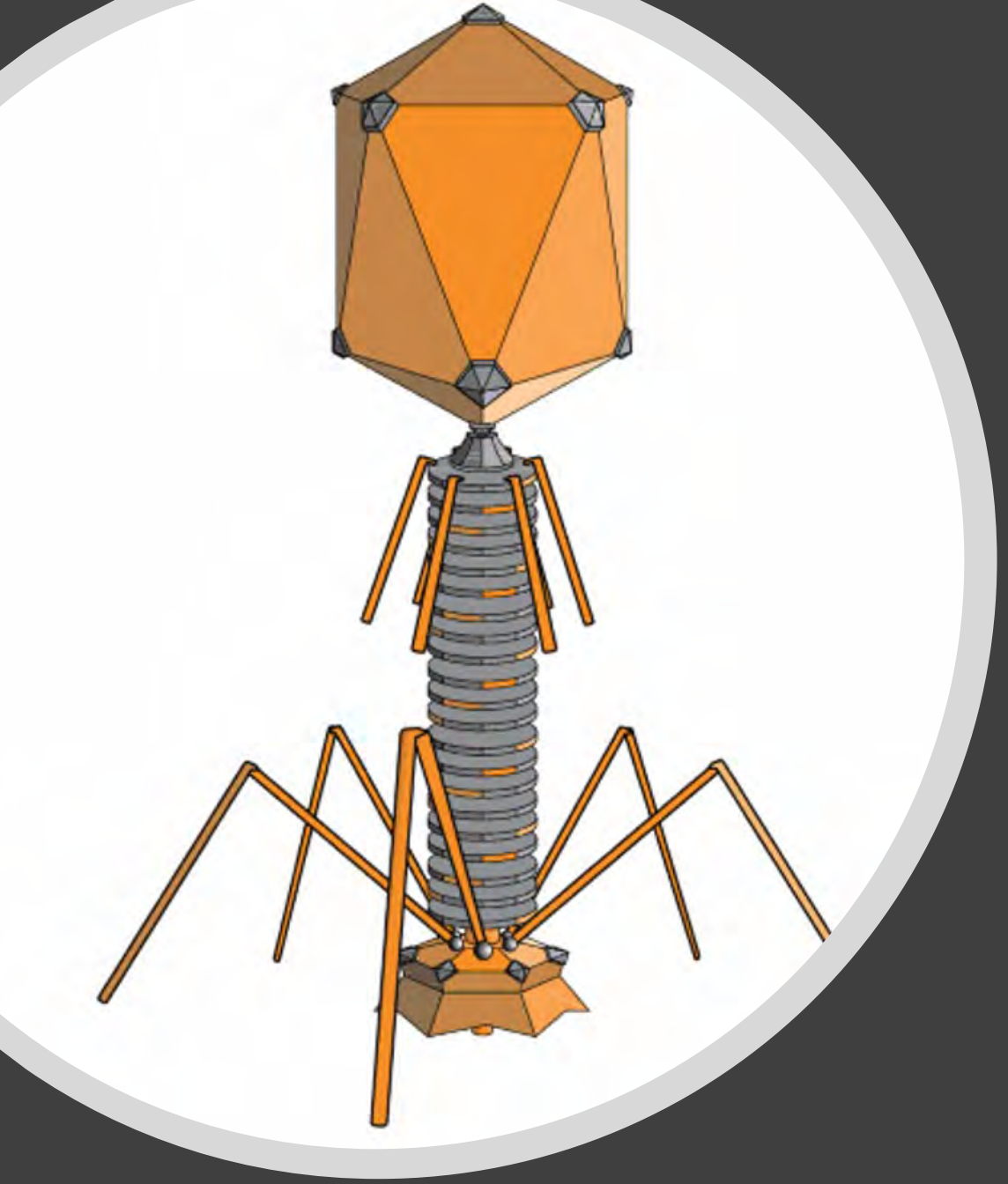

Genome Editing I

CRISPR: As an Adaptive Immune System in Bacteria

CRISPR: It's all about Yogurt

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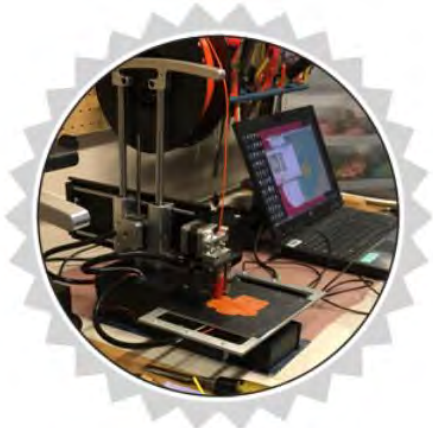


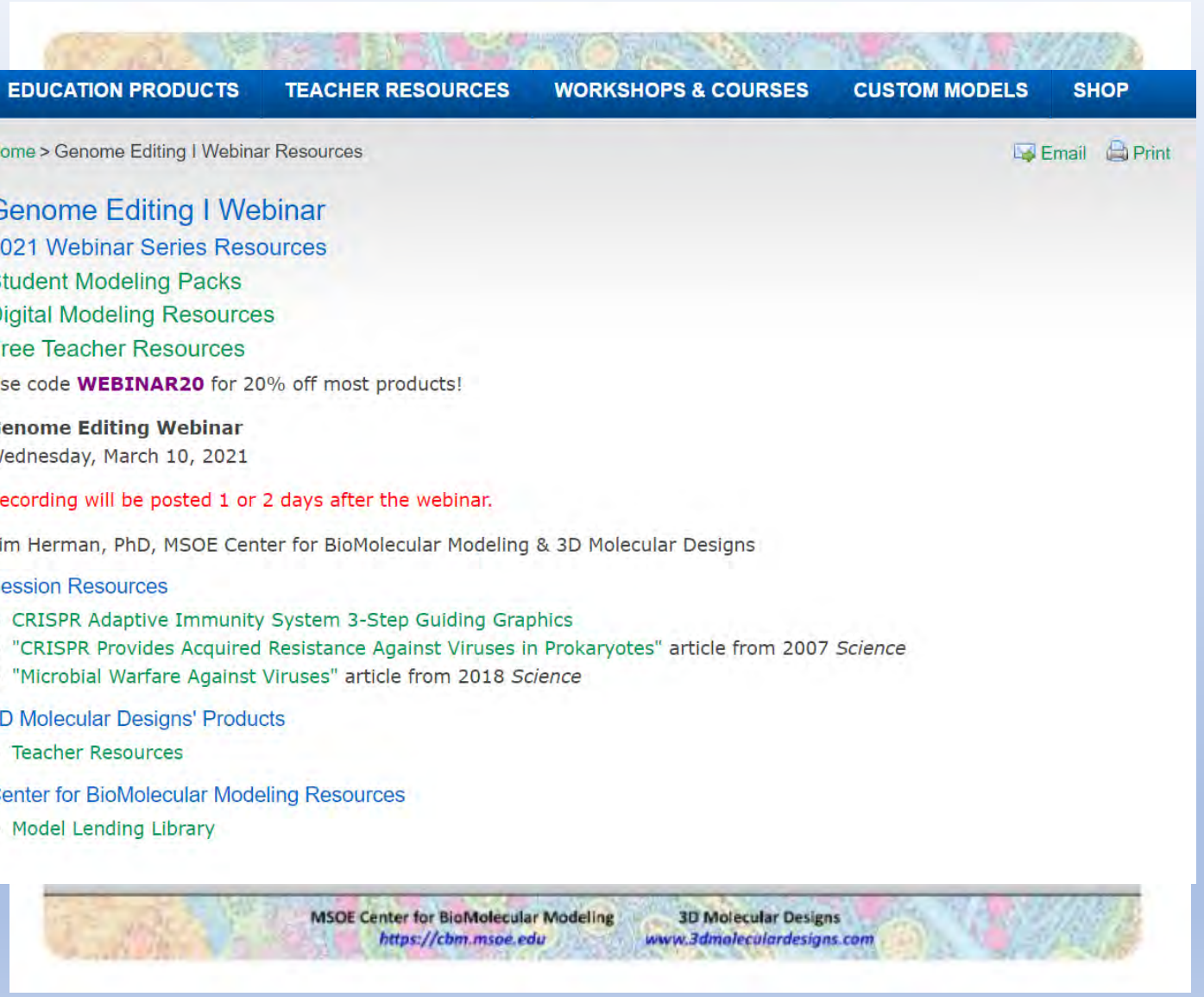
3D Printing for the Bioscience Classroom

