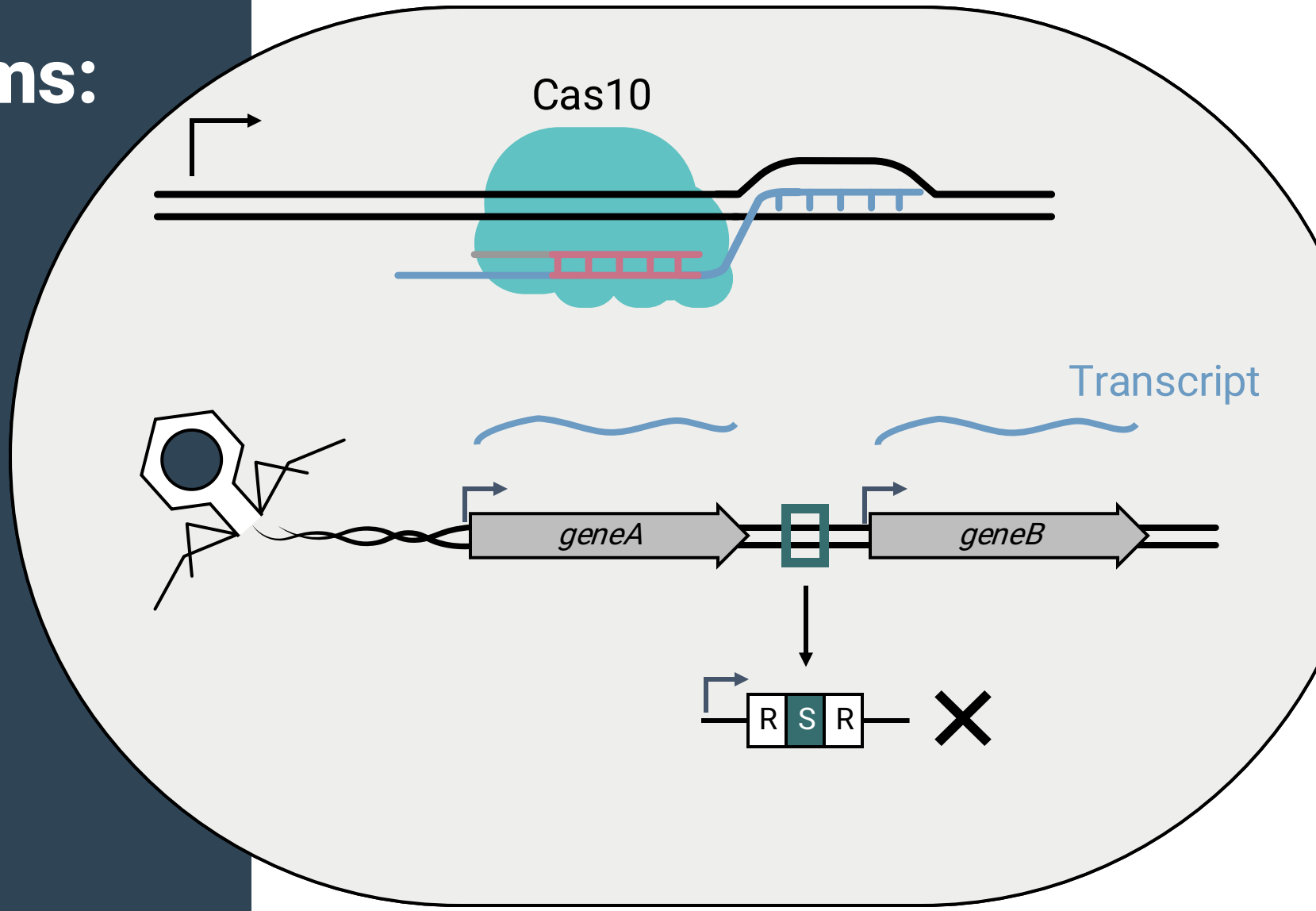

