Bacterial Viruses Thwart CRISPR-Cas

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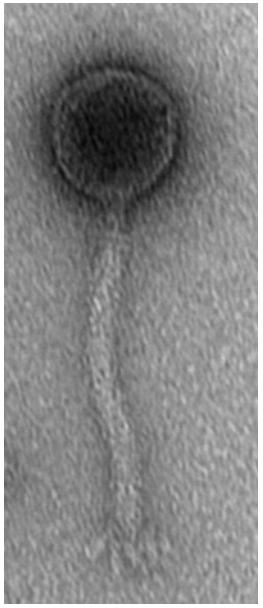
UCSF Sandler Faculty Fellow

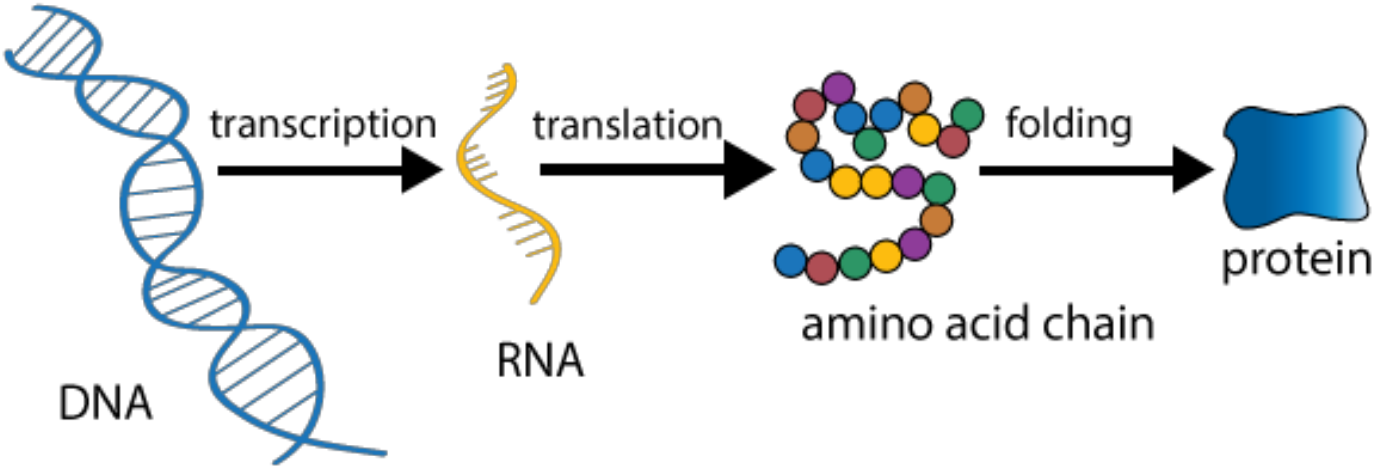


Outline

1. **Introduction:**
   -What is CRISPR?
   -What are phages (bacterial viruses)?

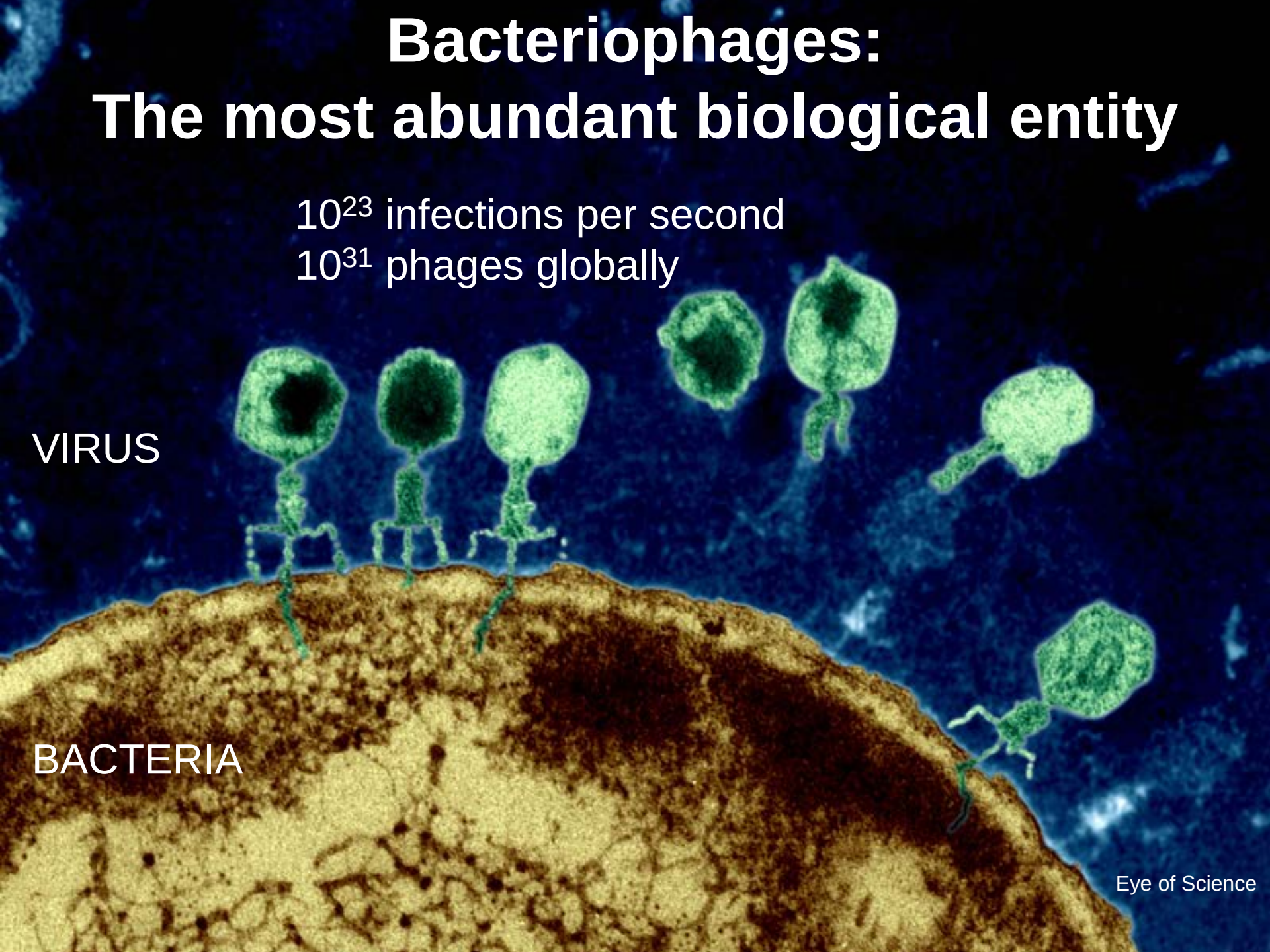
2. **Discovery of phage genes that inhibit CRISPR-Cas function**

