The transformative CRISPR-Cas9 technology: A bacterial mechanism

Emmanuelle Charpentier's Laboratory

Washington, December 1, 2015

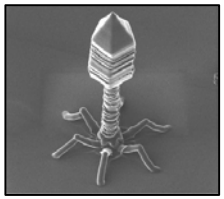
CRISPR-Cas: RNA-mediated adaptive immune system in bacteria and archaea

CRISPR: clustered, regularly interspaced short palindromic repeats

Cas: CRISPR-associated

Infection of bacteria by invading genomes

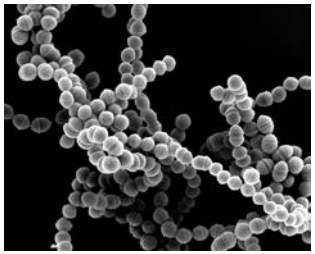
Mobile Genetic Elements



attack

defence

Bacterial Host



Infection of bacteria by invading genomes

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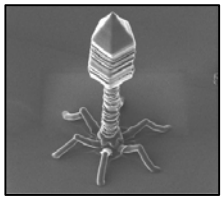

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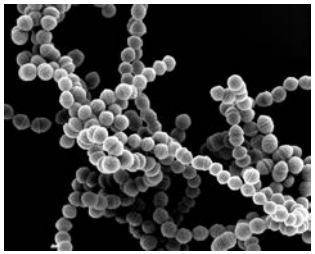
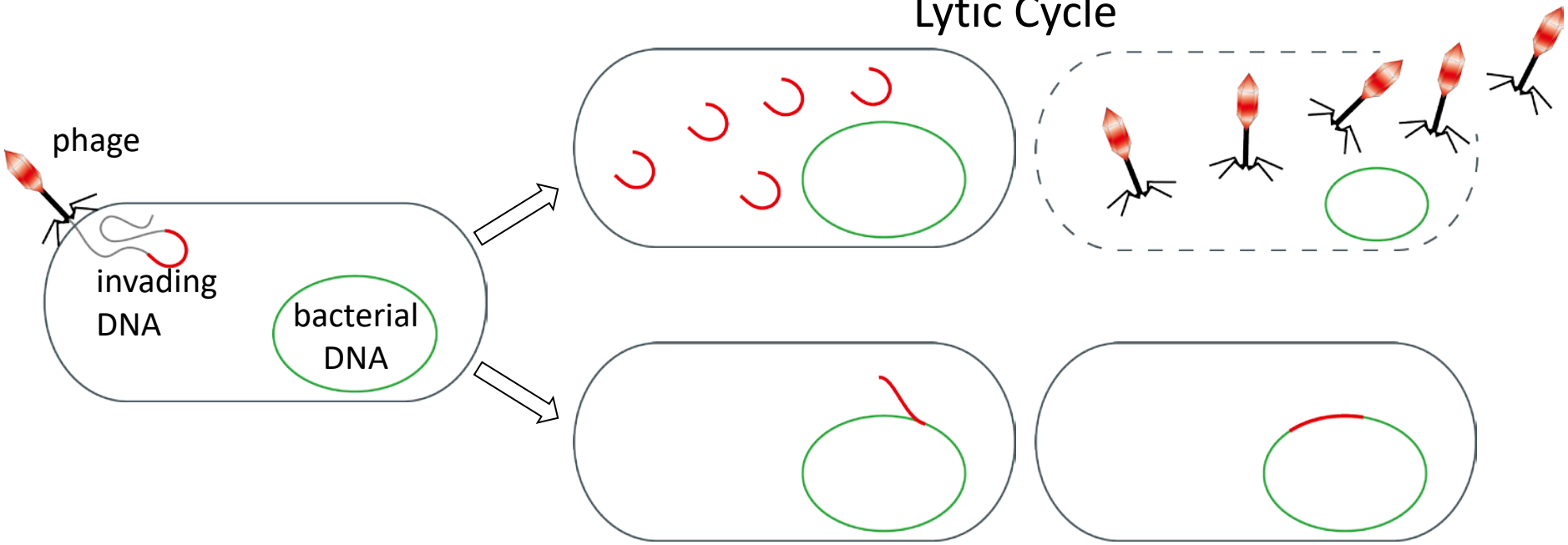
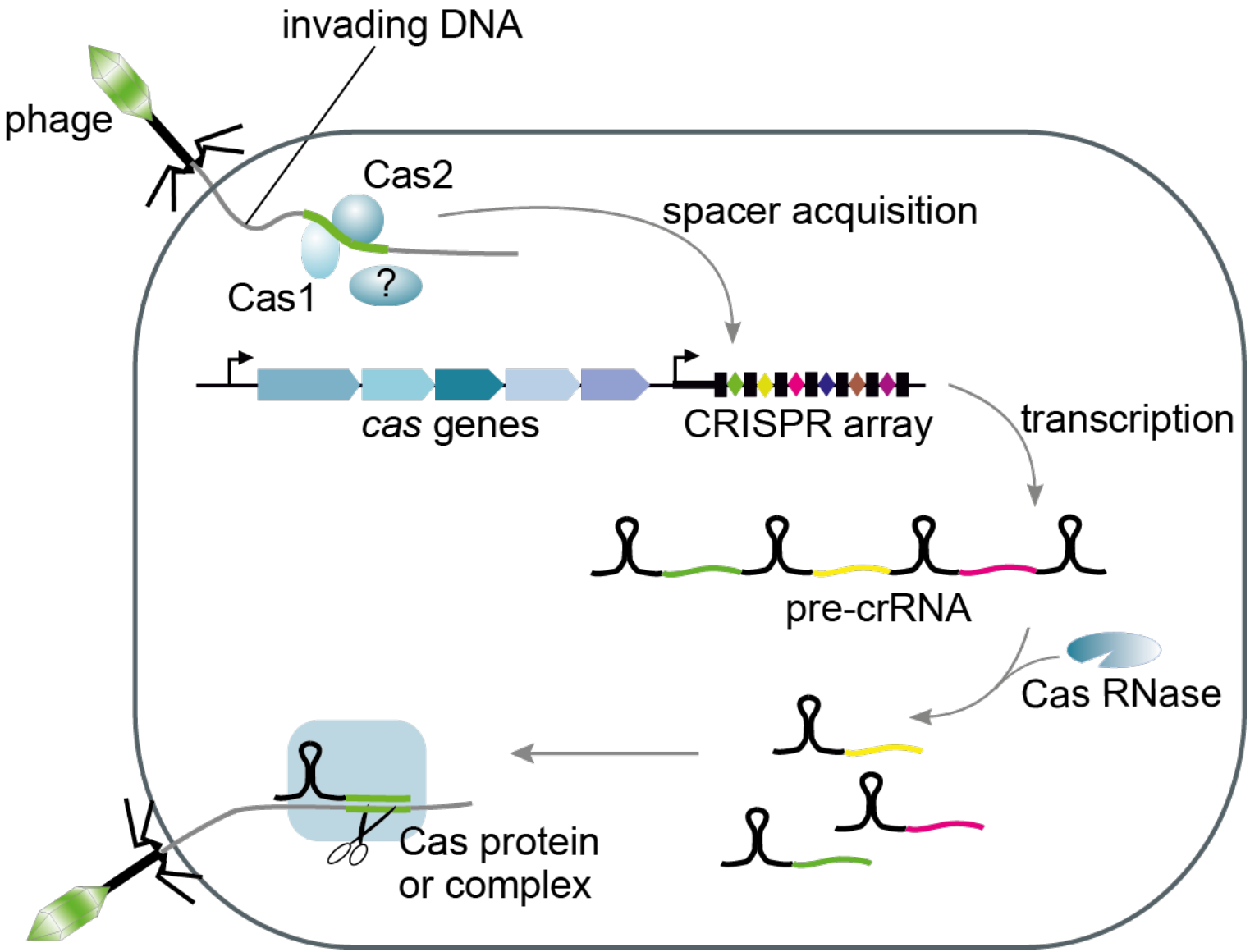


