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Discovering Virally Encoded Proteins That Block Type IV CRISPR Immune Systems

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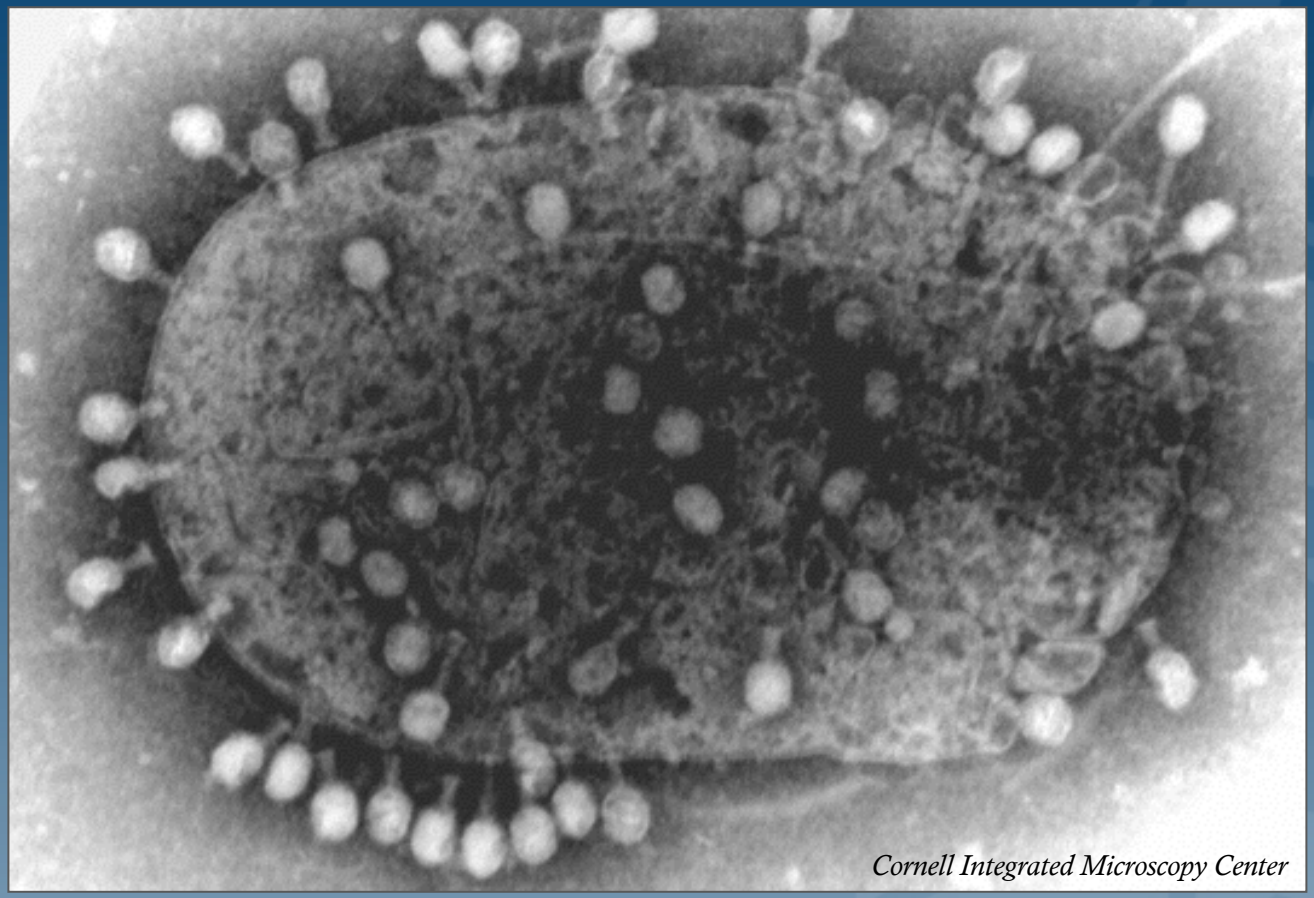
Discovering Virally Encoded Proteins that Block Type IV CRISPR Immune Systems