Learn to 3D print physical models of molecular structures in your very own classroom.



The Science and Ethics of Genome Editing

Dive into cutting-edge genome editing science with models of DNA, chromosomes, CRISPR and more.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Genome Editing Webinar
Wednesday, March 10, 2021
Recording will be posted 1 or 2 days after the webinar.
Tim Herman, PhD, MSOE Center for BioMolecular Modeling & 3D Molecular Designs
Session Resources
CRISPR Adaptive Immunity System 3-Step Guiding Graphics
"CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes" article from 2007 Science
"Microbial Warfare Against Viruses" article from 2018 Science
3D Molecular Designs' Products
Teacher Resources
Center for BioMolecular Modeling Resources
Model Lending Library
MSOE Center for BioMolecular Modeling
https://cbm.msoe.edu
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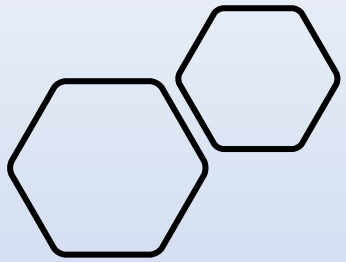
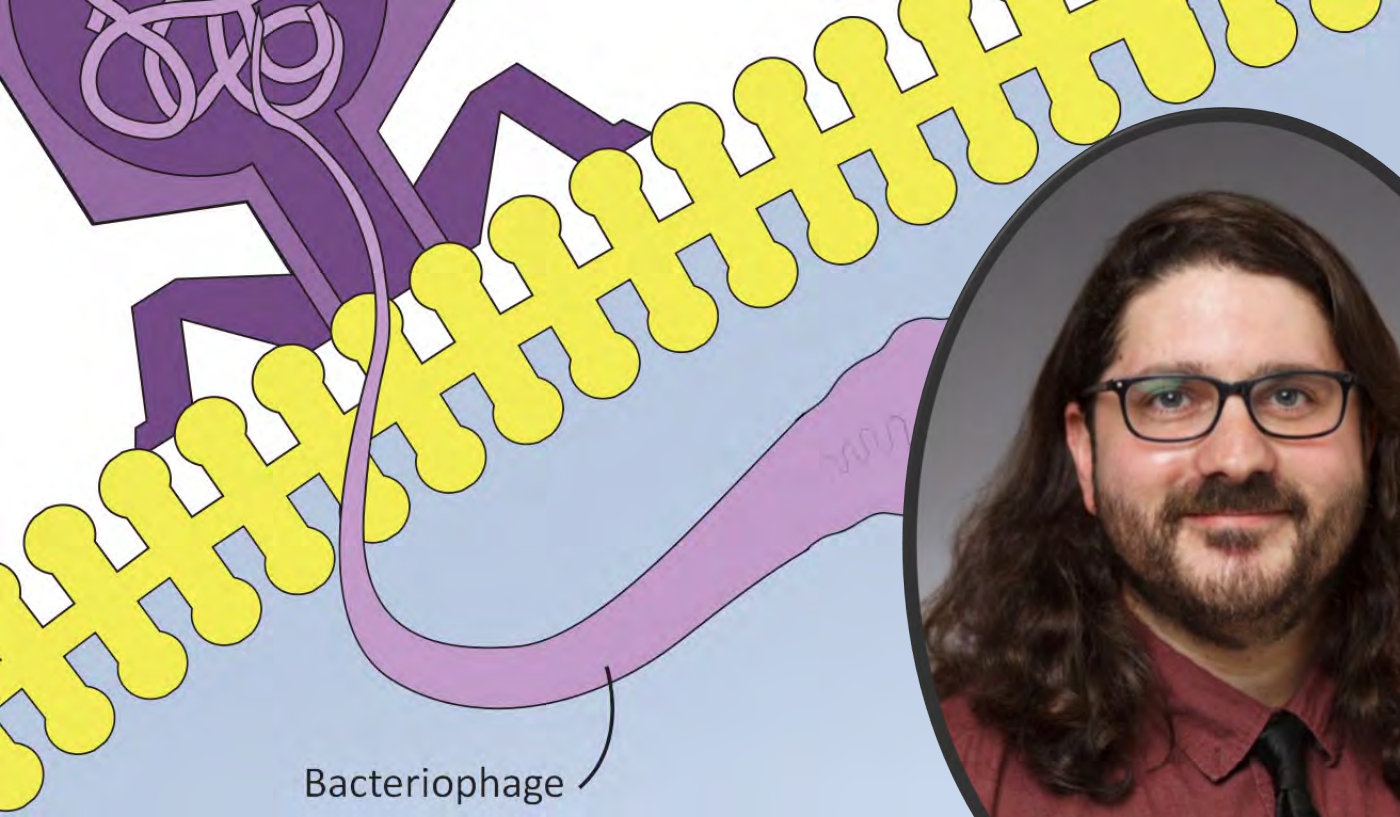