Lytic Cycle



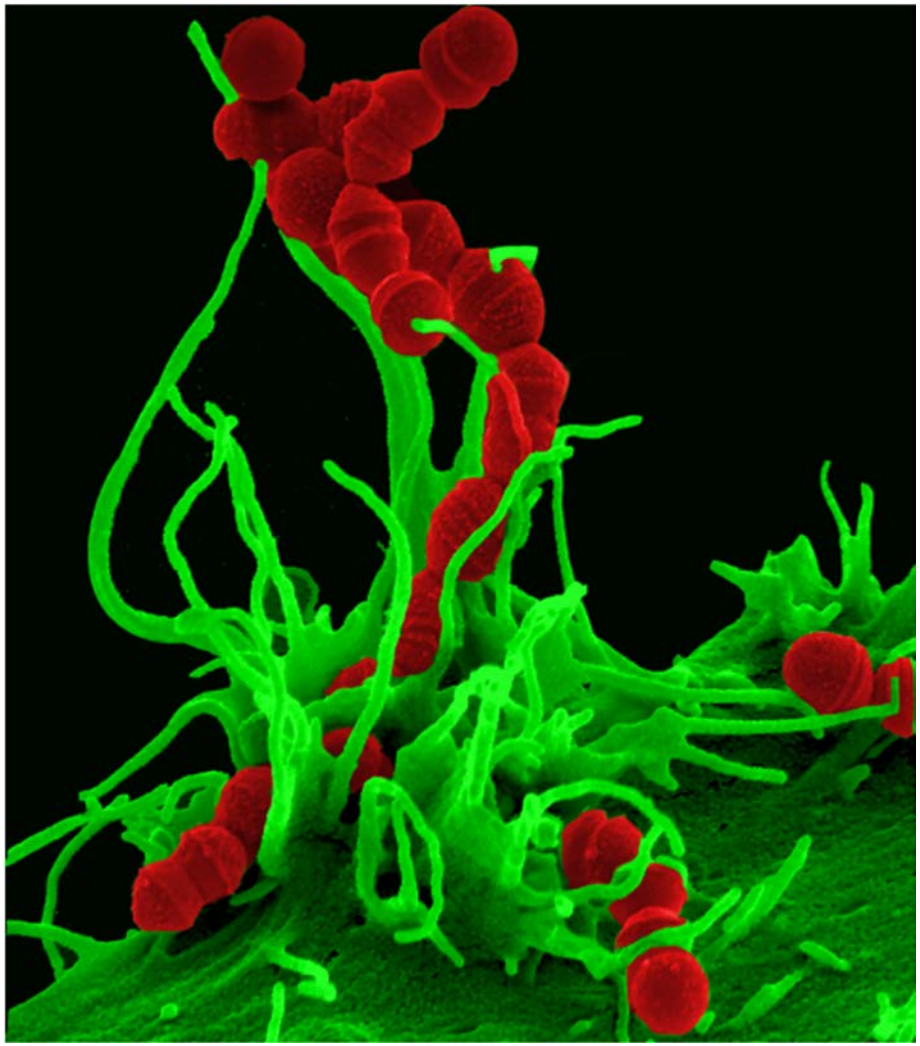
Lysogenic Cycle

The CRISPR-Cas adaptive immune system



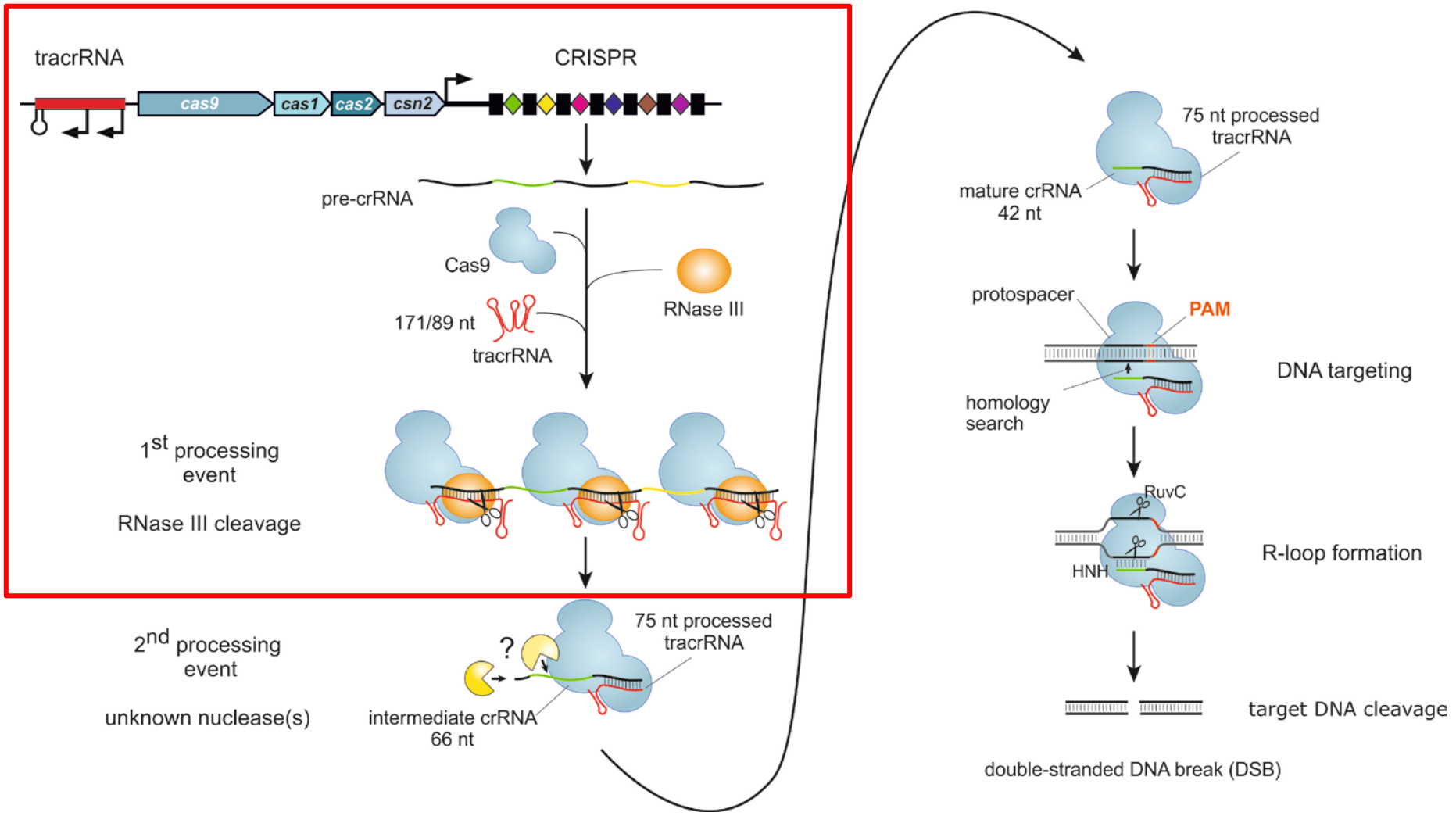
Bacterial pathogens