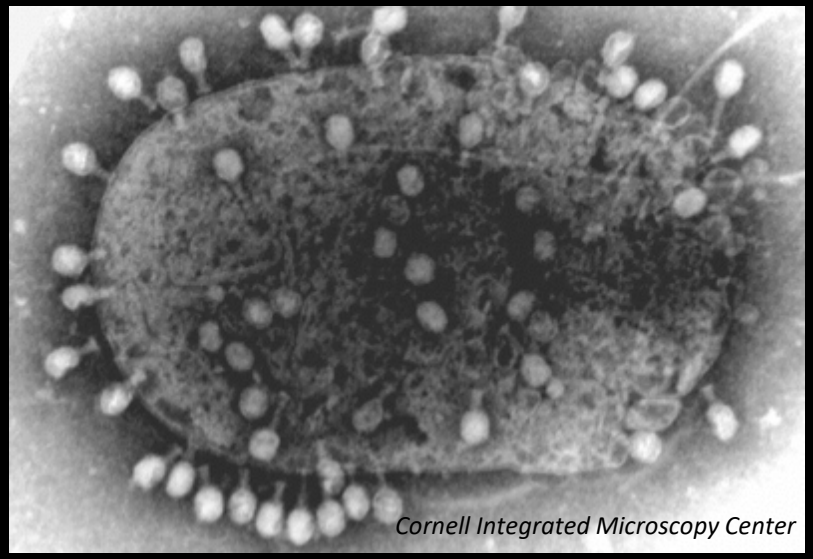


Cornell Integrated Microscopy Center

Andrew Williams
Jackson Lab
Peak Fellow 2021

Utah State University

Viruses are the most abundant biological entities on the planet



1 x10 ^ 31
Viruses on Earth

1 x10 ^ 25
Stars in the universe

How do Bacteria Protect Themselves?

Zimmer, Feb 2013, The Loom, National Geographic.com

Bacteria must defend themselves from phage to propagate

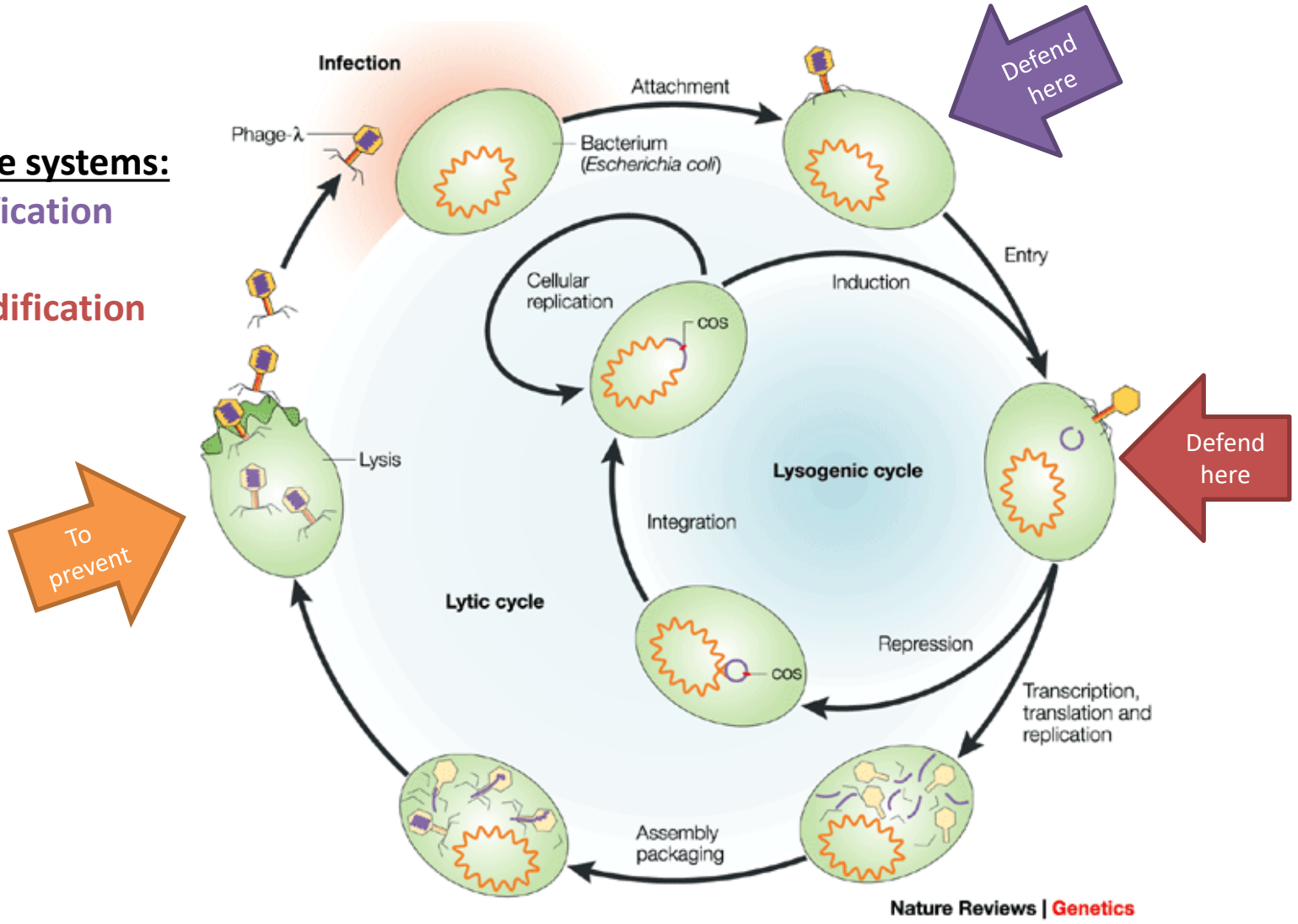
Bacterial immune systems:

