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A Presentation in Six Panels:

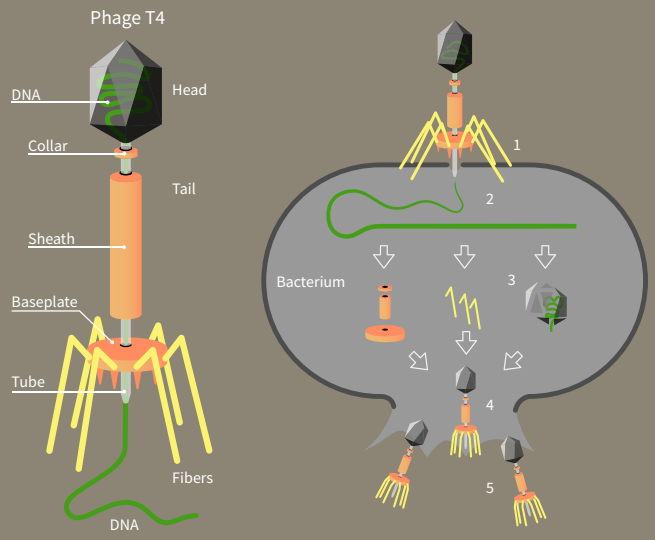
Introduction to CRISPR-Cas9 Biology

Origin and Function of CRISPR-Cas9 Technology
Panel – 1 –

Recently developed CRISPR-Cas9 technology provides scientists with unprecedented possibilities and challenges. This technique can potentially exert a considerable impact on human society. It is therefore essential for a broad audience to understand the biology of CRISPR-Cas9 and to participate in the discussion of its use. This introduction to CRISPR-Cas9 biology and application is aimed towards the interested public as well as students in the life sciences.

The term CRISPR refers to a genomic locus that is found in bacteria and archaea. The CRISPR locus acts as an adaptive immune system that provides a defense mechanism against repeated viral infections. A virus that can infect a bacterium is called a phage. The structure of a typical phage «T4» is shown on the left. This phage can infect the bacterium *Escherichia coli*. A cycle of an infection is shown on the right.

Infection Cycle of a Phage



An infection cycle starts when a phage's fibers attach to the surface of a susceptible bacterium (1). The sheath of the phage's tail contracts, and a hole is punched in the bacterial membrane. The phage injects its DNA, stored in the phage head, via the central tube into the cytoplasm of the bacterium (2). The bacterial metabolism is now reprogrammed in favor of the phage. Phage DNA is translated into proteins to produce the phage components head, tail and fibers (3). The DNA of the host bacterium is hydrolyzed, and nucleotides are used to synthesize copies of the phage genome. Phage components are then assembled to form new phages (4). Finally, the phage activates enzymes to lyse the bacterial membrane from the inside. The bacterium bursts, and several hundreds of new infectious phages are released (5). The next slide shows how bacteria have developed a defense mechanism against repeated phage infections.

Reference: Leiman PG et al. *VIROL J* 2010.

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Introduction to CRISPR Biology:

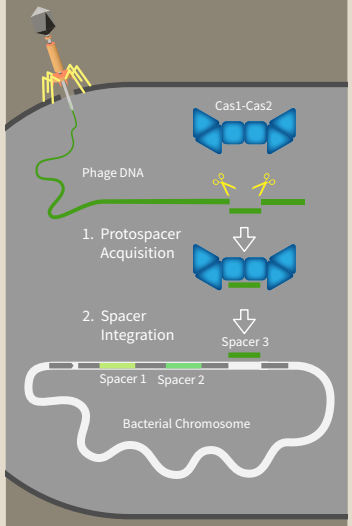
CRISPR-Cas9 Confers Adaptive Immunity

Origin and Function of CRISPR-Cas9 Technology
Panel – 2 –

The CRISPR genetic locus provides bacteria a defense mechanism to protect themselves against repeated phage infections. This immunity is based on the following principle. The bacterium acquires a DNA sequence sample of the phage in the initial infection. This sample sequence is then stored along with other samples in the CRISPR locus. If a secondary infection occurs, this can be detected by comparing the invading DNA with samples stored in the CRISPR genetic locus. If the sequences match, the DNA of the invading phage is destroyed.