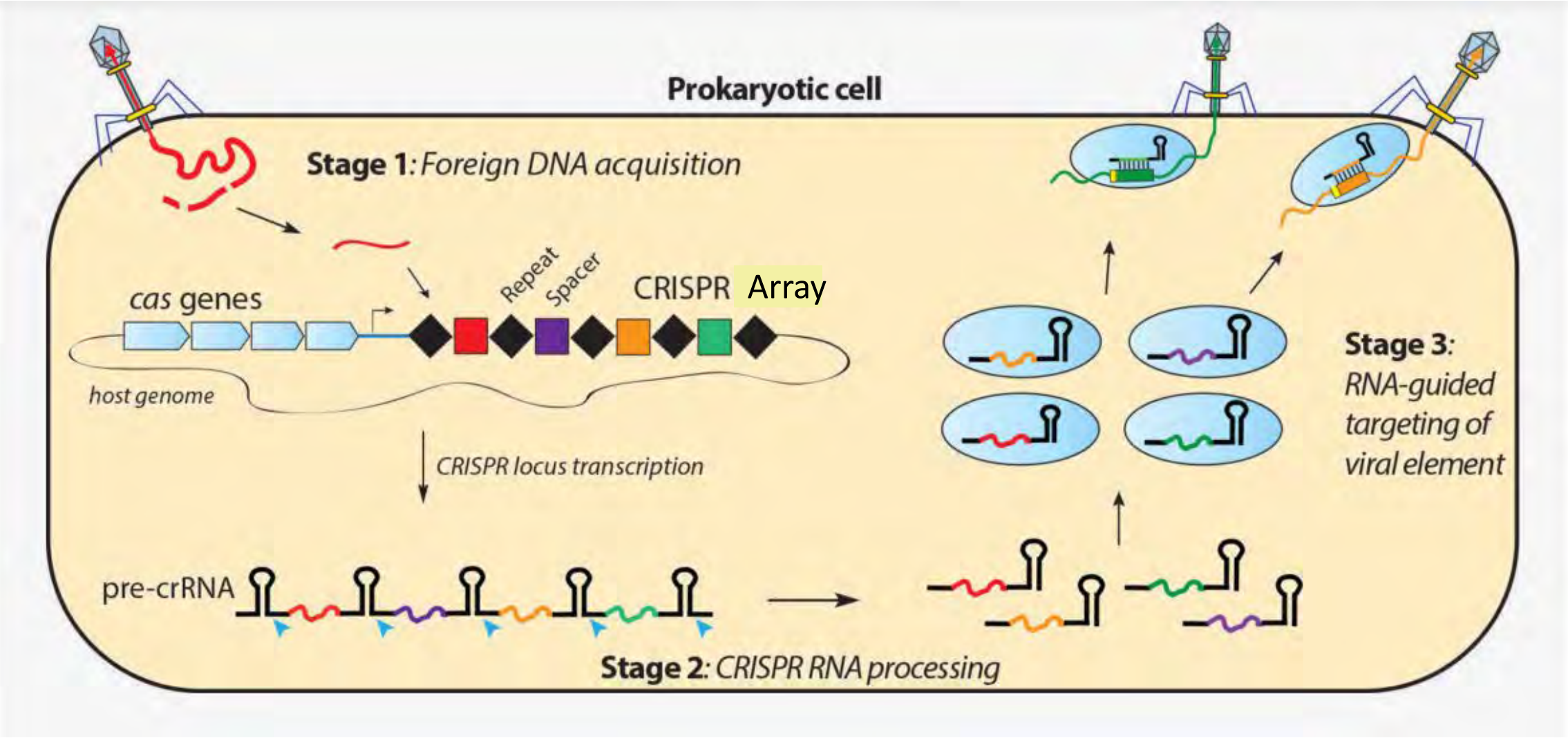

Q&A







CRISPR Locus of the Bacterial Genome



CRISPR – **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

~~Who discovered CRISPR?~~

How was CRISPR discovered?

JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5429–5433
0021-9193/87/125429-05$02.00/0
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Vol. 169, No. 12

Nucleotide Sequence of the *iap* Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in *Escherichia coli*, and Identification of the Gene Product

YOSHIZUMI ISHINO, HIDEO SHINAGAWA, KOZO MAKINO, MITSUKO AMEMURA, AND ATSUO NAKATA*

Department of Experimental Chemotherapy, The Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

Received 1 May 1987/Accepted 22 August 1987

The *iap* gene in *Escherichia coli* is responsible for the isozyme conversion of alkaline phosphatase. We analyzed the 1,664-nucleotide sequence of a chromosomal DNA segment that contained the *iap* gene and its flanking regions. The predicted *iap* product contained 345 amino acids with an estimated molecular weight of 37,919. The 24-amino-acid sequence at the amino terminus showed features characteristic of a signal peptide. Two proteins of different sizes were identified by the maxicell method, one corresponding to the Iap protein and the other corresponding to the processed product without the signal peptide. Neither the isozyme-converting activity nor labeled Iap proteins were detected in the osmotic-shock fluid of cells carrying a multicopy *iap* plasmid. The Iap protein seems to be associated with the membrane.

In the last paragraph of the Discussion…..

An unusual structure was found in the 3′-end flanking region of *iap* (Fig. 5). Five highly homologous sequences of 29 nucleotides were arranged as direct repeats with 32 nucleotides as spacing. The first sequence was included in the putative transcriptional termination site and had less homology than the others. Well-conserved nucleotide sequences containing a dyad symmetry, named REP sequences, have been found in *E. coli* and *Salmonella typhimurium* (28) and may act to stabilize mRNA (18). A dyad symmetry with 14 nucleotide pairs was also found in the middle of these sequences (underlining, Fig. 5), but no homology was found between these sequences and the REP sequence. So far, no sequence homologous to these has been found elsewhere in procaryotes, and the biological significance of these sequences is not known.

This is how science often happens…….

Could you have discovered CRISPR?

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATA
TCCATCATCGCATCCAGTGCGCCCGGTTTATCCCCGCT
GATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACC
GTCGTTGCCGGTTTATCCCTGCTGGCGCGGGGAACTCT
CGGTTCAGGCGTTGCAAACCTGGCTACCGGGCGGTTTA
TCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCA
CCTATGTCTGAACTCCCGGTTTATCCCCGCTGGCGCGG
GGAACTCG

Could you have discovered CRISPR?

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCCCGGTTT
ATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGCCGGTTTATCCCT
GCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGGCGGTTTATCCCCGCTAAC
GCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCCCGGTTTATCCCCGCTGGCGCGGGG
AACTCG

Could you have discovered CRISPR?

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC
CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC
CGGTTTATCCCCGCTGGCGCGGGGAACTCG

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC
CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC
CGGTTTATCCCCGCTGGCGCGGGGAACTCG

29 nucleotide
“short palindromic repeat”

32 nucleotide
“interspersed spacer”

Could you have discovered CRISPR?

GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC
CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC
CGGTTTATCCCCGCTGGCGCGGGGAACTCG

29 nucleotide “short palindromic repeat”	32 nucleotide “interspersed spacer”

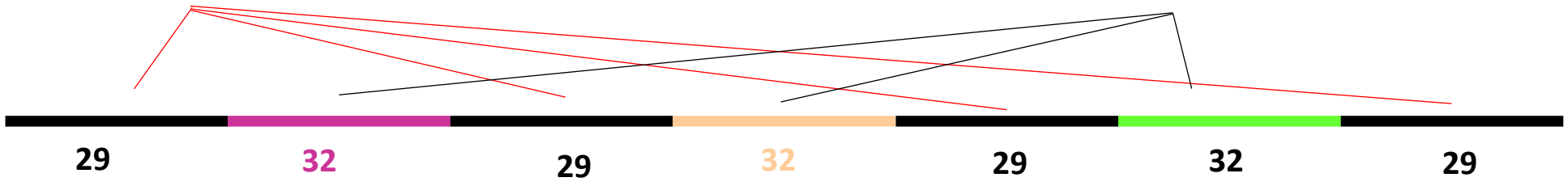
GGAGTTCTACCGCAGAGGCGGGGGAACTCCAAGTGATATCCATCATCGCATCCAGTGCGCC
CGGTTTATCCCCGCTGATGCGGGGAACACCAGCGTCAGGCGTGAAATCTCACCGTCGTTGC
CGGTTTATCCCTGCTGGCGCGGGGAACTCTCGGTTCAGGCGTTGCAAACCTGGCTACCGGG
CGGTTTATCCCCGCTAACGCGGGGAACTCGTAGTCCATCATTCCACCTATGTCTGAACTCC
CGGTTTATCCCCGCTGGCGCGGGGAACTCG

CRISPR repeats (29)

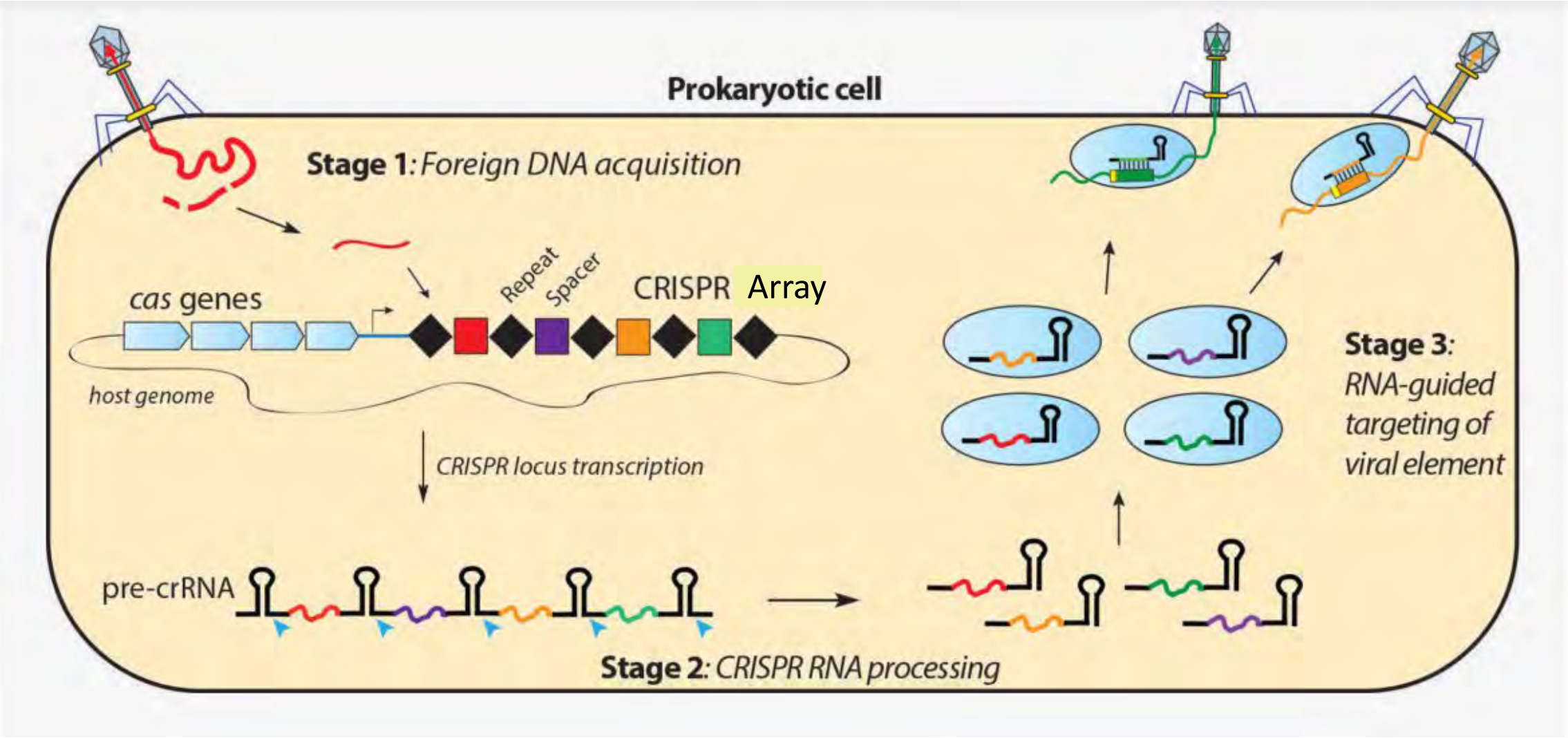
Clustered Regularly Interspersed Palindromic Repeats

Spacer sequences (32)

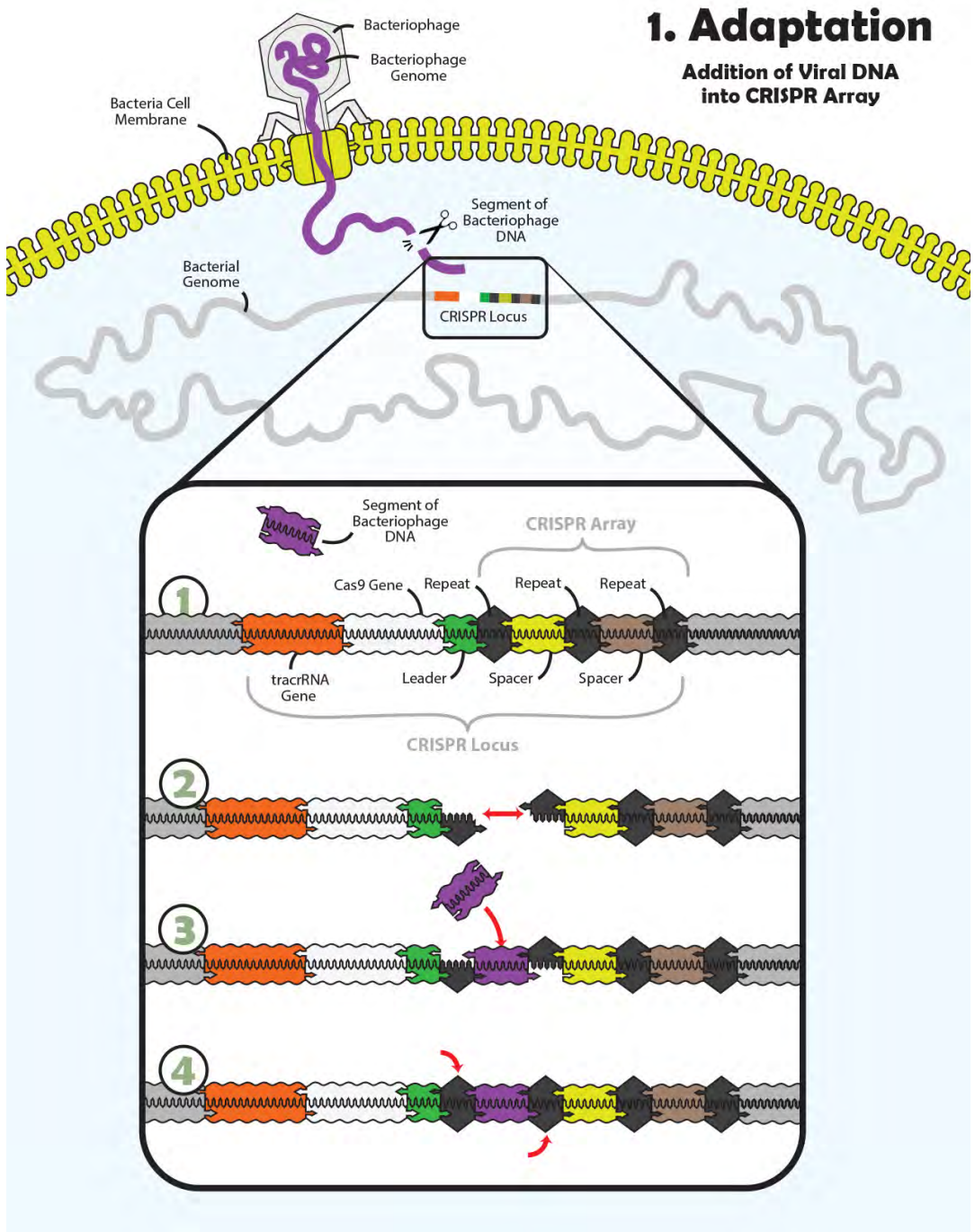
(later shown to be foreign DNA)