Streptococcus pyogenes = Group A streptococcus



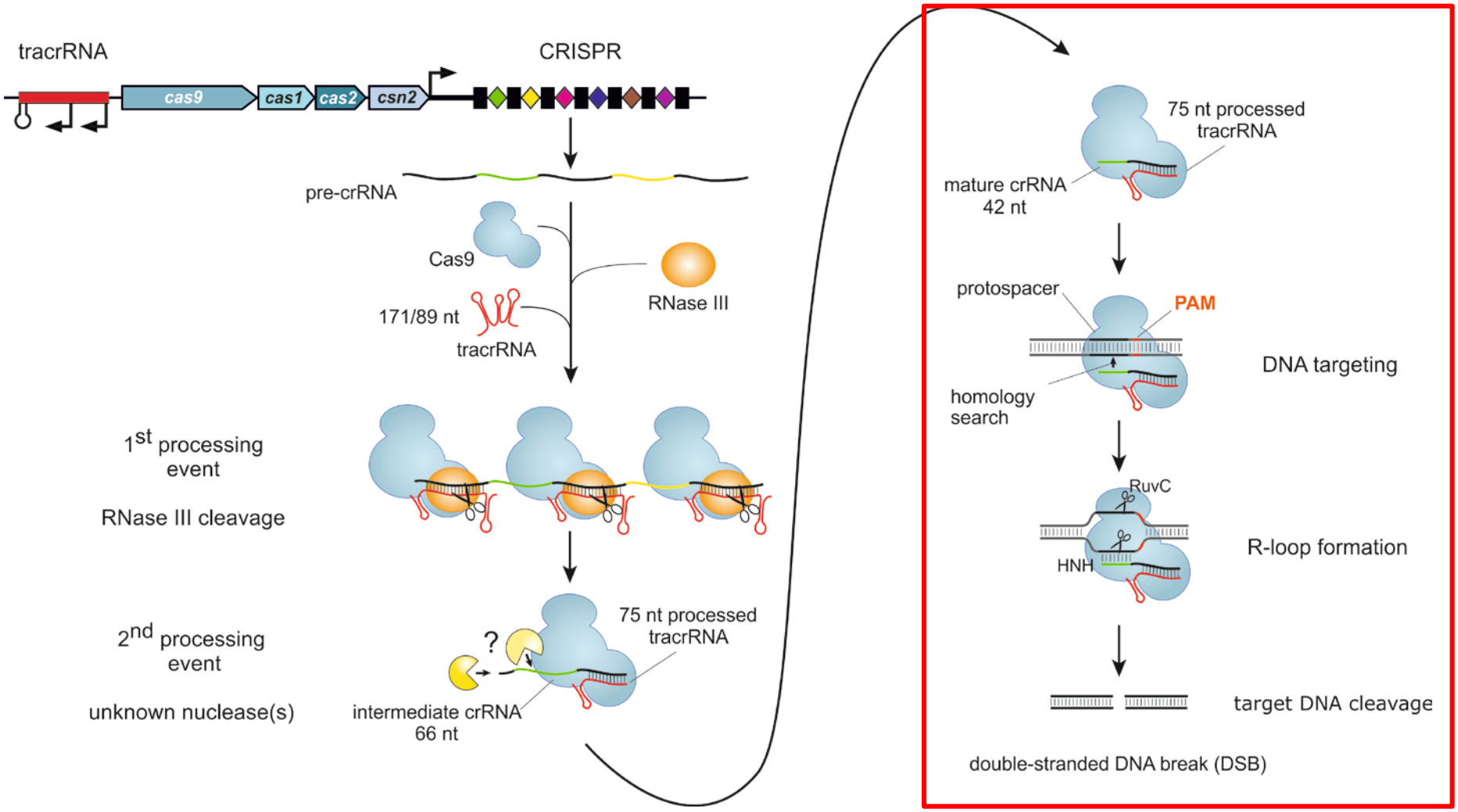
Manfred Rohde (HZI, Braunschweig)

Type II CRISPR-Cas: crRNA maturation



Deltcheva et al., 2011 (Nature)