Receptor modification

Restriction-modification

CRISPR-Cas

etc.



Campbell. 2003. Nature Reviews Genetics.

Basic Research on Prokaryotic Immune Systems has led to groundbreaking discoveries

NOBELPRISET I KEMI 2020
THE NOBEL PRIZE IN CHEMISTRY 2020

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Emmanuelle Charpentier

Jennifer A. Doudna

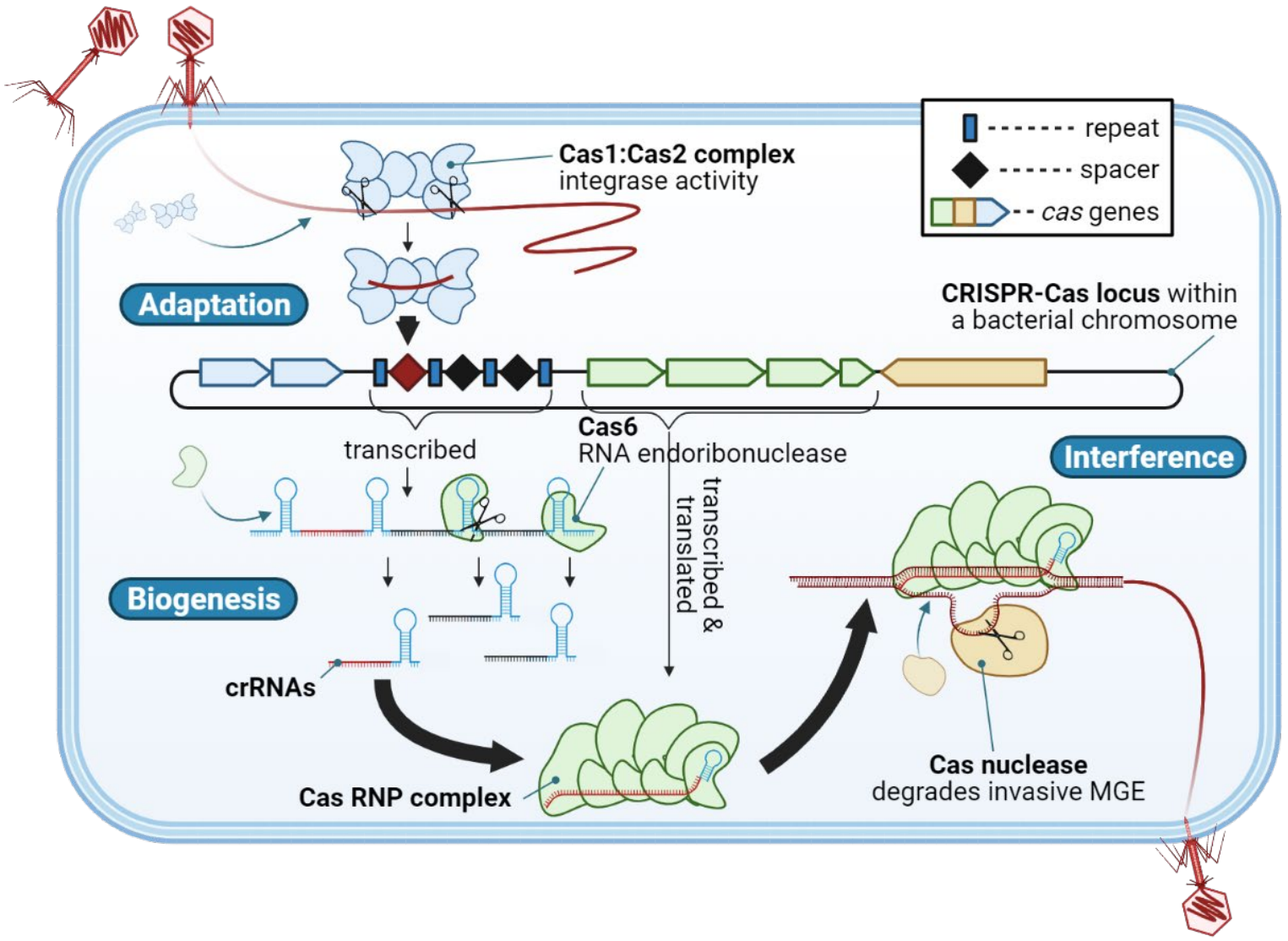
"för utveckling av en metod för genomeditering"