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

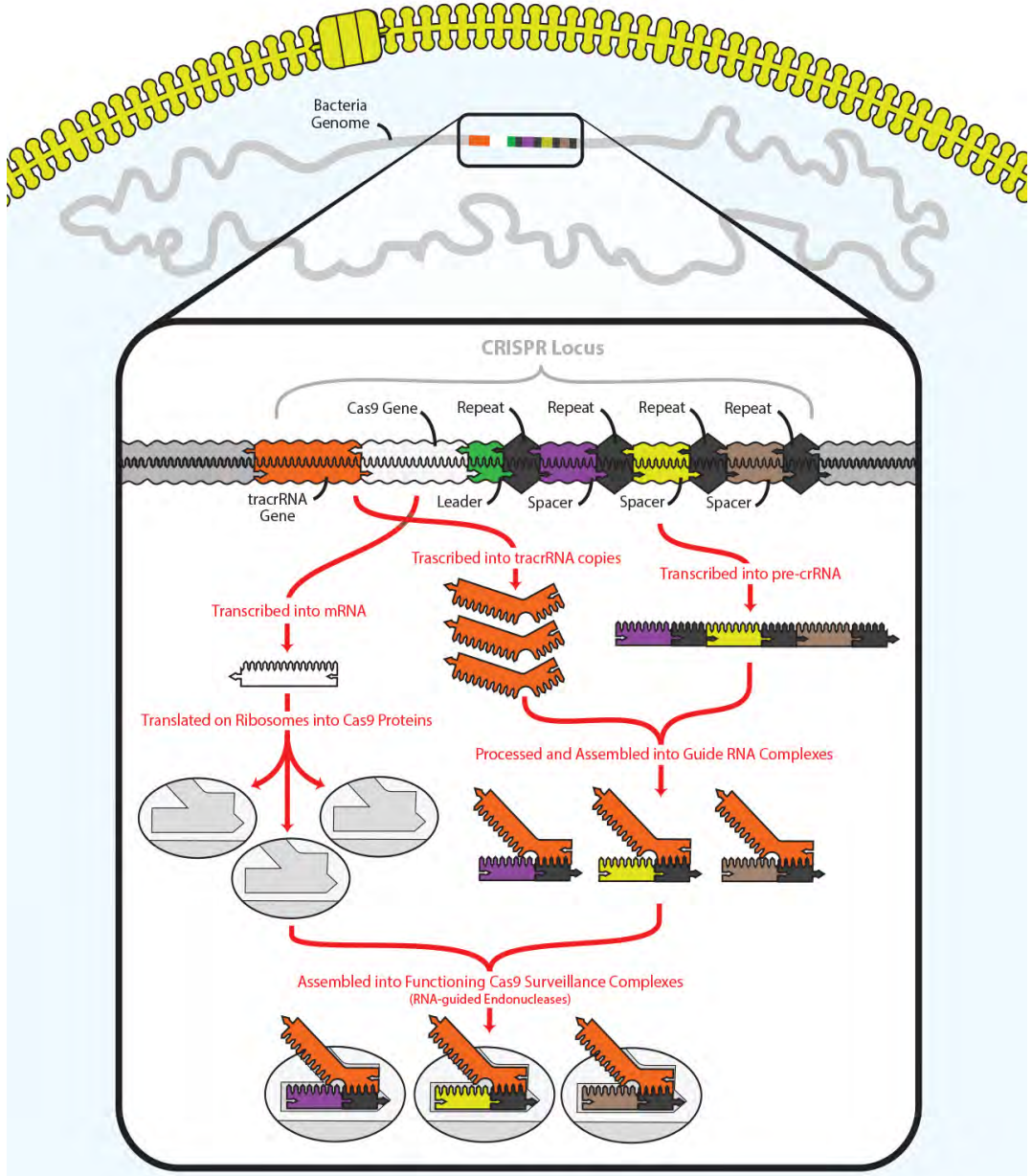


CRISPR – **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats


1. Adaptation
Addition of Viral DNA into CRISPR Array
Bacteriophage
Bacteriophage Genome
Bacteria Cell Membrane
Segment of Bacteriophage DNA
Bacterial Genome
CRISPR Locus
Segment of Bacteriophage DNA
CRISPR Array
Cas9 Gene
Repeat
Repeat
Repeat
1
tracrRNA Gene
Leader
Spacer
Spacer
CRISPR Locus
2
3
4