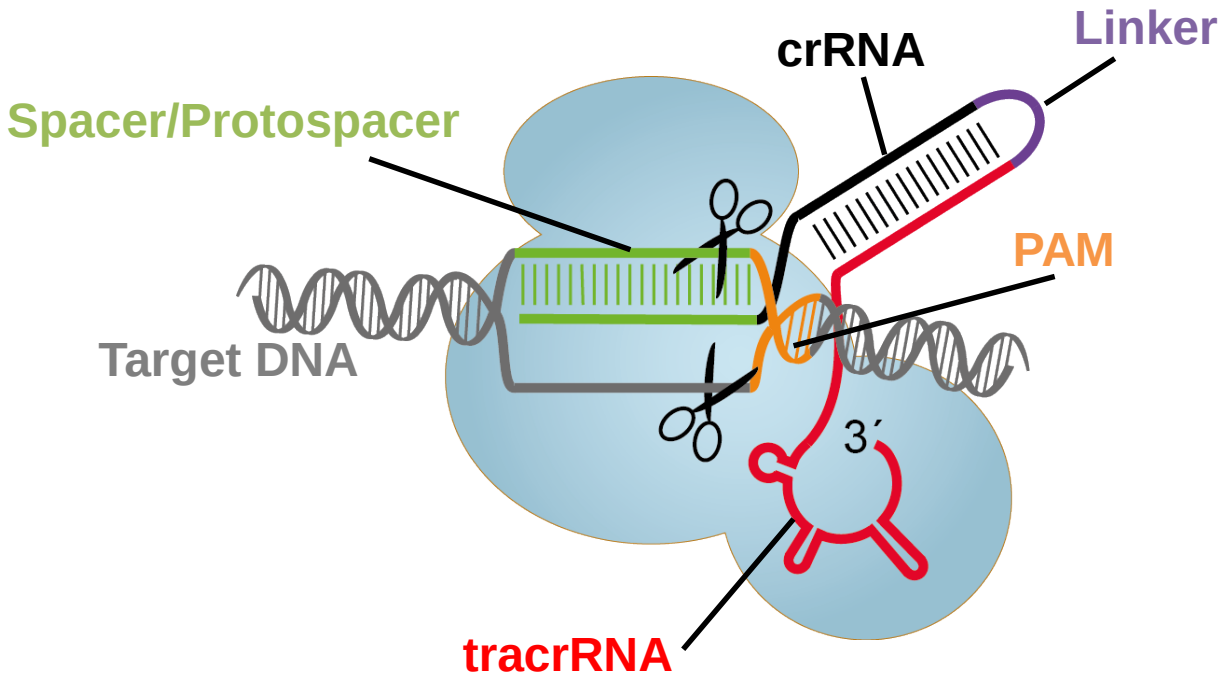
Type II CRISPR-Cas: DNA interference



Jinek, Chylinski et al., 2012 (Science)

CRISPR-Cas9 technology: a two-component system

The enzyme Cas9 is programmed with a guide RNA to target and cleave specifically DNA



Deltcheva et al., 2011 (Nature); Jinek, Chylinski et al., 2012 (Science)

Important milestones towards gene therapy

Fundamental genetics

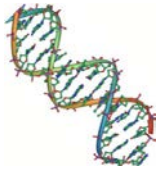
"Rules"

1859
Darwin "On the Origin of Species"

1865
Mendel – Laws of segregation of alleles

1871
Miescher – Isolation of DNA

Structure



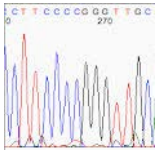
1953
Watson, Crick, Franklin & Wilkins
Structure of double-helix of DNA

1966
Genetic code is revealed

Genetic tools

"Basics"

Mid-1970's
Restriction enzymes
Recombinant DNA



1977
Sanger Sequencing

1983-88
Invention and development of PCR

Gene editing

1996
Zinc finger nucleases

2010
TALENs

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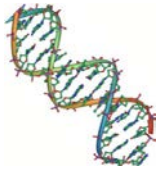

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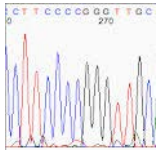

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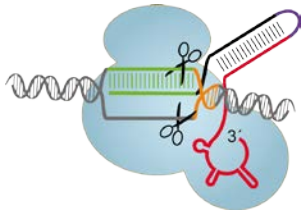
Sanger Sequencing