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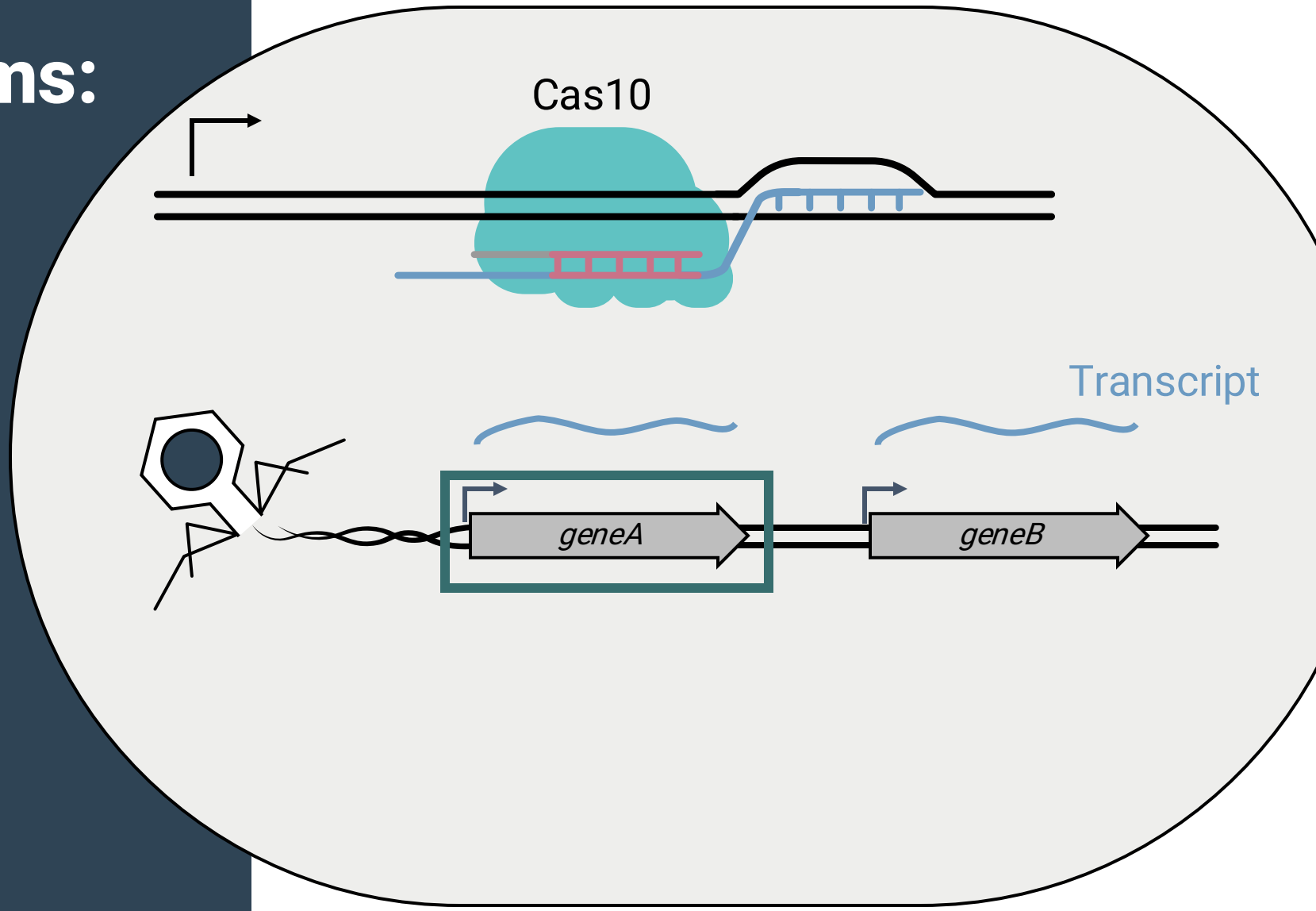