3. **Applications and mechanisms of “anti-CRISPRs”**



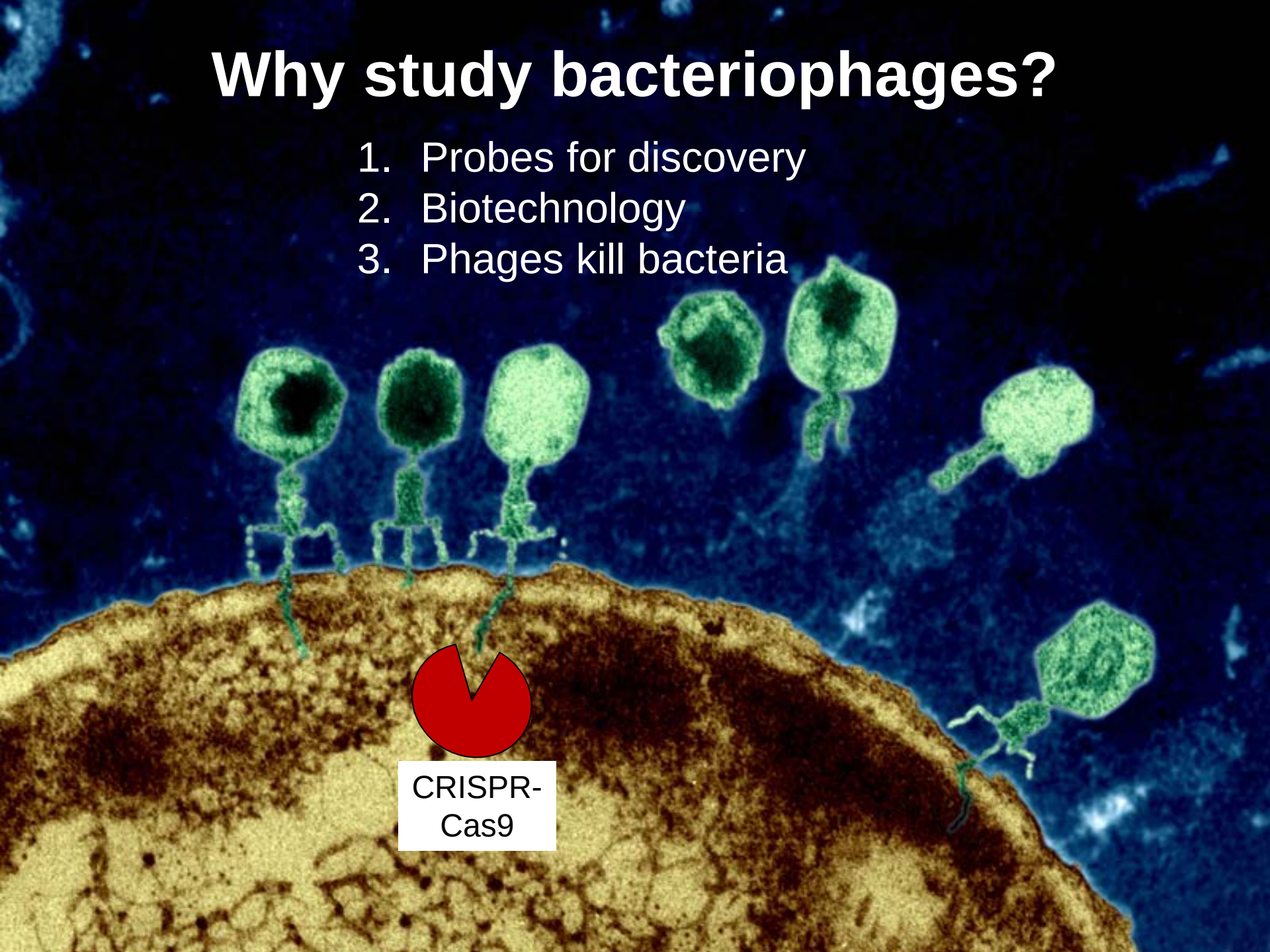
Bacteriophages:
The most abundant biological entity

10^{23} infections per second
10^{31} phages globally



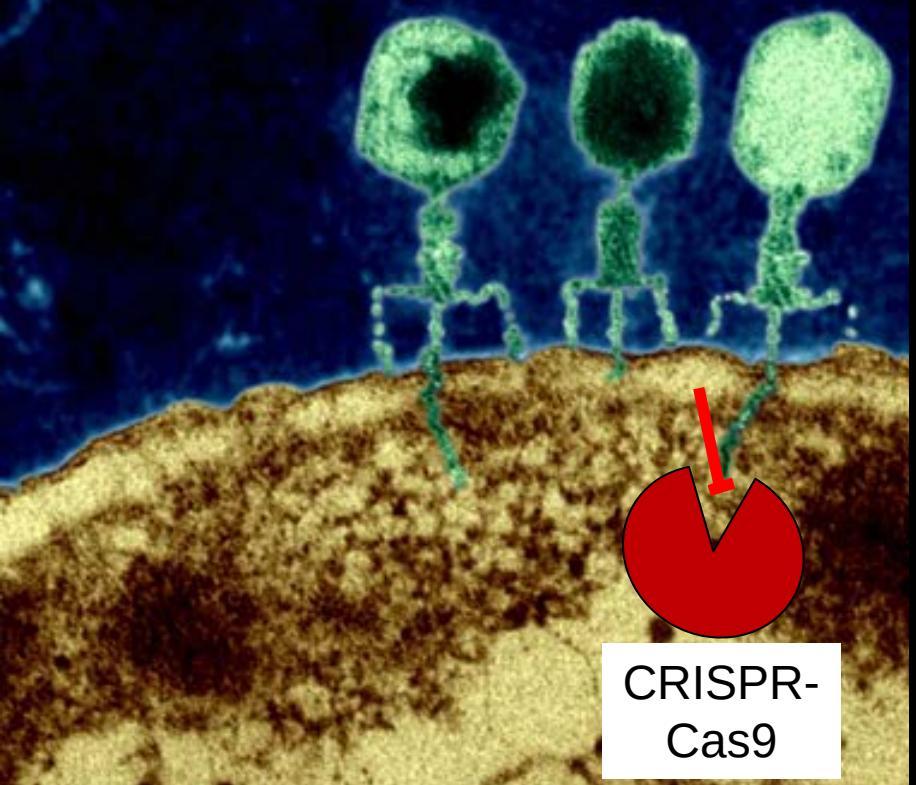
Why study bacteriophages?