"for the development of a method for genome editing"

#nobelprize

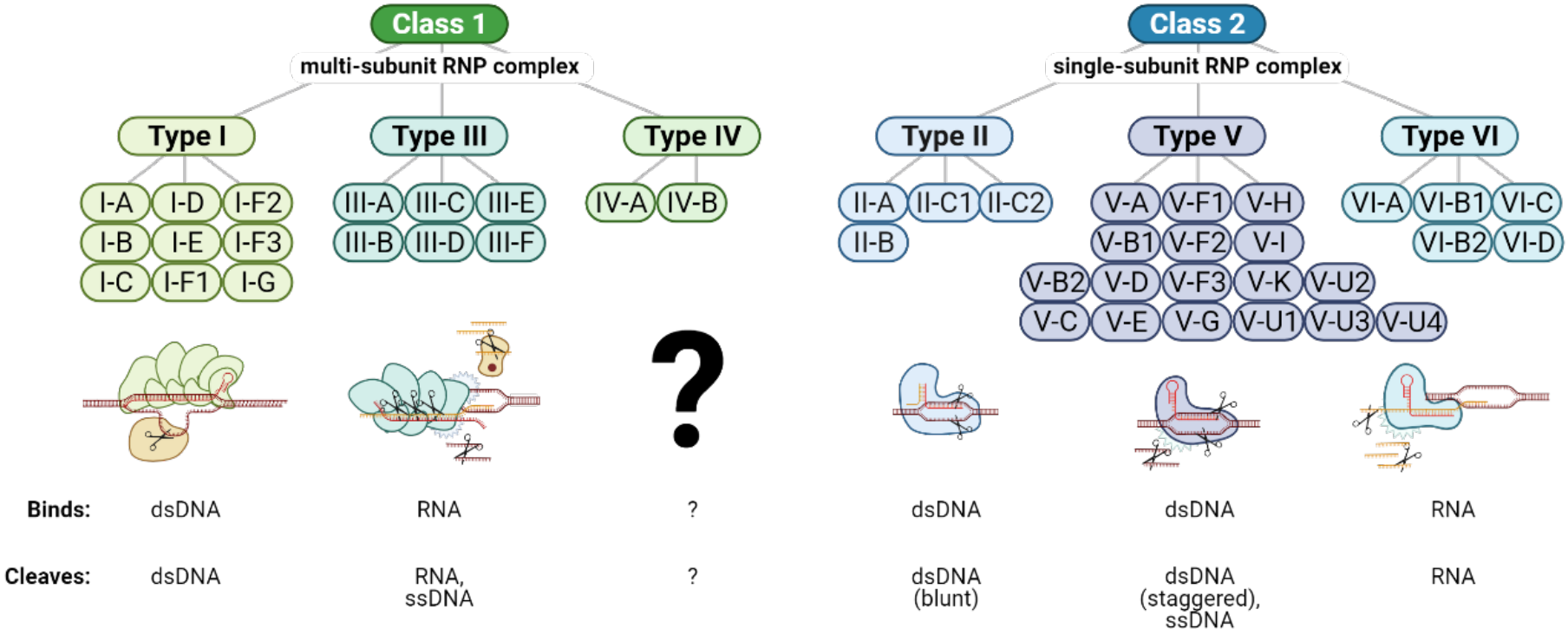
THE NOBEL PRIZE

CRISPR-associated Adaptive Immunity



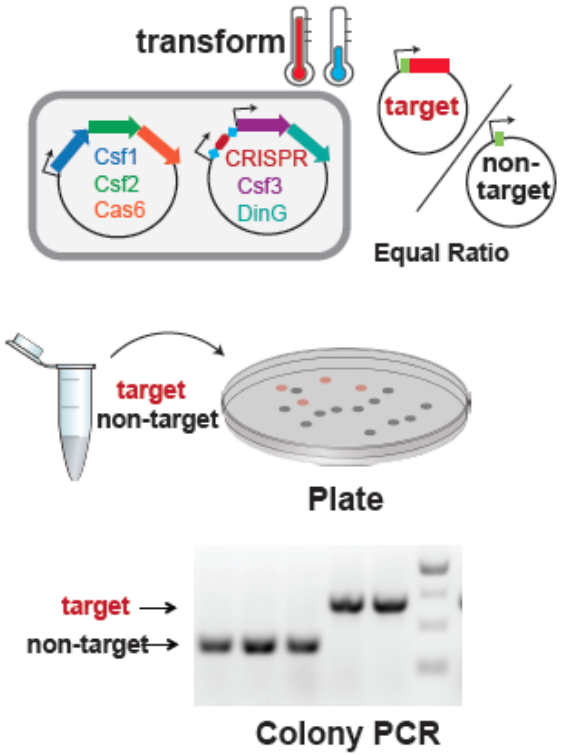
Credit: Hannah Taylor Ph.D.

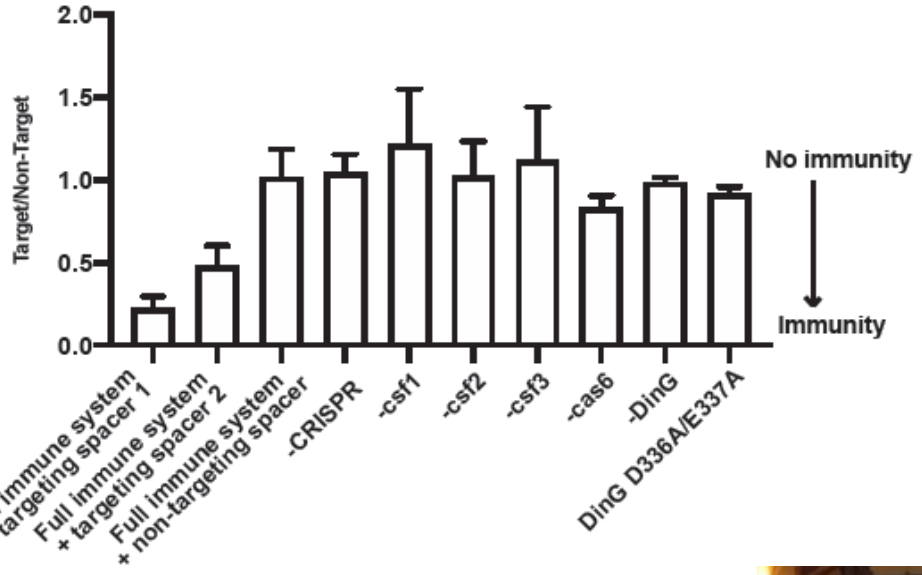
CRISPR-systems are Diverse



Credit: Hannah Taylor, Ph.D.