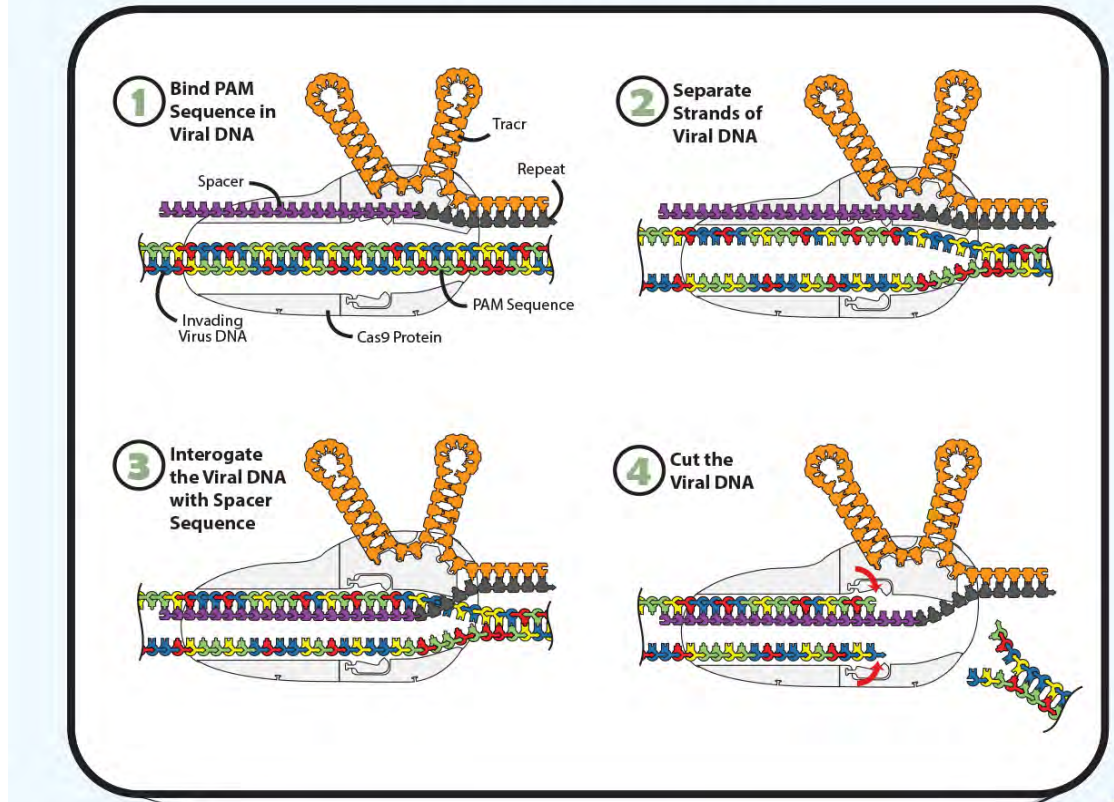
2. Expression of CRISPR Locus Genes

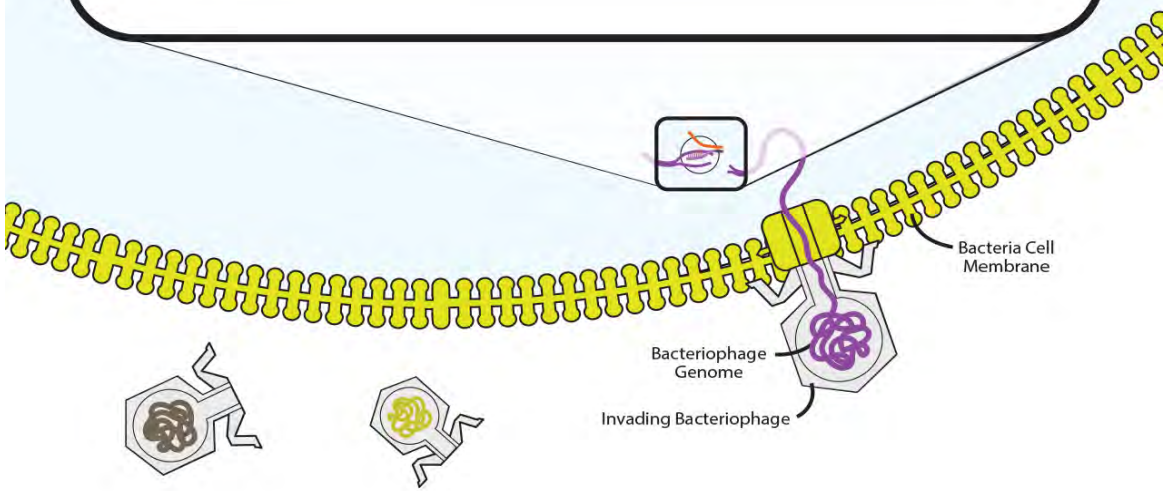
Building Cas9 Surveillance Complexes



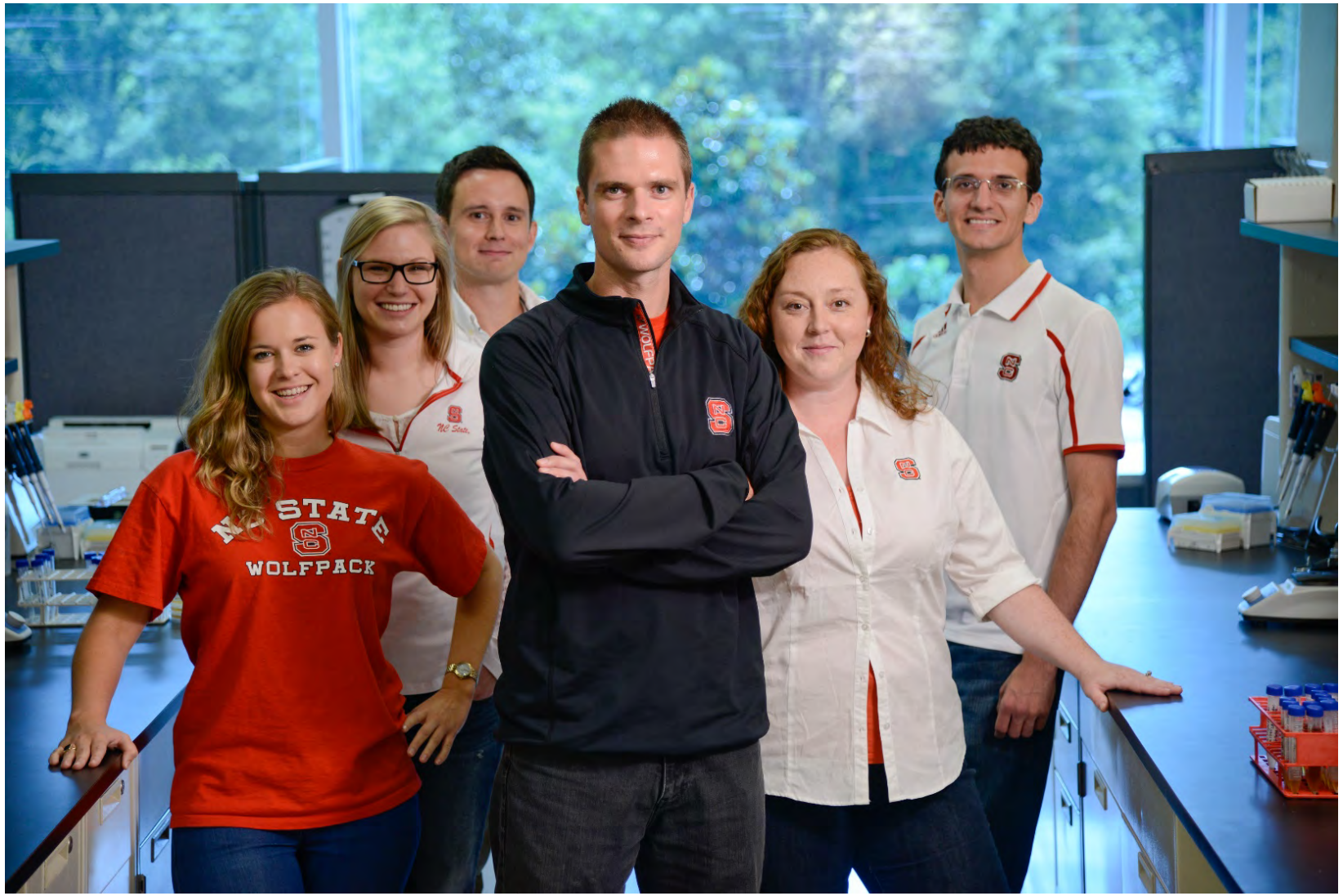
3. Invader Silencing

Cutting the DNA of an Incoming Virus





Rodolphe Barrangou & Research Team at NC State CRISPR Lab





Rodolphe Barrangou, Jennifer Doudna, Philippe Horvath, Emmanuelle Charpentier, & Virginijus Siksnys

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]

Clustered regularly interspaced short palindromic repeats (CRISPR) are a distinctive feature of the genomes of most Bacteria and Archaea and are thought to be involved in resistance to bacteriophages. We found that, after viral challenge, bacteria integrated new spacers derived from phage genomic sequences. Removal or addition of particular spacers modified the phage-resistance phenotype of the cell. Thus, CRISPR, together with associated cas genes, provided resistance against phages, and resistance specificity is determined by spacer-phage sequence similarity.