1. Probes for discovery
2. Biotechnology
3. Phages kill bacteria



Bondy-Denomy Lab Questions

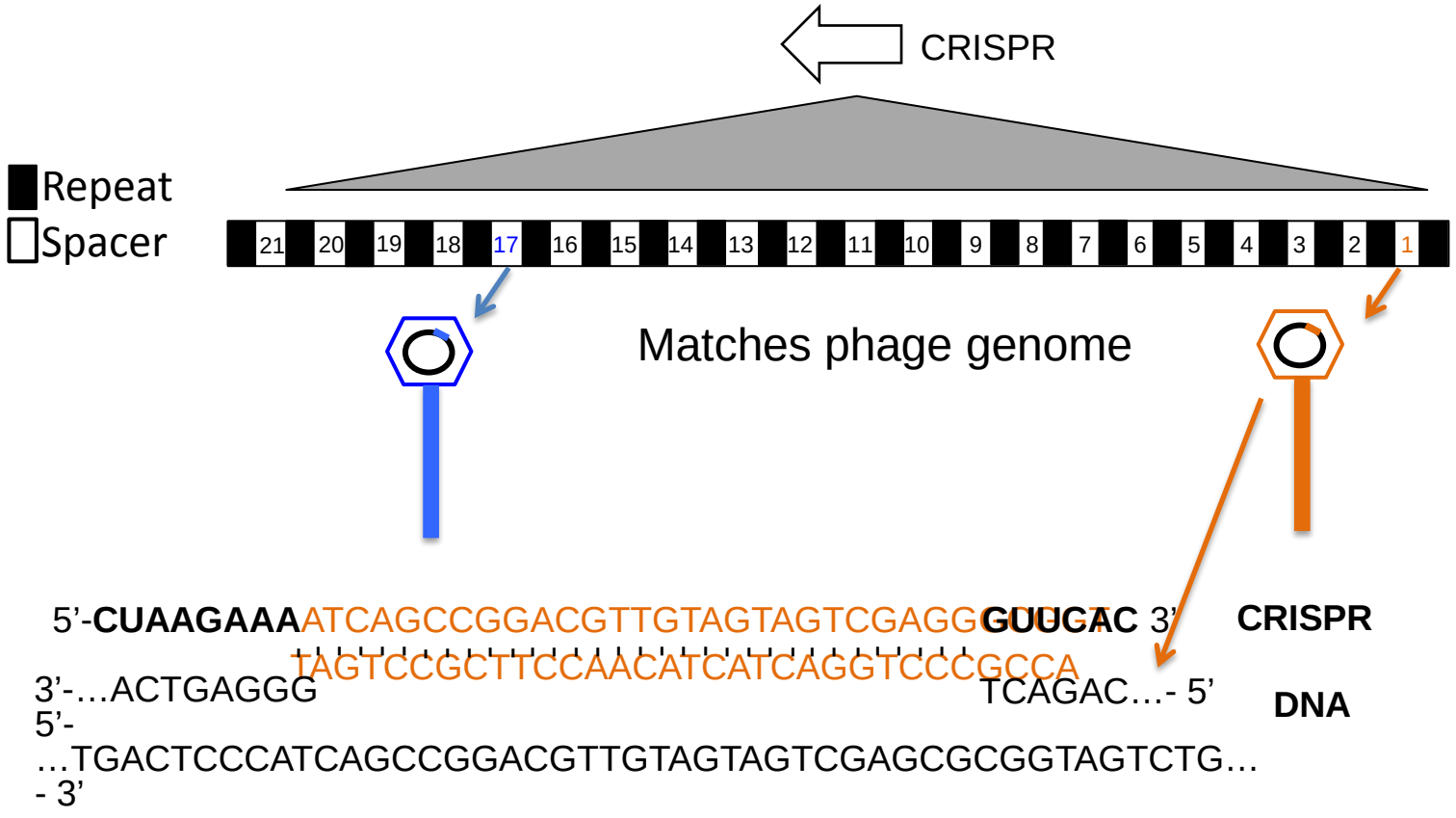
1. How do bacteria defend themselves from phage attack?
2. How do <u>phages</u> defend themselves from <u>bacterial</u> immunity?