The type IV-A system is an active immune system

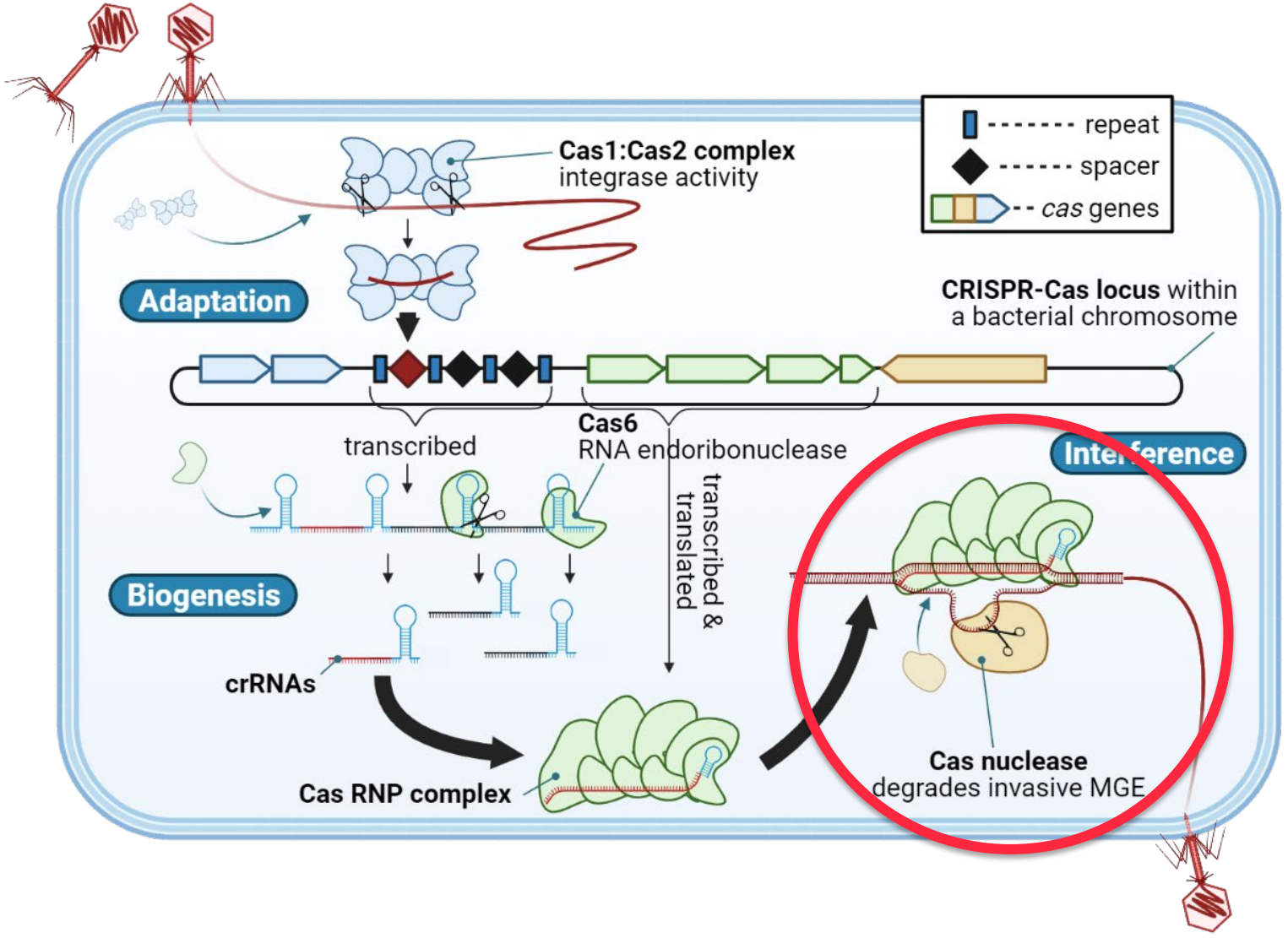




Val Crowley, Ph.D.

Crowley et. al., 2019, the CRISPR Journal

