FOKUS methods lecture

CRISPR-Cas systems

Prof. Dr. Chase Beisel

18 January, 2022

HIRI: the first institute for RNA-based infection research

Lars Barquist

Integrative Informatics for Infection Biology

Chase Beisel

RNA Synthetic Biology

Neva Caliskan

Recoding Mechanisms in Infections

Mathias Munschauer

Viral-host interactions

Emmanuel Saliba

Single-Cell Analysis

Redmond Smyth

Genome Architecture and Evolution of RNA Viruses

Jörg Vogel

RNA Biology of Bacterial Infections

Alexander Westermann

Host-Pathogen-Microbiota Interactions

In this lecture…

- Discovery of CRISPR
- Types and mechanisms
- Cas9 and the sgRNA

Learning objectives

- Define CRISPR, Cas, and other basic terms
- Identify the three steps of adaptive immunity by CRISPR-Cas systems
- Explain how a CRISPR nuclease selects its target
- Explain why CRISPR-Cas systems were readily co-opted as genome-editing technologies

What have you heard about CRISPR?

(Comment using chat function)

CRISPR for genome surgery

Home / News & Opinion

US Companies Launch CRISPR Clinical Trial

The Germany-based study will test an ex vivo genome-editing therapy for the inherited blood disorder β-thalassemia.

Sep 3, 2018
CATHERINE OFFORD

TheScientist
EXPLORING LIFE, INSPIRING INNOVATION

NEWS & TECHNOLOGY 30 May 2017, updated 7 June 2017

Boom in human gene editing as 20 CRISPR trials gear up

NewScientist

Scientists Precisely Edit DNA In Human Embryos To Fix A Disease Gene

August 2, 2017 · 1:09 PM ET
Heard on All Things Considered

npr

AUG 4, 2017 @ 11:37 AM 8,821 12 Stocks to Buy I

Gene Editing Breakthrough: How Far Are We From Fixing And Designing Babies?

Forbes

European court ruling raises hurdles for CRISPR crops

By Erik Stokstad | Jul. 25, 2018 , 4:40 PM

Science

How will we keep controversial gene drive technology in check?