CRISPR-Cas: Immunity to phages

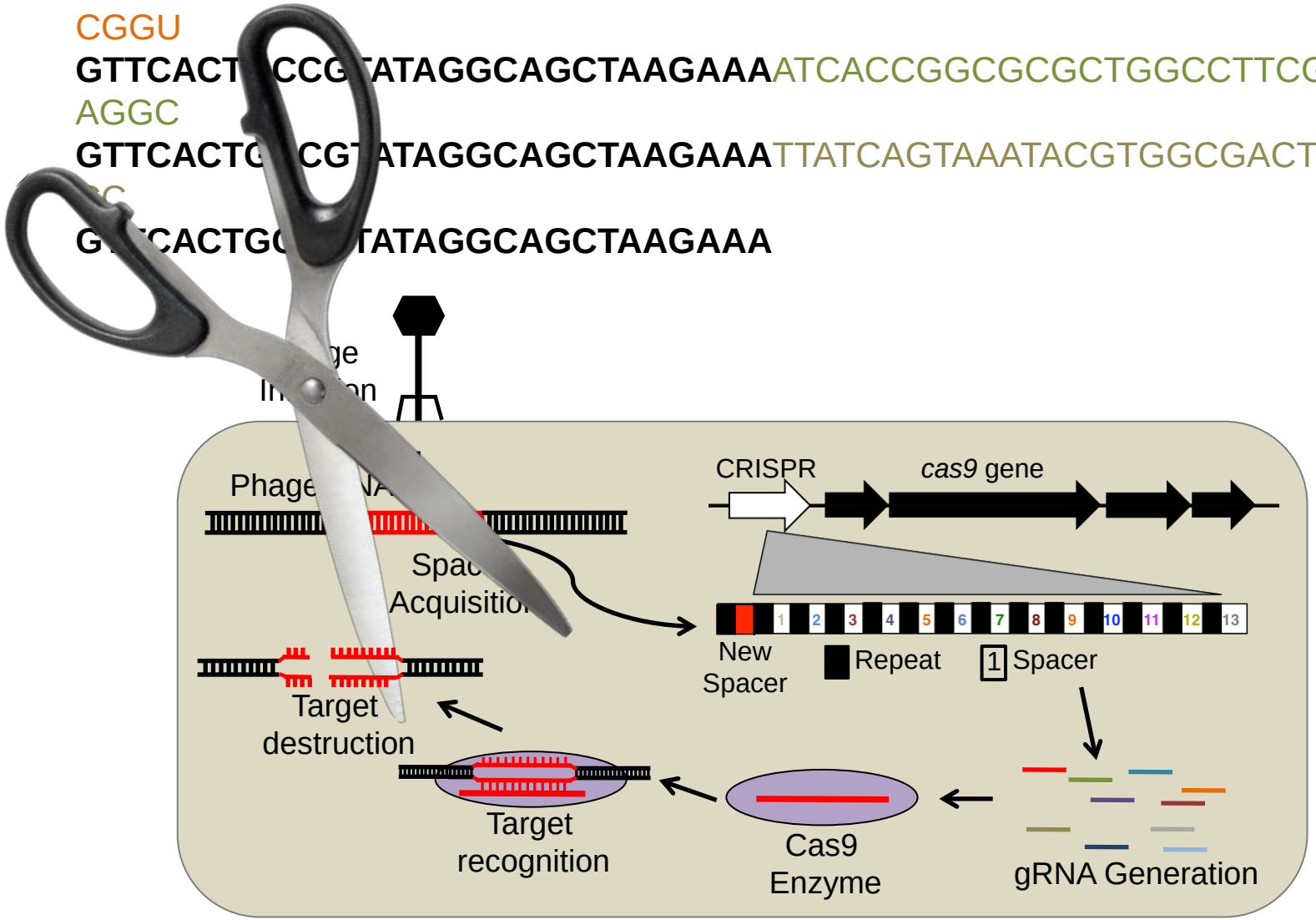
GTTCACTGCCGTATAGGCAGCTAAGAAAAUCAGCCGGACGUUGUAGUAGUCGAGCG
CGGU
GTTCACTGCCGTATAGGCAGCTAAGAAAATCACCGGCGCGCTGGCCTTCGGCGCCC
AGGC
GTTCACTGCCGTATAGGCAGCTAAGAAATTATCAGTAAATACGTGGCGACTTGGCCAC
CC
GTTCACTGCCGTATAGGCAGCTAAGAAA

Clustered Regularly Interspaced Short Palindromic Repeats

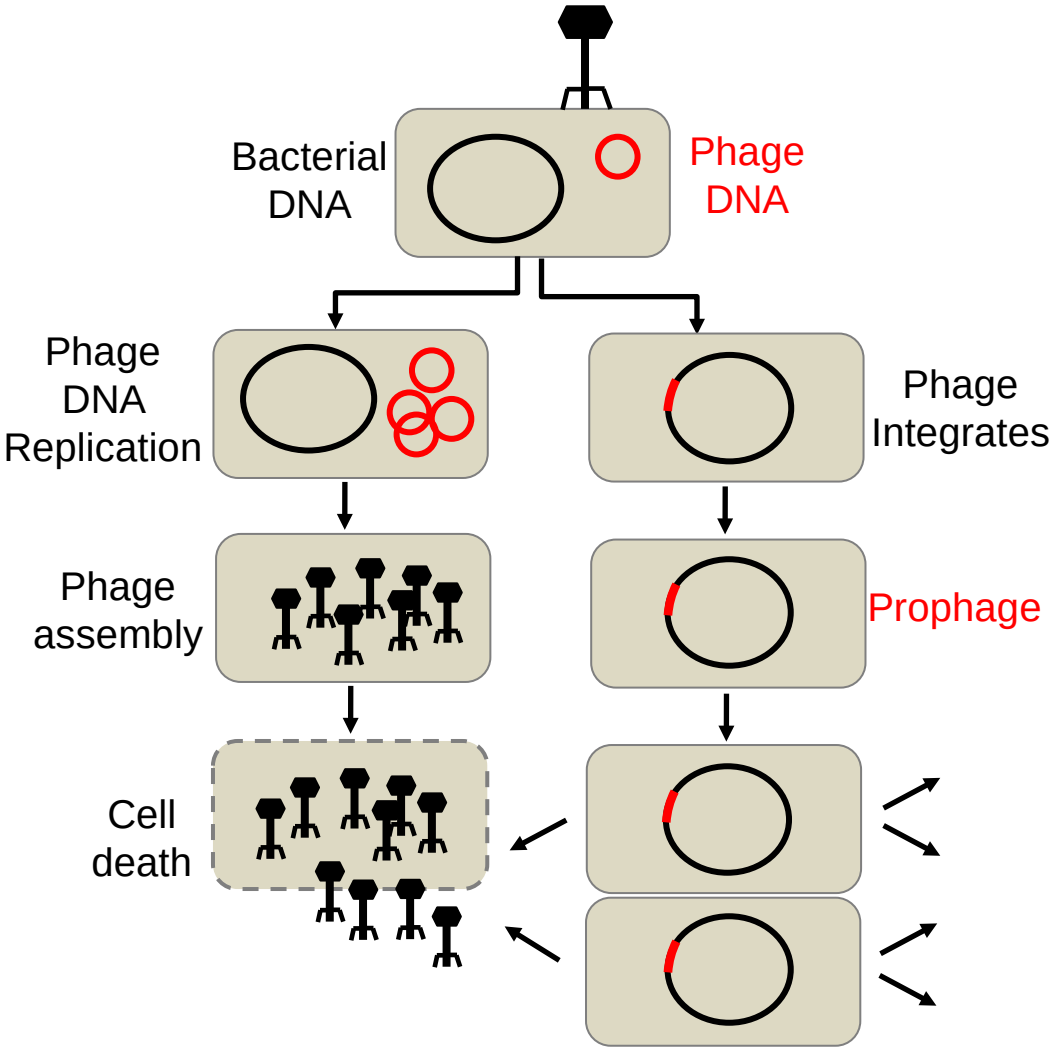


CRISPR-Cas: Adaptive immunity to phage

GTTCACTGCCGTATAGGCAGCTAAGAAAAUCAGCCGGACGUUGUAGUAGUCGAGCG
CGGU
GTTCACT CCG TATAGGCAGCTAAGAAAATCACCGGCGCGCTGGCCTTCGGCGCCC
AGGC
GTTCACTG CG TATAGGCAGCTAAGAAATTATCAGTAAATACGTGGCGACTTGGCCAC
C
G CACTG TATAGGCAGCTAAGAAA