23 MARCH 2007 VOL 315 SCIENCE

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2Danisco France SAS, Boîte Postale 10, F-86220 Dangé-Saint-Romain, France.

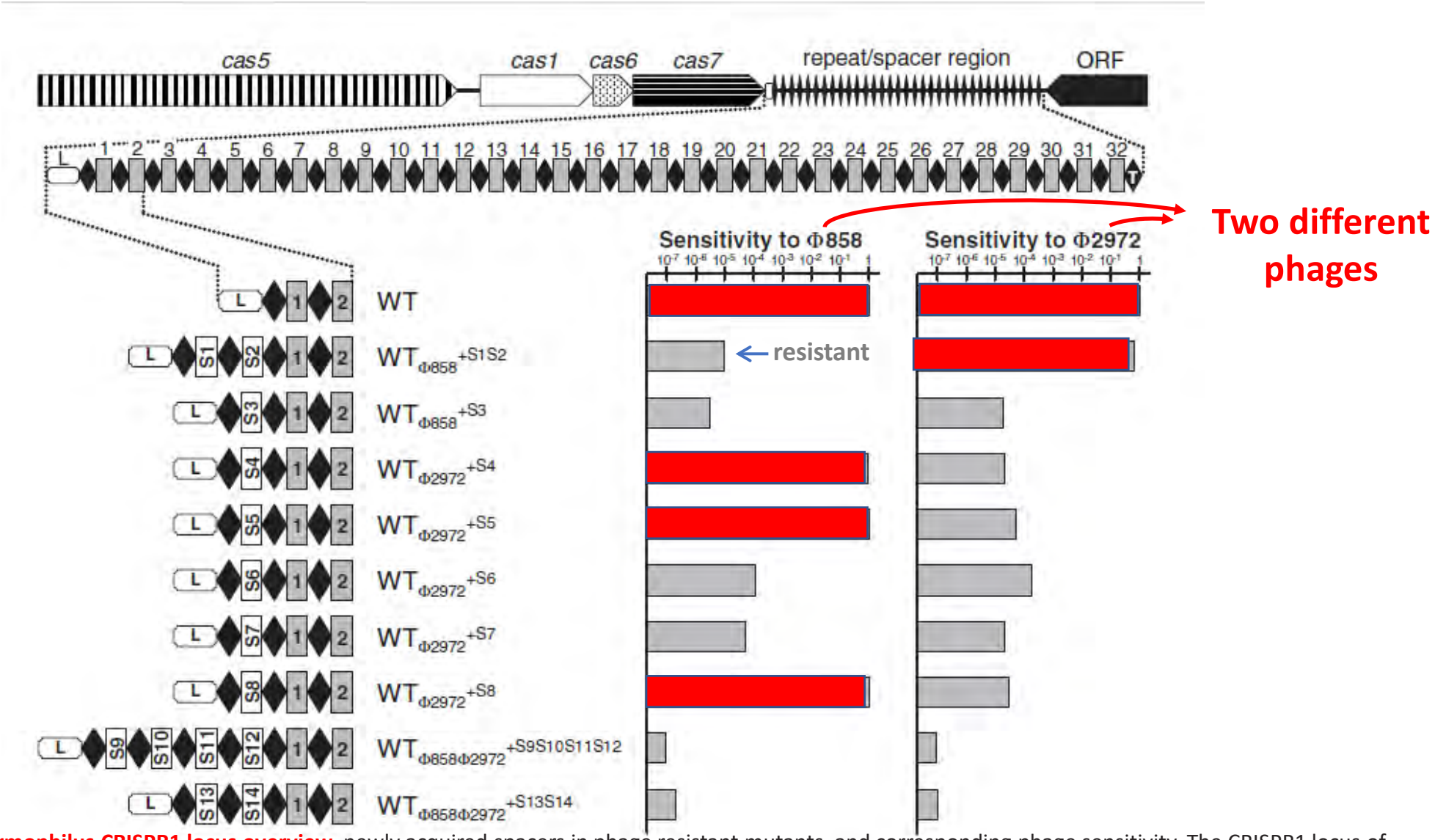


Fig. 1. **Streptococcus thermophilus CRISPR1 locus overview**, newly acquired spacers in phage resistant mutants, and corresponding phage sensitivity. The CRISPR1 locus of DGCC7710 (WT) is at the top. The repeat-spacer region of WT is in the middle: repeats (black diamonds), spacers (numbered gray boxes), leader (L, white box), and terminal repeat (T, black diamond). (Bottom left) The spacer content on the leader side of the locus in phage-resistant mutants is detailed, with newly acquired spacers (white boxes, S1 to S14). (Bottom right) The sensitivity of each strain to phages 858 and 2972 is represented as a histogram of the efficiency of plaquing (EOP), which is the plaque count ratio of a mutant strain to that of the wild-type.