How do phages continue to survive despite innovative bacterial immunity?



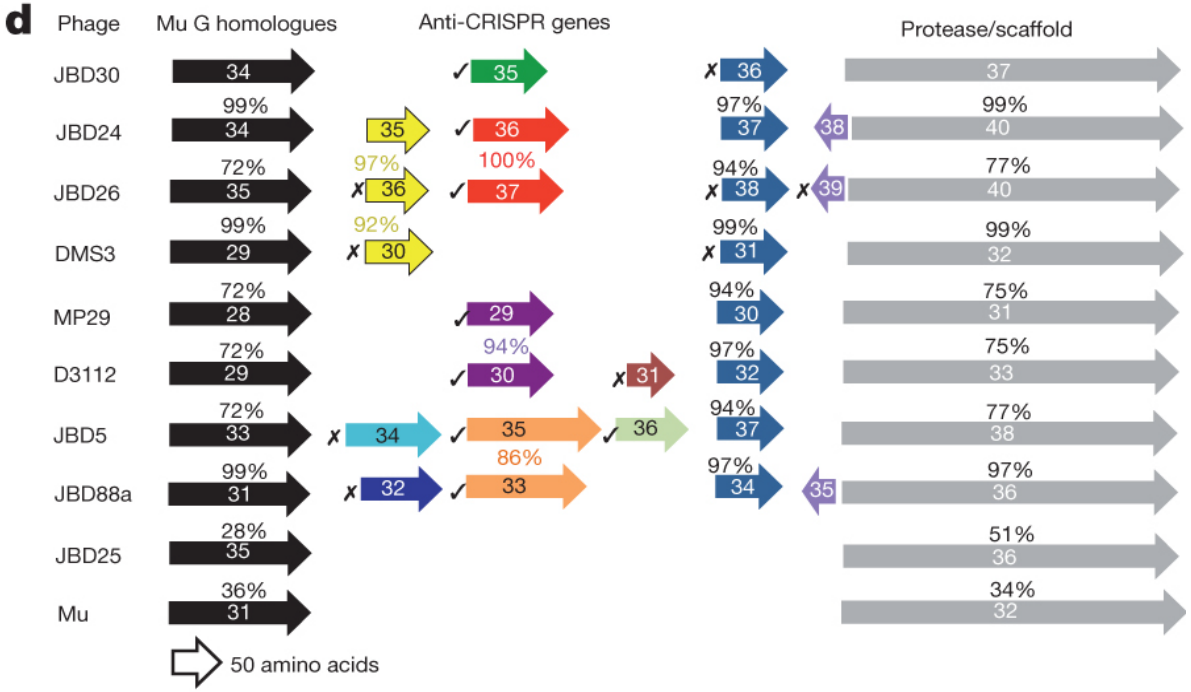
Credit: Hannah Taylor Ph.D.