Phages lead a dual life

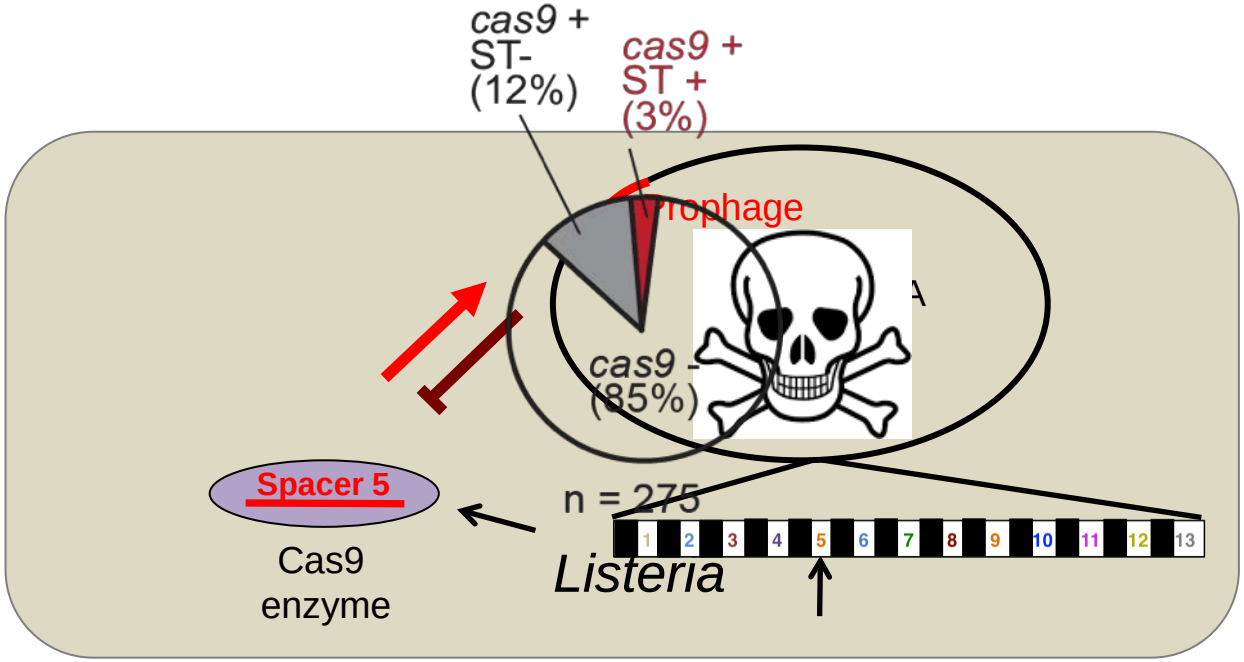


CRISPR-Cas9 was discovered because of a fundamental interest in phage biology.

Are we done discovering tools from phages?

Phages can inhibit many bacterial processes…CRISPR inhibition?

A bioinformatics approach to find CRISPR-Cas9 inhibitors



“Self-Targeting”