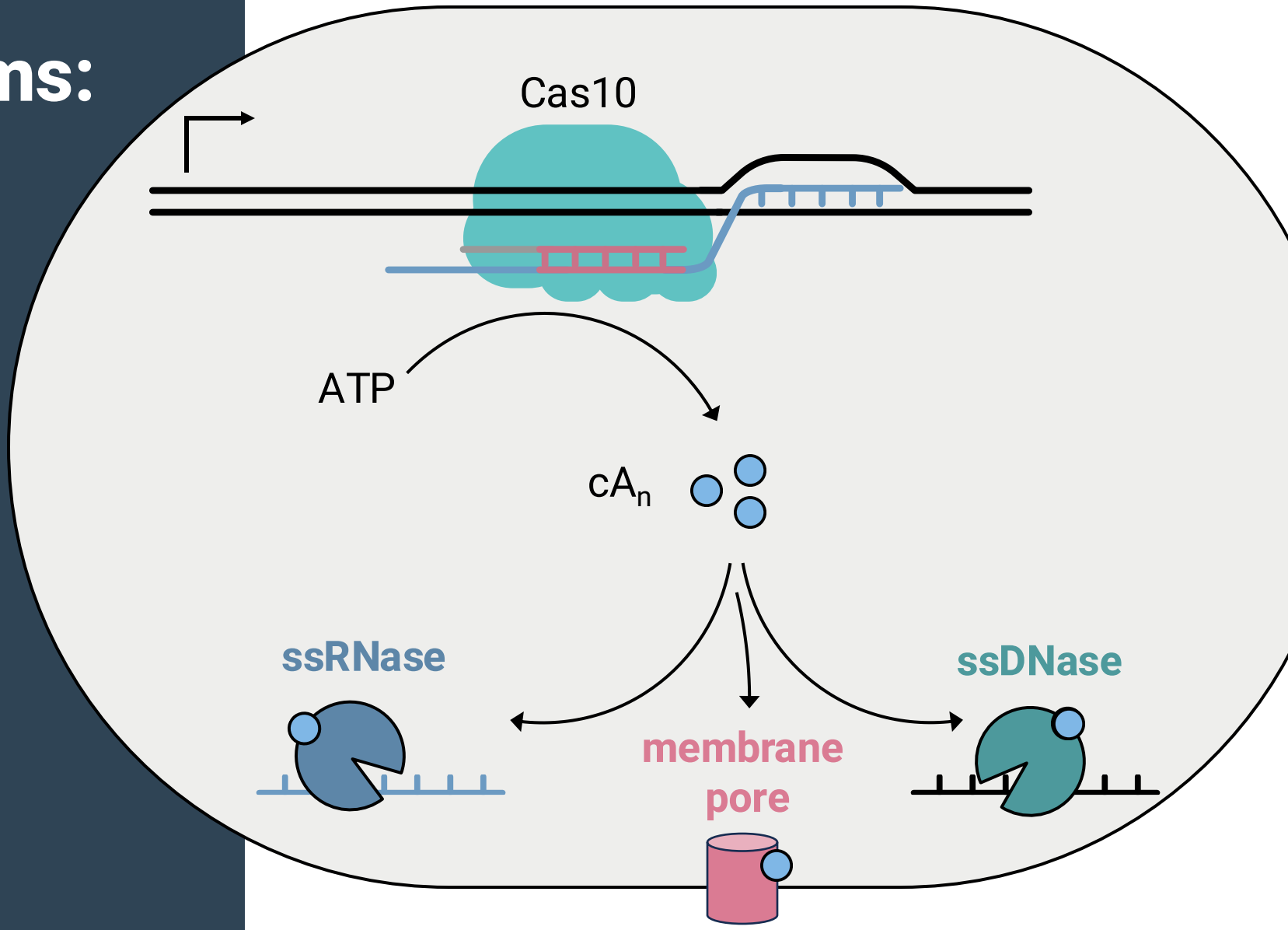