1983-88
Invention and development of PCR

Possible to introduce changes, but time-consuming and difficult

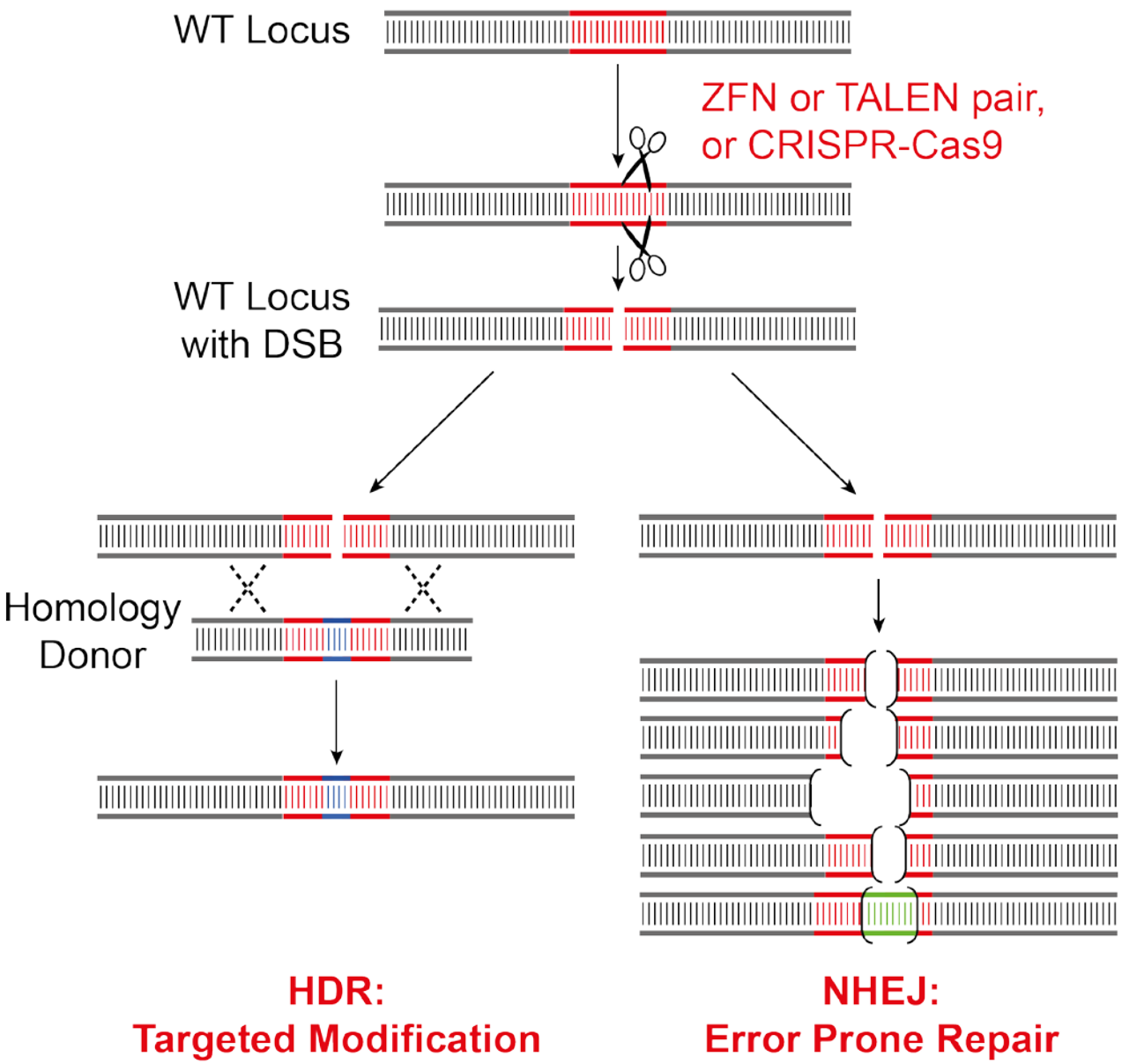
Gene editing



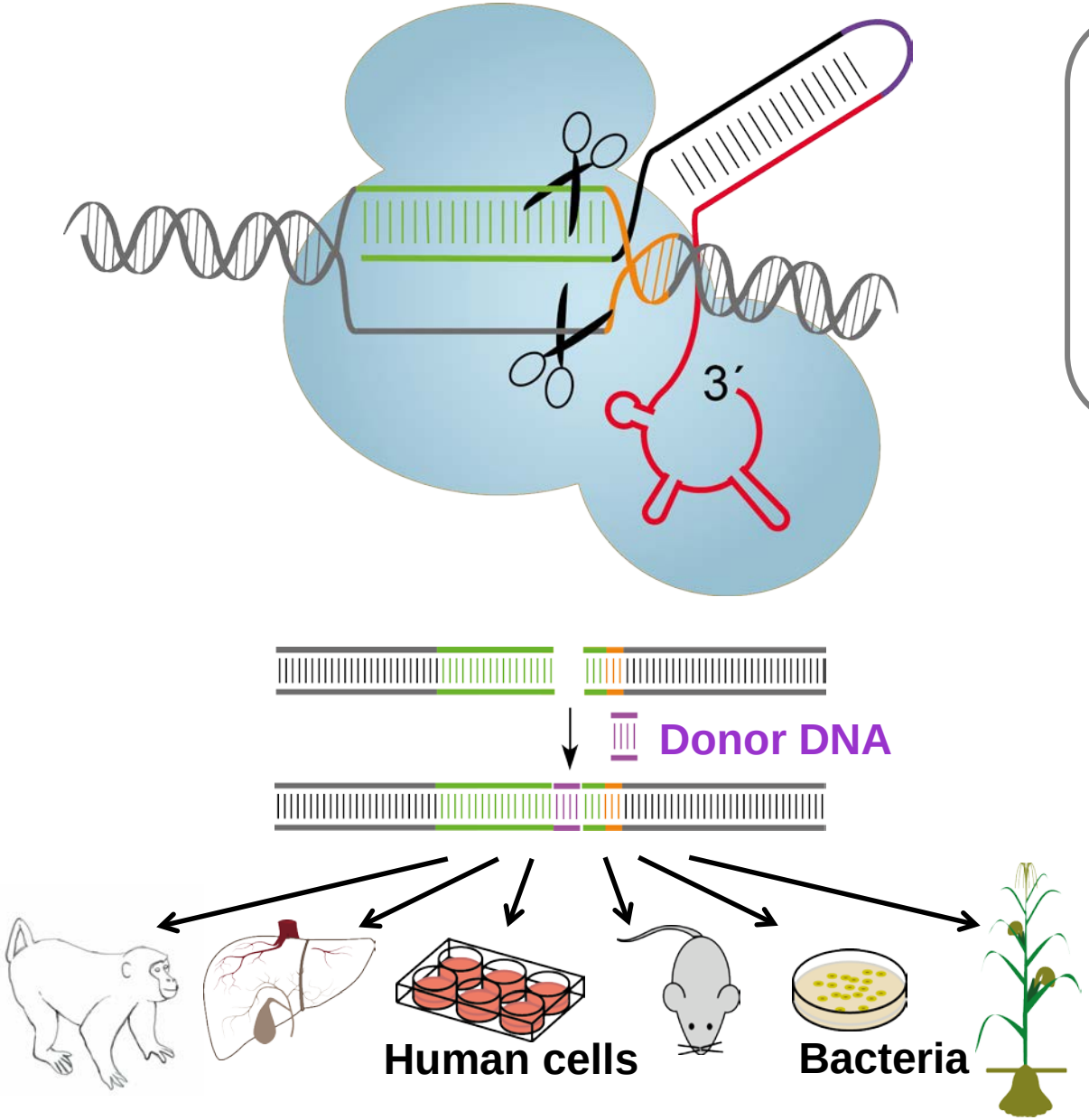
2012
CRISPR-Cas9

Fast and easy tool needed

Genome editing with sequence-specific nucleases