5’-**CTAAGAAA**ATCAGCCGGACGTTGTAGTAGTCGAGCGCGGT**GTTCAC** 3’ **CRISPR**

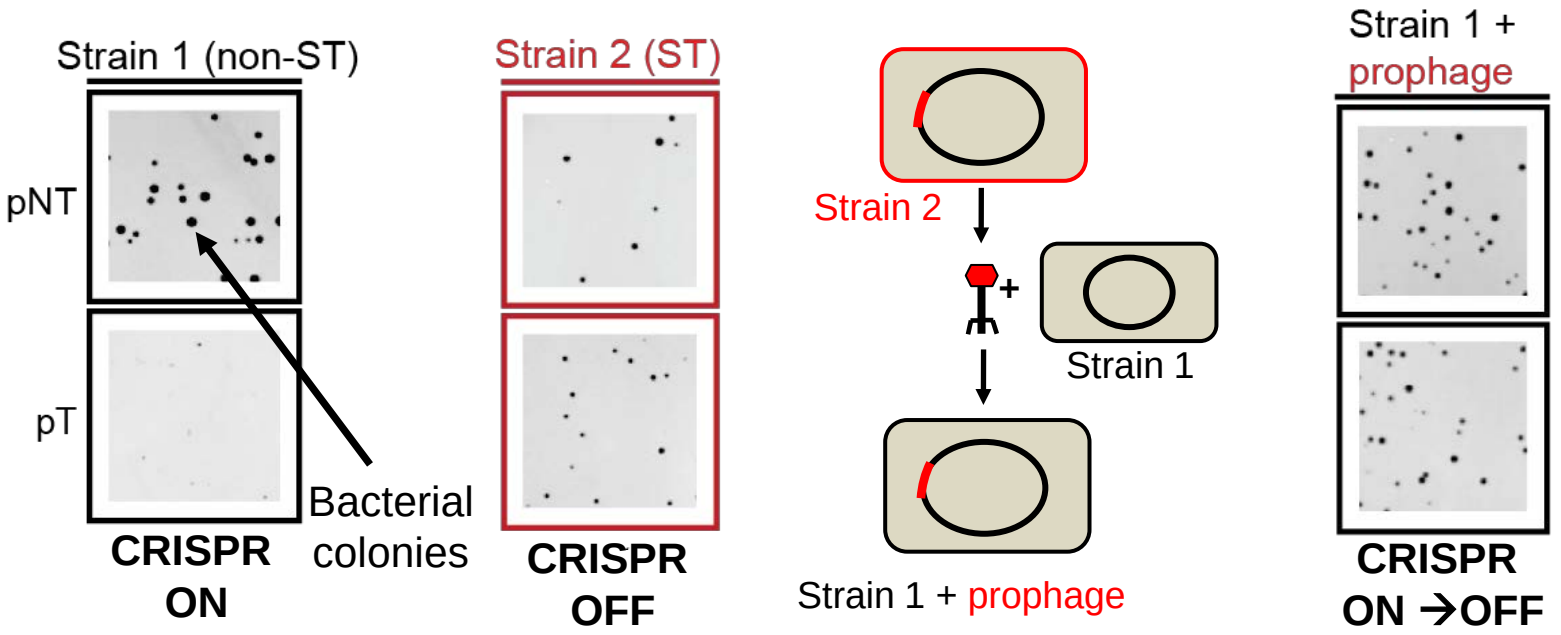
TAGTCCGCTTCCAACATCATCAGGTCCCGCCA

3’-…ACTGAGGG TCAGAC…- 5’ **DNA**

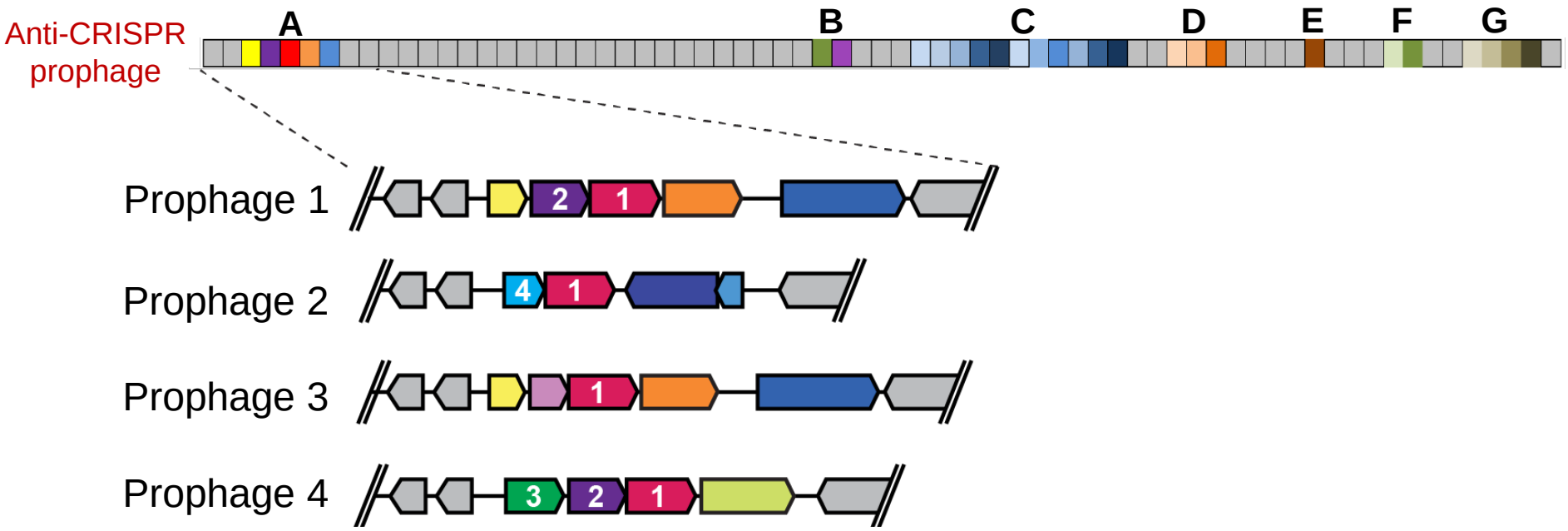
5’-
…TGACTCCCATCAGCCGGACGTTGTAGTAGTCGAGCGCGGTAGTCTG…
- 3’