Anti-CRISPR genes are phages' response to CRISPR immunity

Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system

Joe Bondy-Denomy, April Pawluk, Karen L. Maxwell & Alan R. Davidson

16 December 2012



J B-Denomy *et al. Nature* **000**, 1-4 (2012) doi:10.1038/nature11723

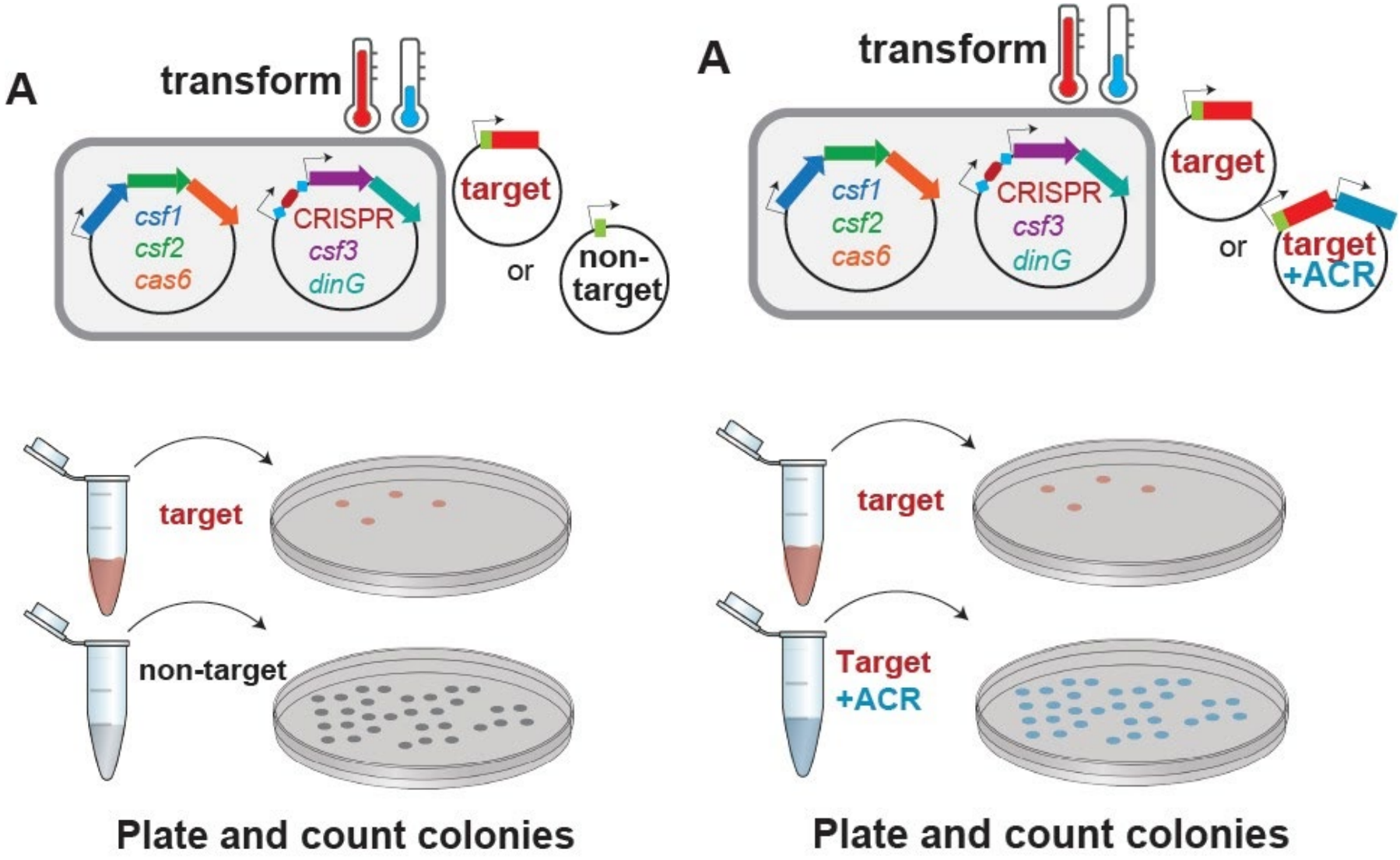
Project flow

Insertion of individual genes into plasmids through Gibson assembly.