By Kelly Servick | Jul. 19, 2017 , 4:00 PM

Science

The Nobel prize for CRISPR

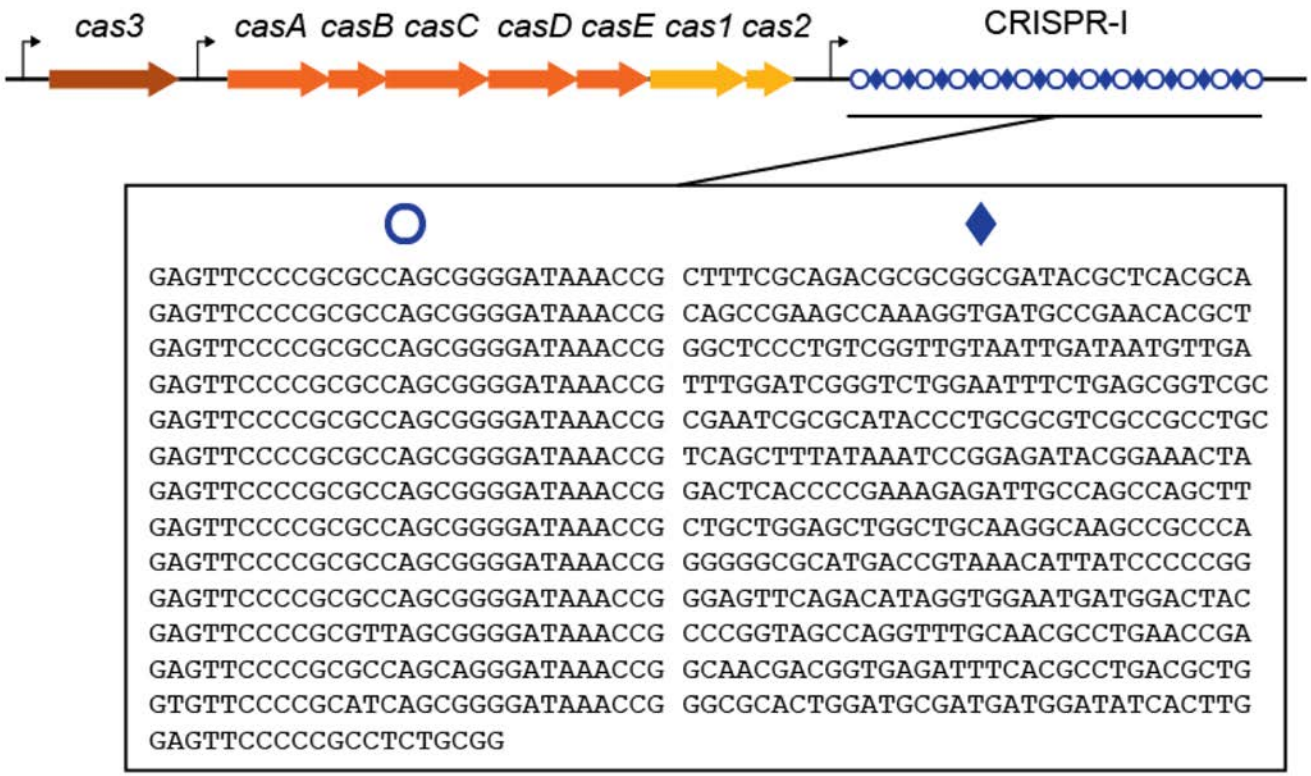
2020 Nobel prize in chemistry

Emmanuelle Charpentier
(Max Planck)

Jennifer Doudna
(UC Berkeley)

The mystery of CRISPR and Cas

CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats
Cas – CRISPR associated