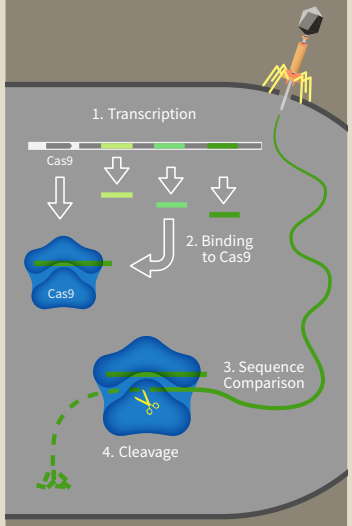
There are different types of CRISPR systems in different bacteria. The one depicted here is the Type II CRISPR-Cas9 system derived from *Streptococcus pyogenes*. All of the following information refers to Cas9 since this is the protein that has been successfully converted into a tool for scientists.

Acquisition of Phage Sequences



Adaptive immunity of the CRISPR system starts with the acquisition of a DNA sequence of an invading phage. After a phage has injected its DNA into the bacterial cytoplasm, a protein complex forms consisting of Cas1 and Cas2. A sample sequence, termed protospacer, is then acquired from the phage DNA by the Cas1-Cas2 complex (1). This sequence is then integrated into the CRISPR locus (2). The sequence is now called a spacer. Several spacers from earlier contacts with phages are stored in the CRISPR genetic locus.

Immunity against Reinfection



The CRISPR-Cas9 system provides a defense mechanism against reinfection with the same type of phage. Spacer sequences are transcribed as RNA, and Cas9 protein is synthesized (1). The RNA is integrated into the Cas9 protein to form a surveillance and interference complex (2). The complex then scans intracellular DNA for matching sequences (3). If a matching sequence is found, Cas9 destroys the DNA by cleavage (4).

Reference: Marraffini L *NATURE* 2015.

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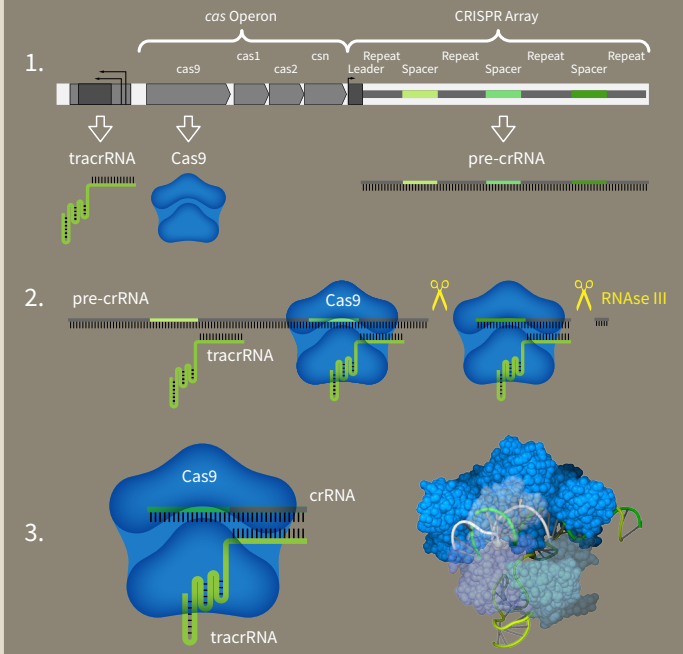
Introduction to CRISPR Biology:

CRISPR Locus Codes for a Surveillance Complex

Origin and Function of CRISPR-Cas9 Technology
Panel – 3 –

From Transcription to Complex Formation



1. CRISPR Genetic Locus Transcripts
At the genetic level, the CRISPR locus of *S. pyogenes* consists of a *cas* operon, a CRISPR array, and genes coding for trans-activating crRNA (tracrRNA). The CRISPR array consists of acquired sequences (spacers) matching phage DNA. Each of these sequences is flanked by repeats. These repeats are frequently palindromic, meaning that the sequence of the bases is the same forwards and backwards. This characteristic accounts for the name CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats. tracrRNA, Cas9 RNA and pre-crRNA are transcribed from the CRISPR genetic locus. The tracrRNA forms a tertiary structure with three loops and Cas9 RNA is further translated in protein.

2. Pre-crRNA Maturation
Because pre-crRNA is synthesized as a continuous stretch, a maturation process follows. In this process, tracrRNAs pair with repeat sequences of pre-crRNA. Cas9 protein is recruited to form a ternary complex with the tracrRNA and pre-crRNA. Single interference complexes are then released through cleavage of the crRNA by RNAse III enzymes.

3. Interference Complex
The interference complex consists of Cas9 and a duplex of tracrRNA and crRNA. crRNA contains the spacer sequence for comparison with DNA, and a sequence to form a duplex with tracrRNA. The 3D structure of the complex, based on crystallization data, is shown on the right (PDB sequence 4OO8).

References: Mali P et al. *NATURE METHODS* 2013, Doudna JA and Charpentier E *SCIENCE* 2014, Nishimasu H et al. *CELL* 2014.

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