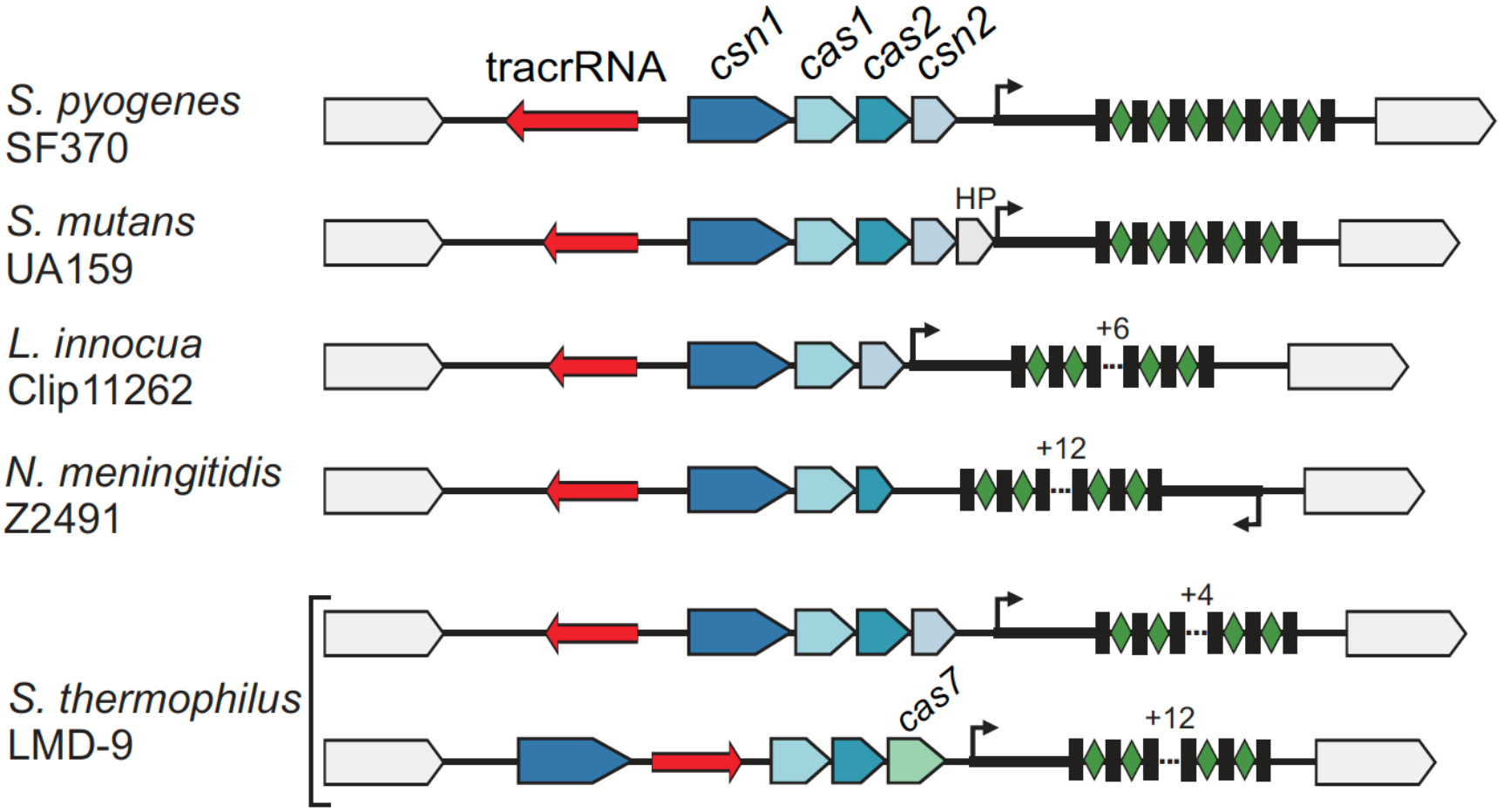
RNA-programmable CRISPR-Cas9



RNA-programmed Cas9 could offer considerable potential for genome editing in cells of the three kingdoms of life for biotechnological, biomedical and gene-therapeutic purposes.