Type III-A systems: Open questions

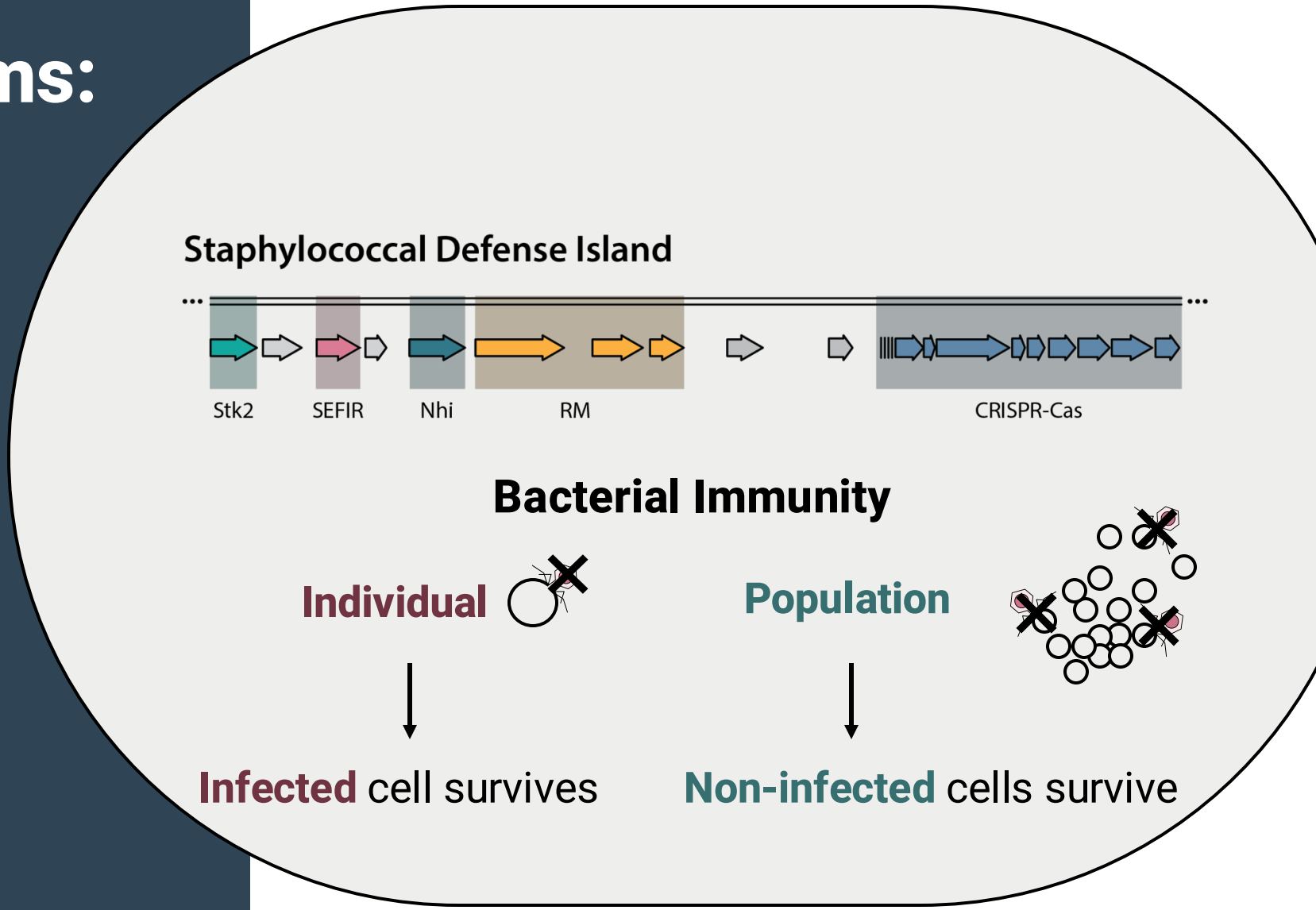
1. How are **functional** spacers selected for acquisition?
2. Why are **multiple cA_n-activated proteins** encoded by individual CRISPR-Cas systems?