E. coli MG1655

In ~40% of bacteria, ~90% of archaea

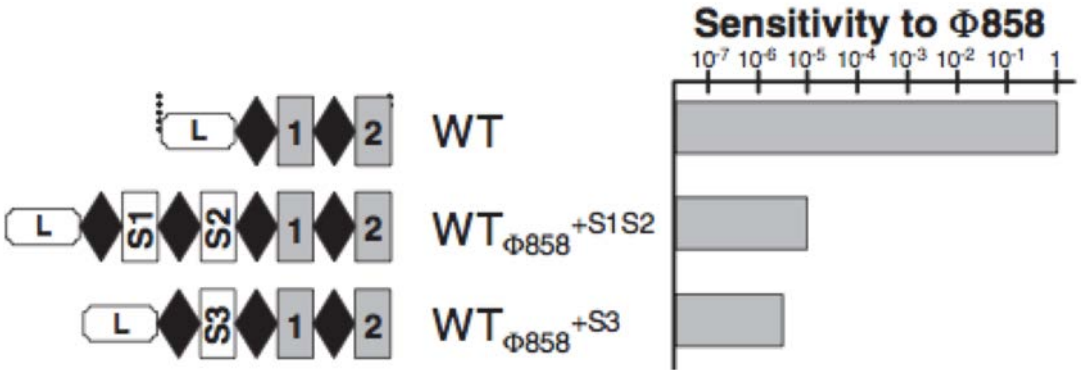
Spacers match invader sequences

Other ID*	Species	No. of phage-matching spacers§					
		Sfi11 (AF158600)	Sfi19 (AF115102)	Sfi21 (AF115103)	DT1 (AF085222)	O1205 (U88974·1)	7201 (AF145054)
CNRZ1066	*S. thermophilus*	7	6	7	4	9	0
LMG18311	*S. thermophilus*	4	4	3	1	4	5
CNRZ302	*S. thermophilus*	2	0	0	1	2	1
CNRZ388	*S. thermophilus*	2	6	5	5	2	6
CNRZ389	*S. thermophilus*	3	2	1	2	2	5
CNRZ1100	*S. thermophilus*	2	4	2	2	2	2
CNRZ1202	*S. thermophilus*	2	5	8	4	3	2
CNRZ703	*S. thermophilus*	1	2	5	1	0	1
CNRZ1575	*S. thermophilus*	2	2	1	1	1	0
CNRZ385	*S. thermophilus*	0	3	3	2	1	3
JIM8229	*S. vestibularis*	0	0	0	0	0	0
JIM8230	*S. vestibularis*	1	1	1	0	1	0
JIM1567	*S. thermophilus*	3	4	1	1	3	2
JIM1560	*S. thermophilus*	1	1	2	0	2	0
JIM1575	*S. thermophilus*	1	1	2	0	2	0
JIM1584	*S. thermophilus*	1	1	1	1	0	0
JIM1588	*S. thermophilus*	1	1	2	0	2	0
JIM70	*S. thermophilus*	2	1	1	1	1	2
JIM71	*S. thermophilus*	1	1	1	1	0	0
JIM72	*S. thermophilus*	2	2	2	2	1	3
JIM76	*S. thermophilus*	10	8	9	6	12	0

Bolotin et al. *Microbiol* (2005)

CRISPR and Cas as adaptive defense systems

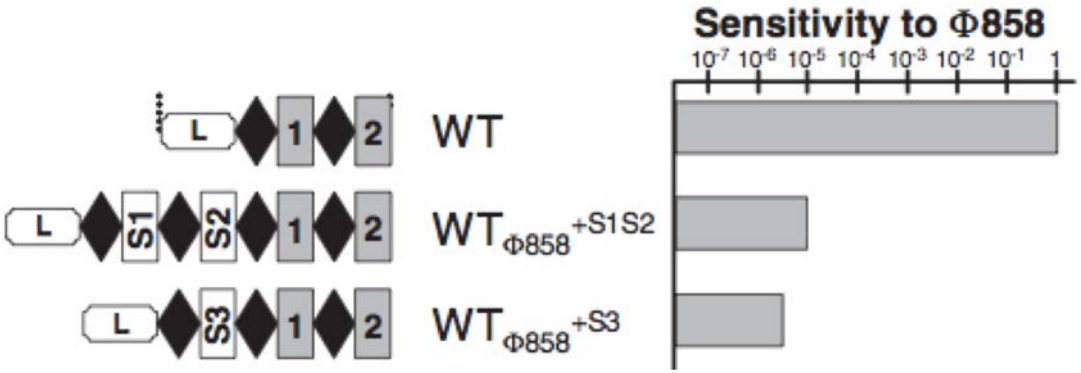
Blocks infection of targeted bacteriophages…



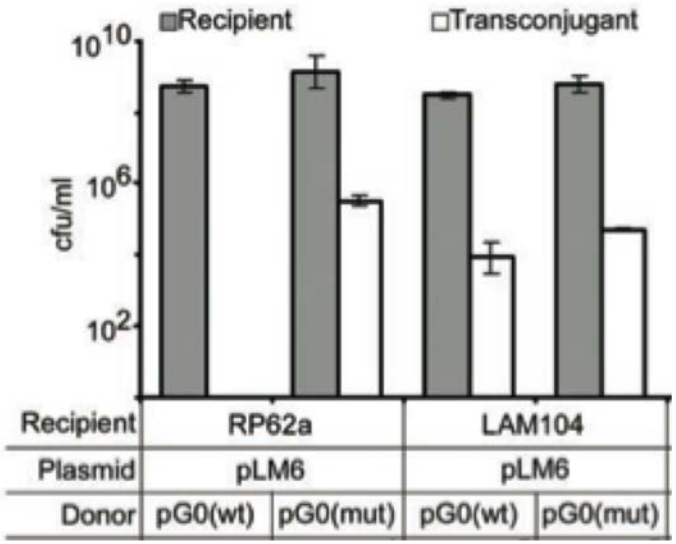
Barrangou et al. *Science* (2007)