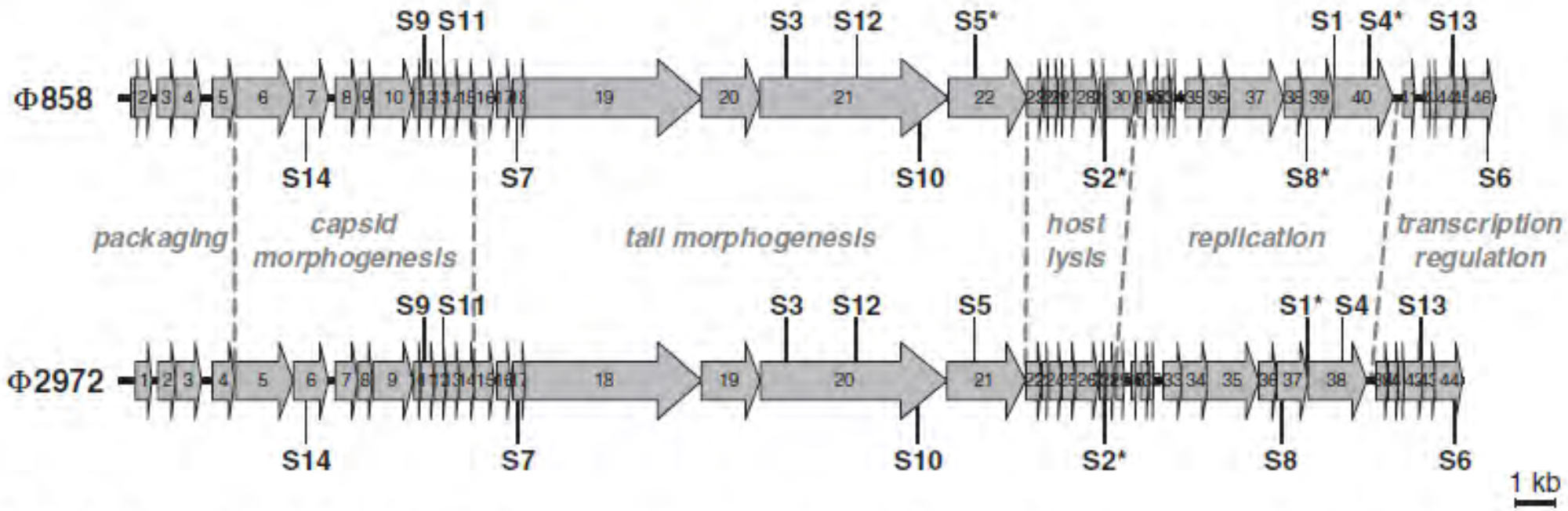


Fig. 2. *S. thermophilus* phage genome maps with the position of sequences similar to the acquired CRISPR1 spacers of the phage-resistant mutants. Spacers shown above and below the genome maps indicate that the spacer matches a sequence on the (+) and on the (–) strand, respectively. An asterisk indicates the existence of a SNP between the spacer sequence and that of the phage genome (fig. S1). The genome sequences of phage 2972 (accession number AY699705) and phage 858 are 93% identical.

MATERIALS AND METHODS

Isolation of phage-resistant mutants and confirmation of CRISPR sequences

Streptococcus thermophilus phage-resistant mutants were obtained by challenging the wild-type host strain DGCC7710 (also called RD534) with phage 2972 and/or phage 858 (1). The host strain was grown at 42ºC in 10 ml of M17 broth supplemented with 0.5% lactose (LM17). When the optical density (600 nm) reached 0.3, phages and calcium chloride 10mM were added at a final concentration of 107 pfu/ml and 50 mM, respectively. The phage-containing culture was incubated at 42ºC for 24 hours and monitored for lysis. Then, 100 µl of the lysate were inoculated into 10 ml of fresh LM17. The remaining lysate was centrifuged and the pellet was inoculated into another tube containing 10 ml of fresh LM17. These two cultures were incubated at 42ºC for 16 hours. Finally, these cultures were diluted and plated on LM17. Isolated colonies were tested for phage sensitivity as previously described (2). The CRISPR loci of the resistant isolates were verified by sequencing PCR products, and using relevant phage genome information (1).

Science 2 MARCH 2018

MICROBIOLOGY

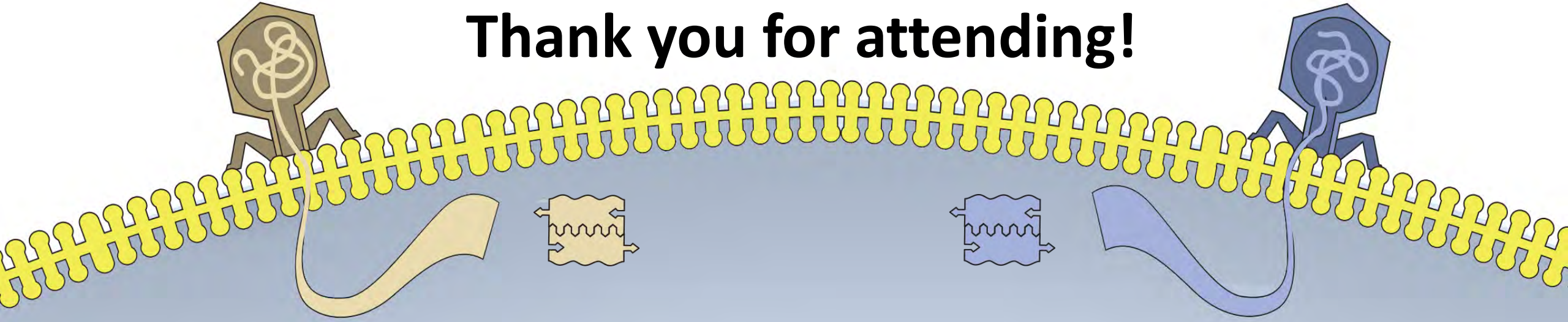
Microbial warfare against viruses

Many new antiviral defense systems are found in bacteria and archaea

Because genes in one immune defense system are often found next to genes in another defense system, forming "defense islands" in microbial genomes, Doron et al. hypothesized that unknown defense systems could be found near already-known antiviral systems.

Dorin et al. identified 9 new phage defense systems in bacteria….

Thank you for attending!



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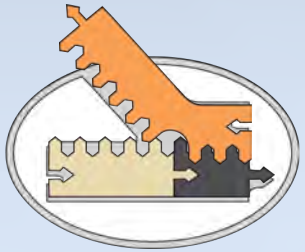

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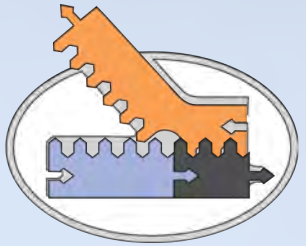

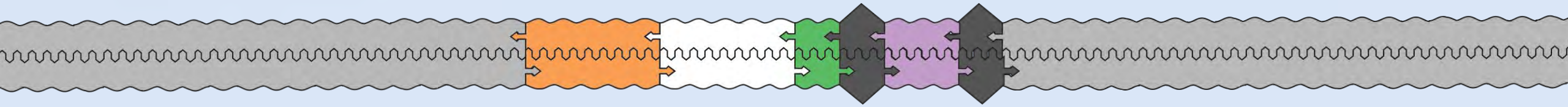

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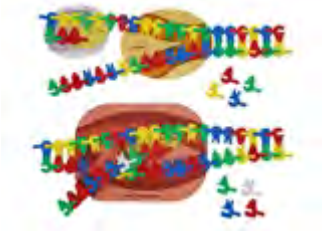
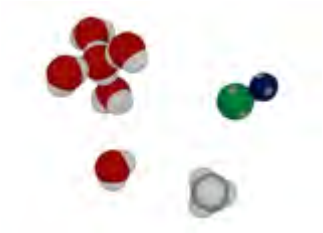


Genome Editing II

CRISPR Cas9 - A Powerful New Tool for Editing the Human Genome

Wednesday, March 17, 2021 at 6:00 PM Central



Celebrate World Water Day

Engage with Water Molecules!

Monday, March 22, 2021 at 6:00PM Central



Membranes & Ion Transport

Exploring the Wonderful World of Cell Membranes: Proteins, Lipids, and Ions, Oh My!

Wednesday, April 14, 2021 at 6:00 PM Central

https://www.3dmoleculardesigns.com/Webinars4.htm