Ben Rauch

Listeria Cas9 cuts DNA



Listeria phage accessory genes inhibit CRISPR-Cas9



>50% of *Listeria* strains with *cas9* possess anti-CRISPR

Anti-CRISPR genes are very common

Ben Rauch & Chris Waters

Outline

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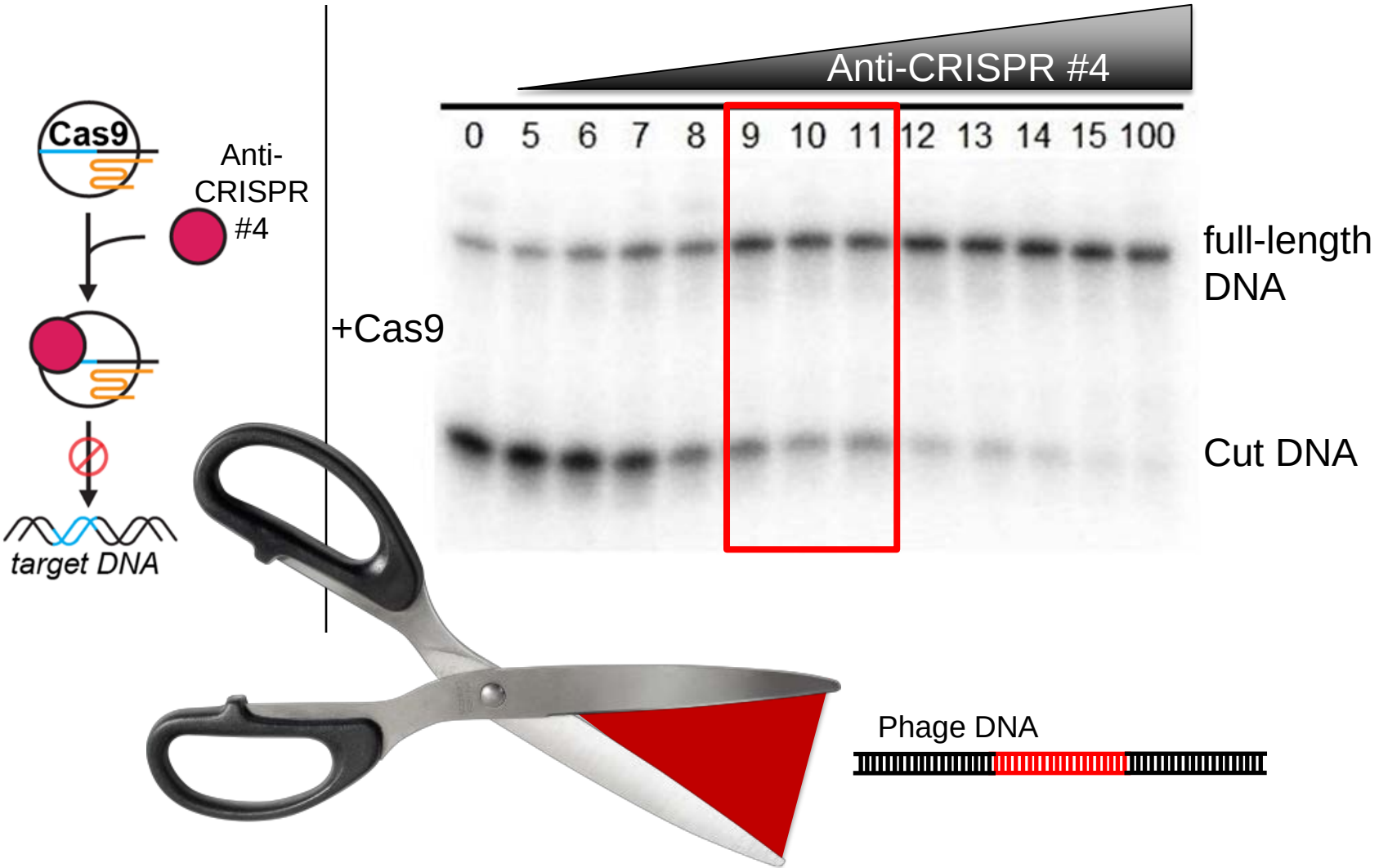
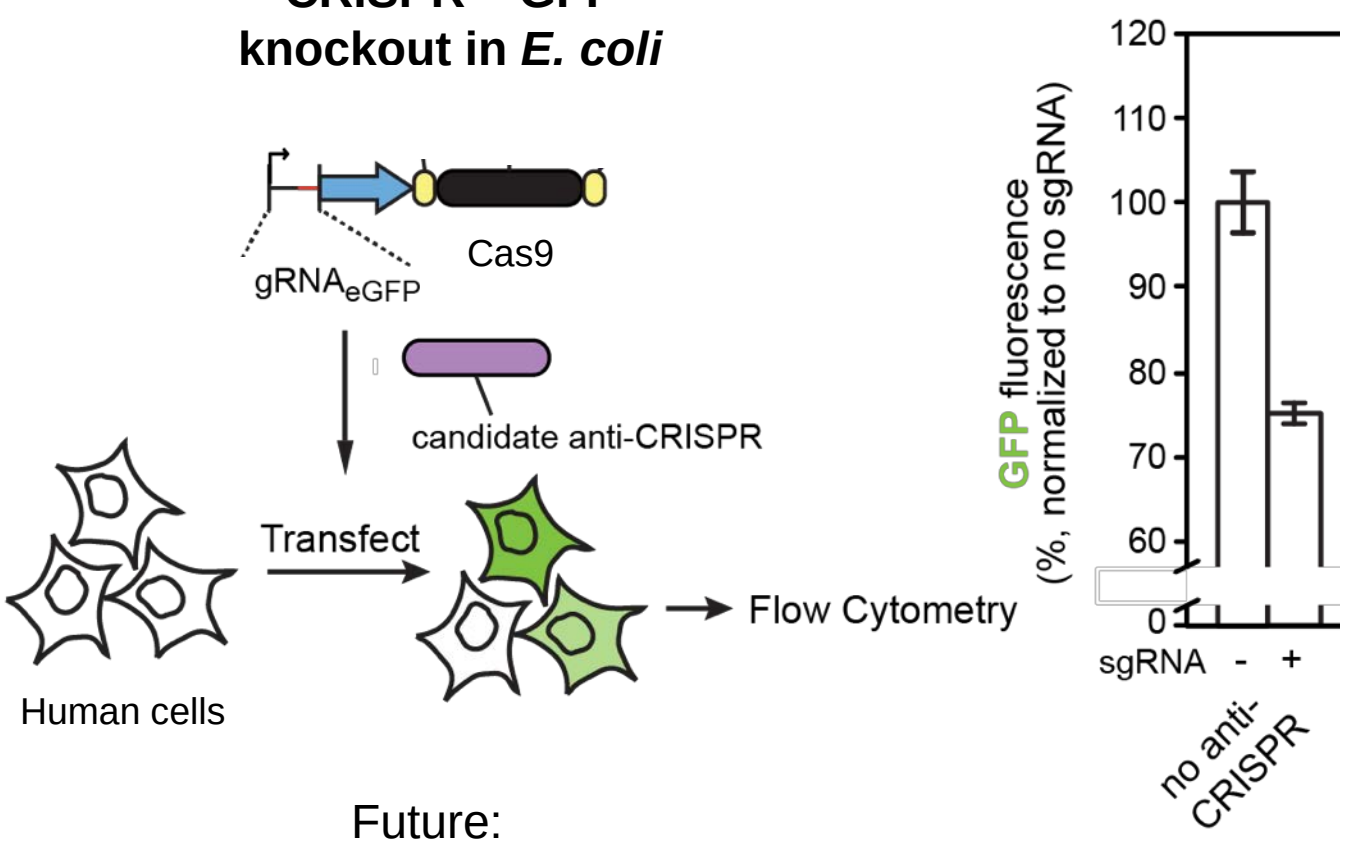
3. **Mechanisms and applications of “anti-CRISPRs”**

Anti-CRISPR #4 blocks Cas9 Cutting *in vitro*



Blocking Cas9 gene editing in human cells

CRISPR – GFP knockout in *E. coli*



Future:

1. Does this prevent off-target gene editing?
2. Can anti-CRISPRs be used to “turn off” gene editing?

Judd Hultquist
(Nevan Krogan lab)