CRISPR and Cas as adaptive defense systems

Blocks infection of targeted bacteriophages…



…and conjugated plasmids

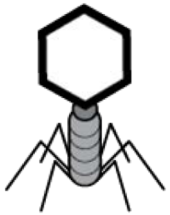


Barrangou et al. *Science* (2007)
Marraffini & Sontheimer. *Science* (2008)

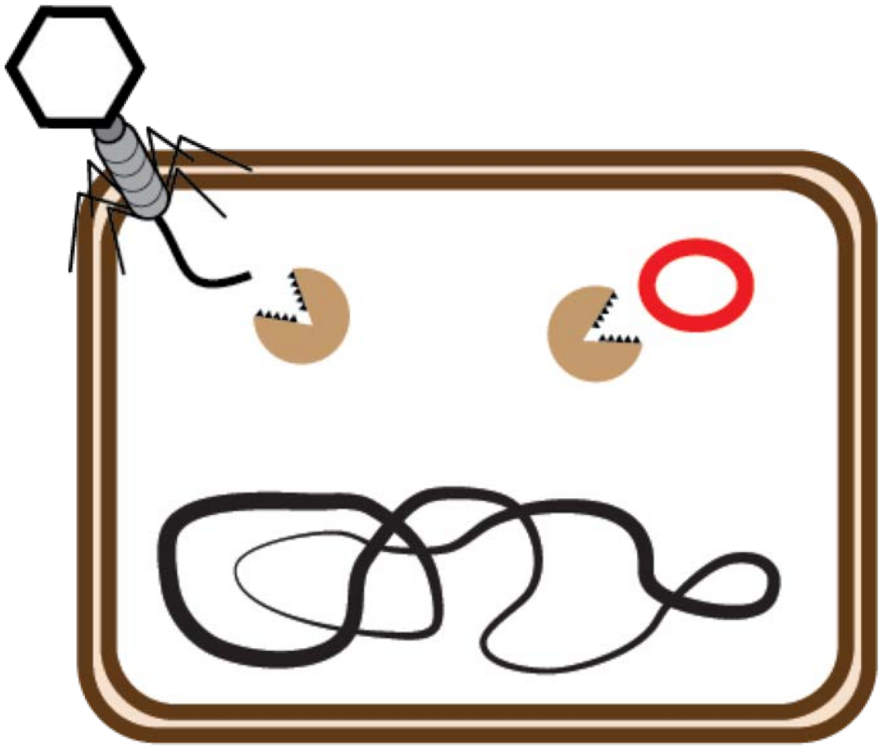
CRISPR and Cas as adaptive defense systems