Type III-A systems: Open questions

1. How are **functional** spacers selected for acquisition?
2. Why are **multiple cA_n-activated proteins** encoded by individual CRISPR-Cas systems?
3. How do CRISPR-Cas systems function in their **natural context**?

Interested? aviramn@mskcc.org



What will we discover next?..

Paper Discussion

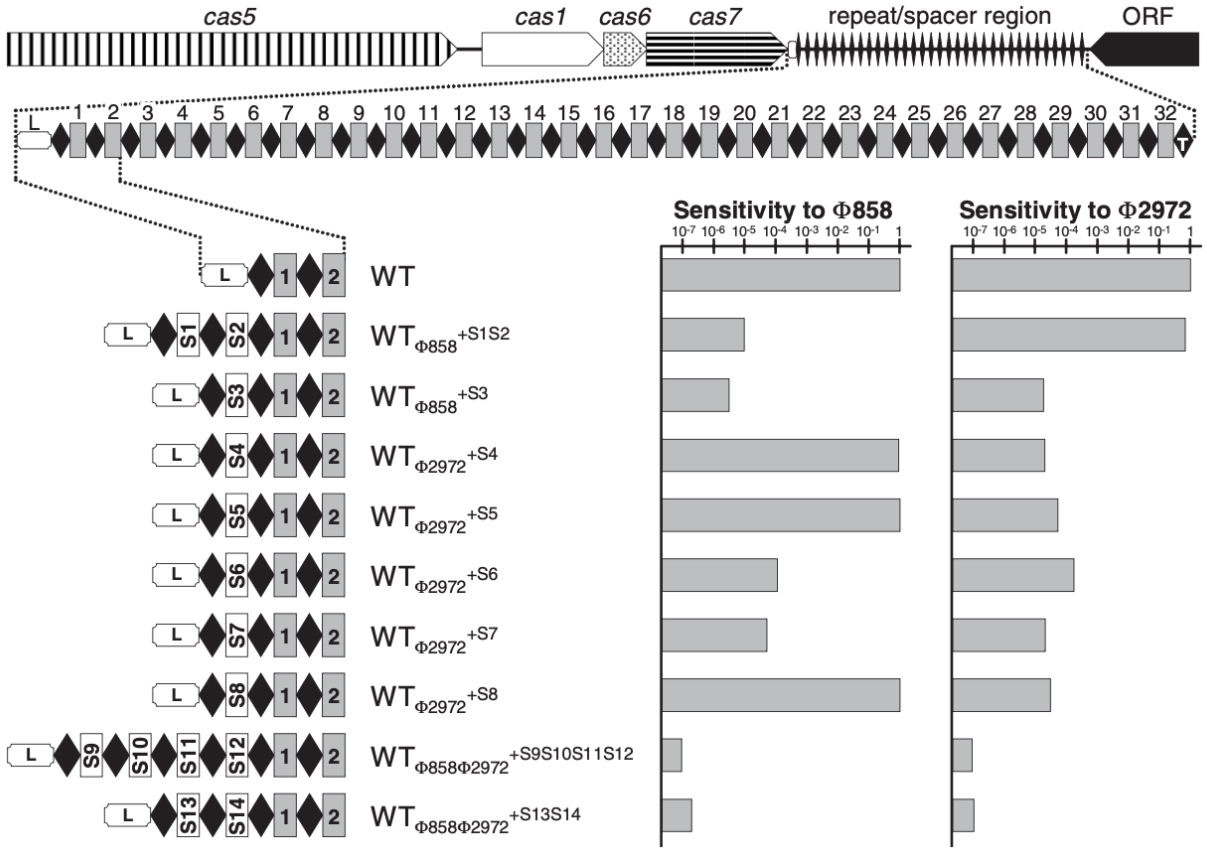
Q1. Conceptual Basics

What was the central question that Barrangou et al. set out to test in this paper? Before this study, people noticed repeats and spacers but didn’t know their function. What hypothesis were the authors testing?