Testing of cloned proteins for immune system suppression through colony forming unit assays and PCR assays.

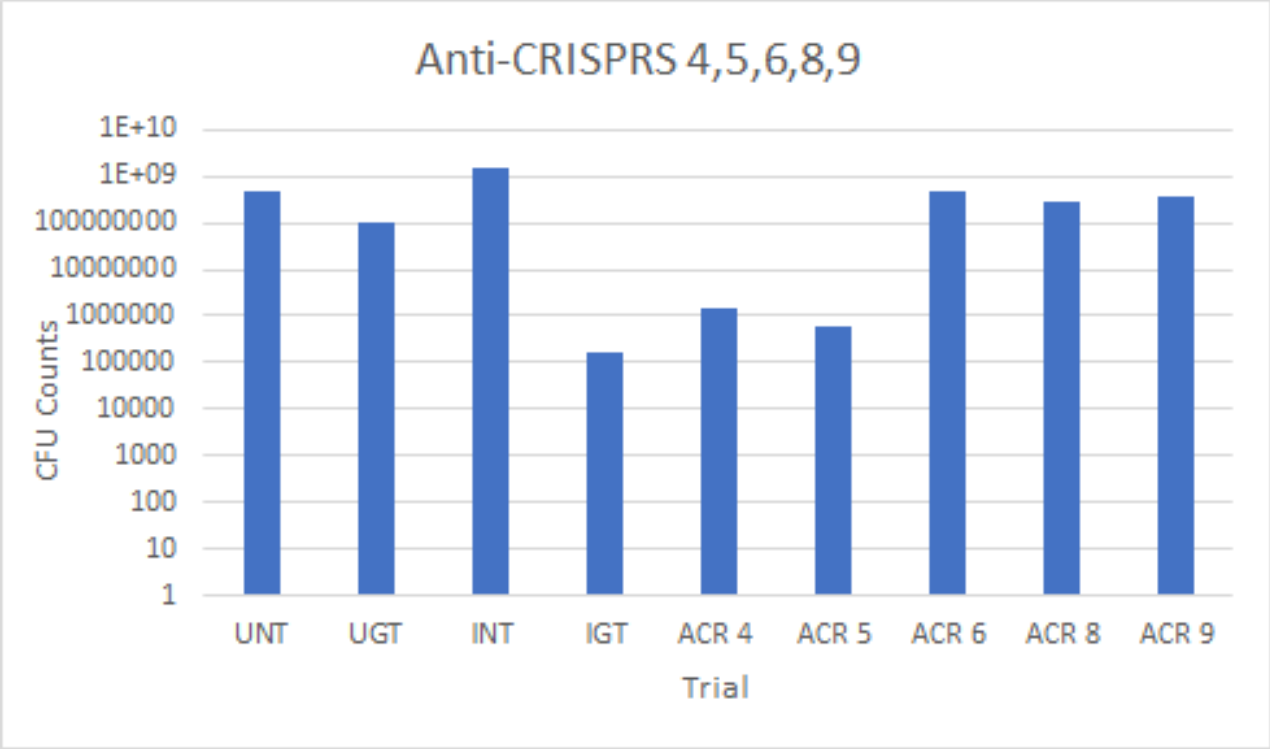
Insertion of identified proteins into expression vectors and subsequent purification and classification.

Assessing anti-CRISPR activity of isolated genes



Credit: Val Crowley, Ph.D.

Several Genes found in Pseudomonas aeruginosa can suppress type IV-A CRISPR immunity



These proteins and others that will be identified likely will shed light on new mechanisms of anti-CRISPR activity that have not been seen before in other systems

Future Directions

- Further identification of hits using retooled assay in new cell line
- Insertion of identified Acr proteins into expression vectors
- Expression and purification of Acr proteins
- Classification by *in vitro* assays using the type IV-A complex

Utah State University

Acknowledgements

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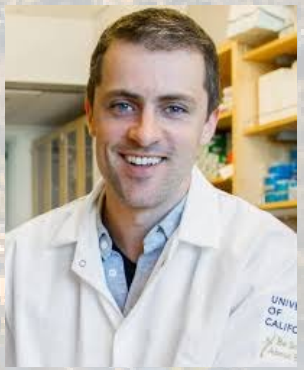

Bondy-Denomy Lab @ UCSF
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Adair Borges
Jennifer Zhang

NIH