Listeria phages encode Cas9 inhibitors

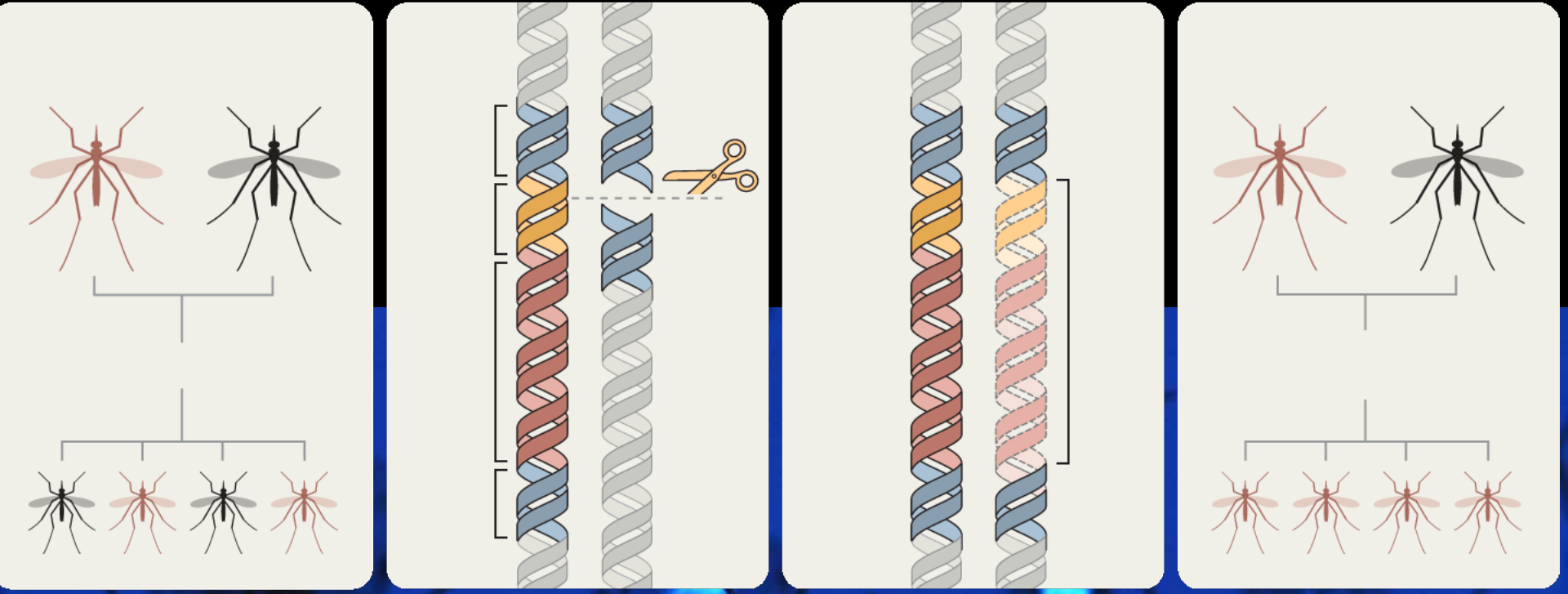
Future Work:

<u>Structure/Biochemistry</u>
Jennifer Doudna (Berkeley)

<u>Applications</u>
-CRISPRi on/off switch
Stanley Qi (Stanford)

-On vs. off-target editing
-Off switch for therapy
Jacob Corn (Berkeley)

Rauch B *et al*. *Cell* (2017)

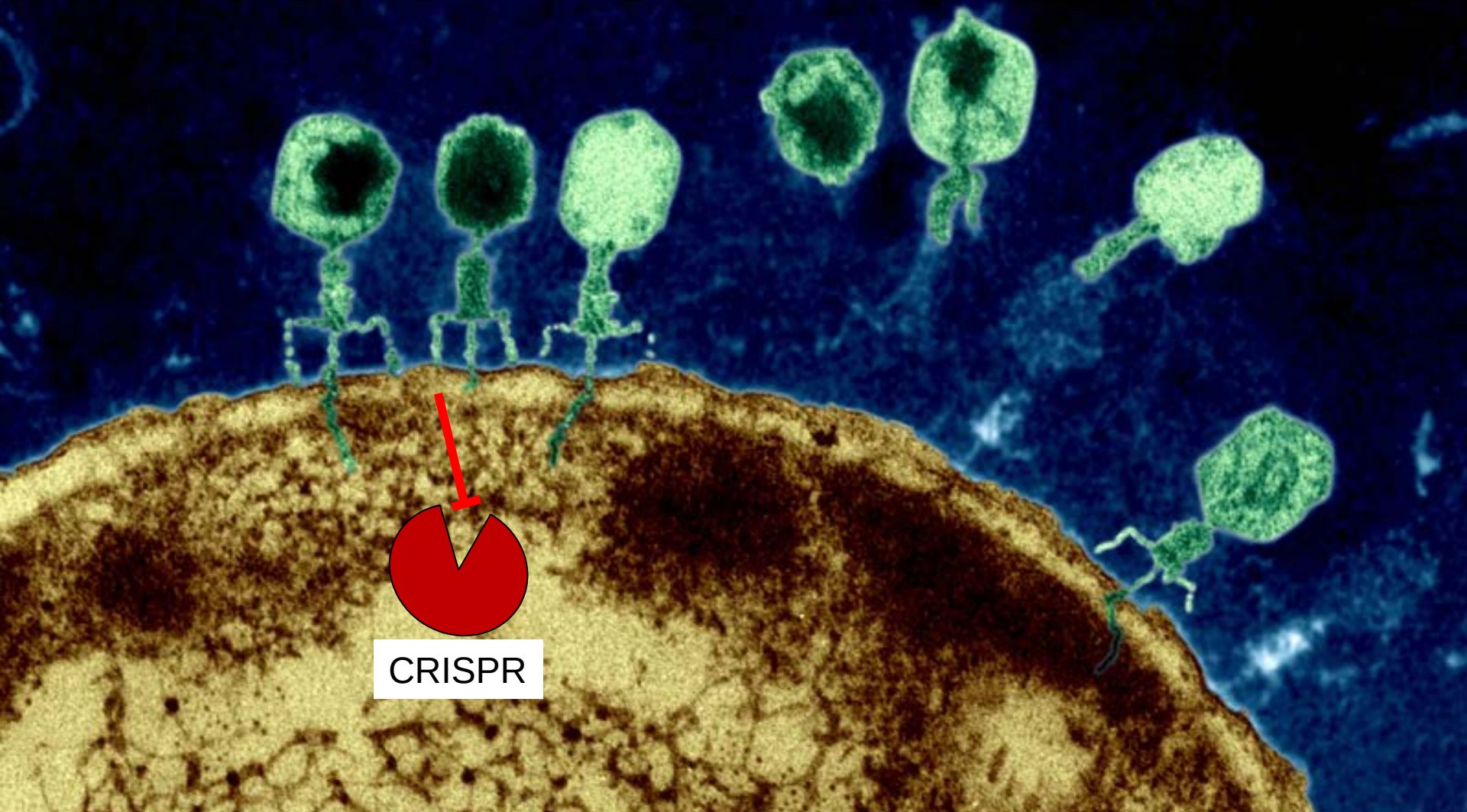


Gene Drive – Coming to a mosquito near you

1. Anti-CRISPR genes are widespread in the phages

2. *in vitro* / *in vivo* mechanisms for Cas9 inhibition

3. Technology – Human gene editing inhibitors (Cas9)



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Bondy-Denomy Lab

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