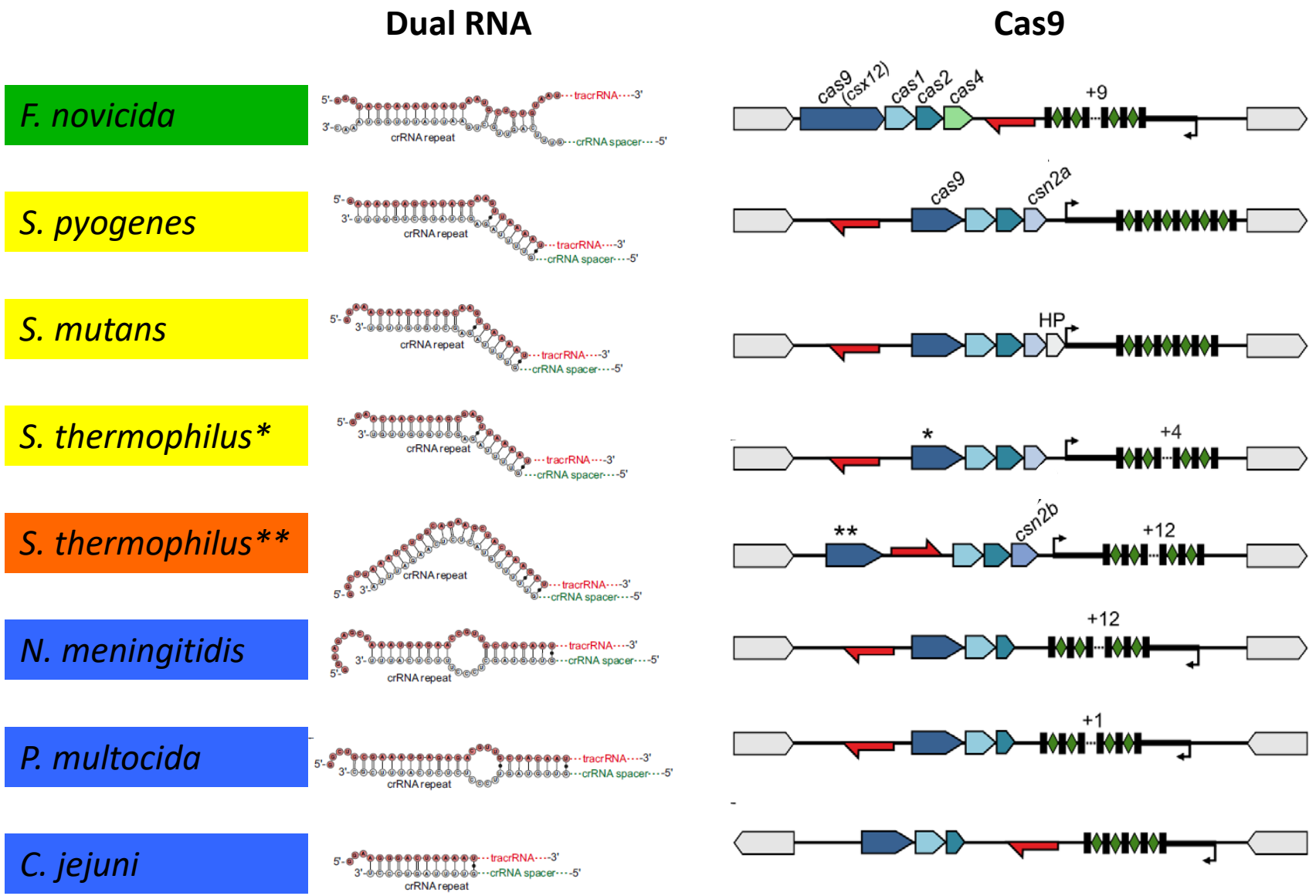
- **Advantages:**
  - Cheap and easy
  - Efficient
  - Versatile
  - Multiplexing
  - Various flavors
- **Improvements:**
  - Specificity (off-target)
  - Toxicity
  - Delivery

tracrRNA is associated to type II CRISPR-Cas



Deltcheva et al., 2011 (Nature)

Interchangeability among dual-RNA-Cas9 orthologs



Fonfara, Le Rhun et al., 2014 (Nucleic Acids Res.)

Applications of CRISPR-Cas9 in biology

Human medicine

Synthetic biology: pathway engineering

Agriculture: crops

Applications of CRISPR-Cas9 in human medicine

Human medicine

- Understanding of functions of genes and unravel new pathways
- Engineering of disease models
- Screening for new targets for therapeutics
- Validation of new therapeutic targets and medicine under development
- Bed to bench approach (e.g. genetic predisposition for diseases)
- Develop direct therapeutics (e.g. gene therapy)

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Washington, December 1, 2015

CRISPR-Cas9: Funding

Austria

CRISPR-Cas9: Funding

Sweden

CRISPR-Cas9: Funding

Sweden

MIMS
Molecular Infection Medicine Sweden
Nordic EMBL Partnership for Molecular Medicine

UMEÅ UNIVERSITET

UCMR
Umeå Centre for Microbial Research

Vetenskapsrådet

EMBL

Germany

MAX-PLANCK-GESELLSCHAFT

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für Bildung
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GENOMICS


CRISPR
THERAPEUTICS