Paper Discussion

Q2. Spacer Acquisition Experiment

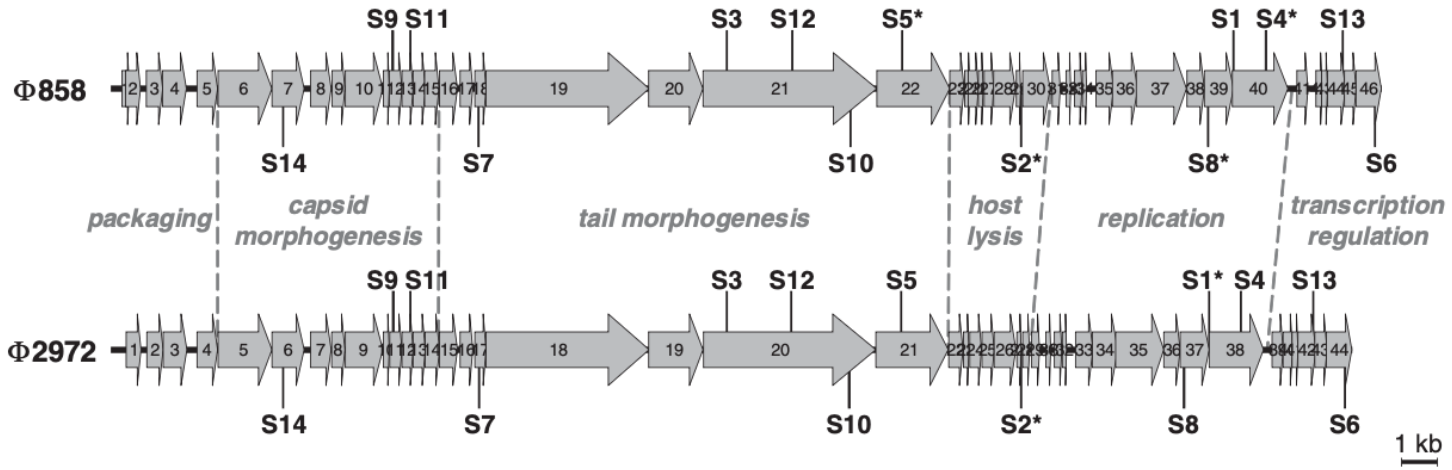
Figure 1 shows bacteria surviving phage challenge gained new spacers in their CRISPR array. How did the authors demonstrate that these new spacers correspond to phage sequences? Why is this result important?



Paper Discussion

Q3. Functional Test of Spacers

In Figure 2, bacteria with newly acquired spacers resisted infection by the corresponding phage. How does this result move from correlation to causation? What control would you want to see?



Paper Discussion

Q4. Conceptual Extension

Why do bacteria acquire new spacers instead of relying only on fixed innate defenses (like restriction enzymes)?

Paper Discussion

Q5. Critical Thinking

Suppose you find a bacterial strain whose CRISPR array matches phages no longer present in its environment. What does this tell you about CRISPR immunity? How might this shape bacterial-phage co-evolution?

Paper Discussion

Q6. Methods

In 2007, Barrangou et al. identified new spacers by sequencing CRISPR arrays after phage challenge. What are the strengths and limitations of this approach? How would you design this experiment today with modern sequencing tools?

Paper Discussion

Q7. Next Experiment

Barrangou et al. showed that new spacers provide immunity, but they didn’t dissect the role of individual Cas proteins. If you were designing the next experiment, how would you test whether Cas1 and Cas2 are required for spacer acquisition? What outcome would you predict?