Fends off foreign invaders

Plasmids

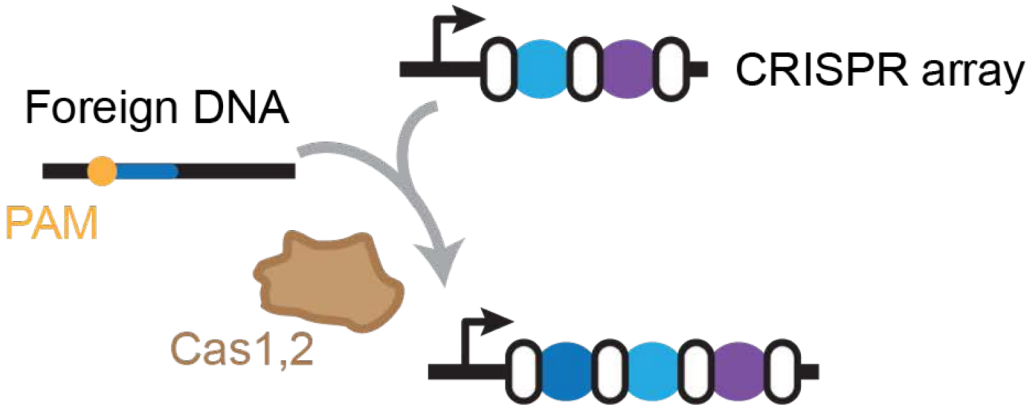


Bacteriophages



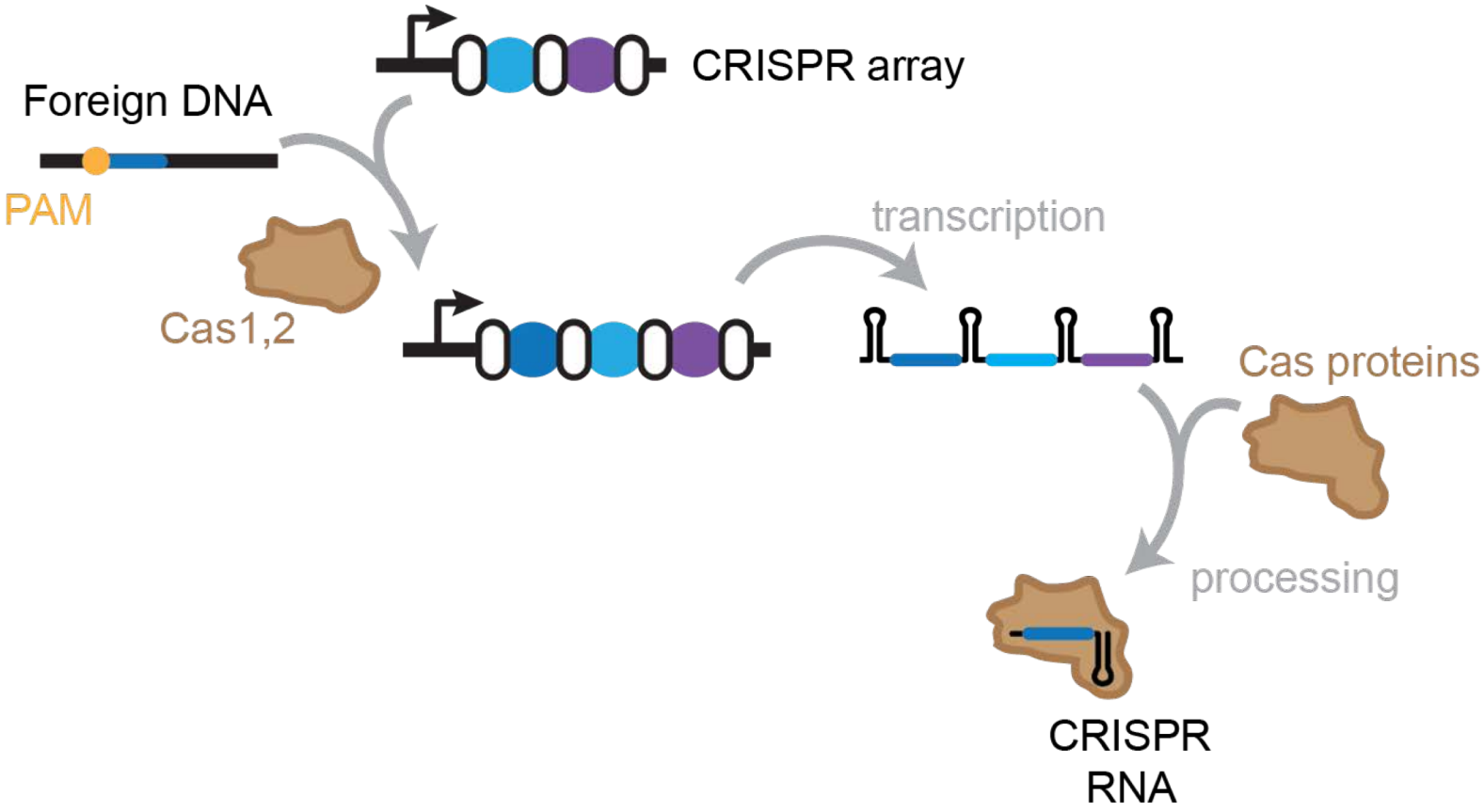
3 steps: Acquisition, Expression, Interference

CRISPR and Cas as adaptive defense systems



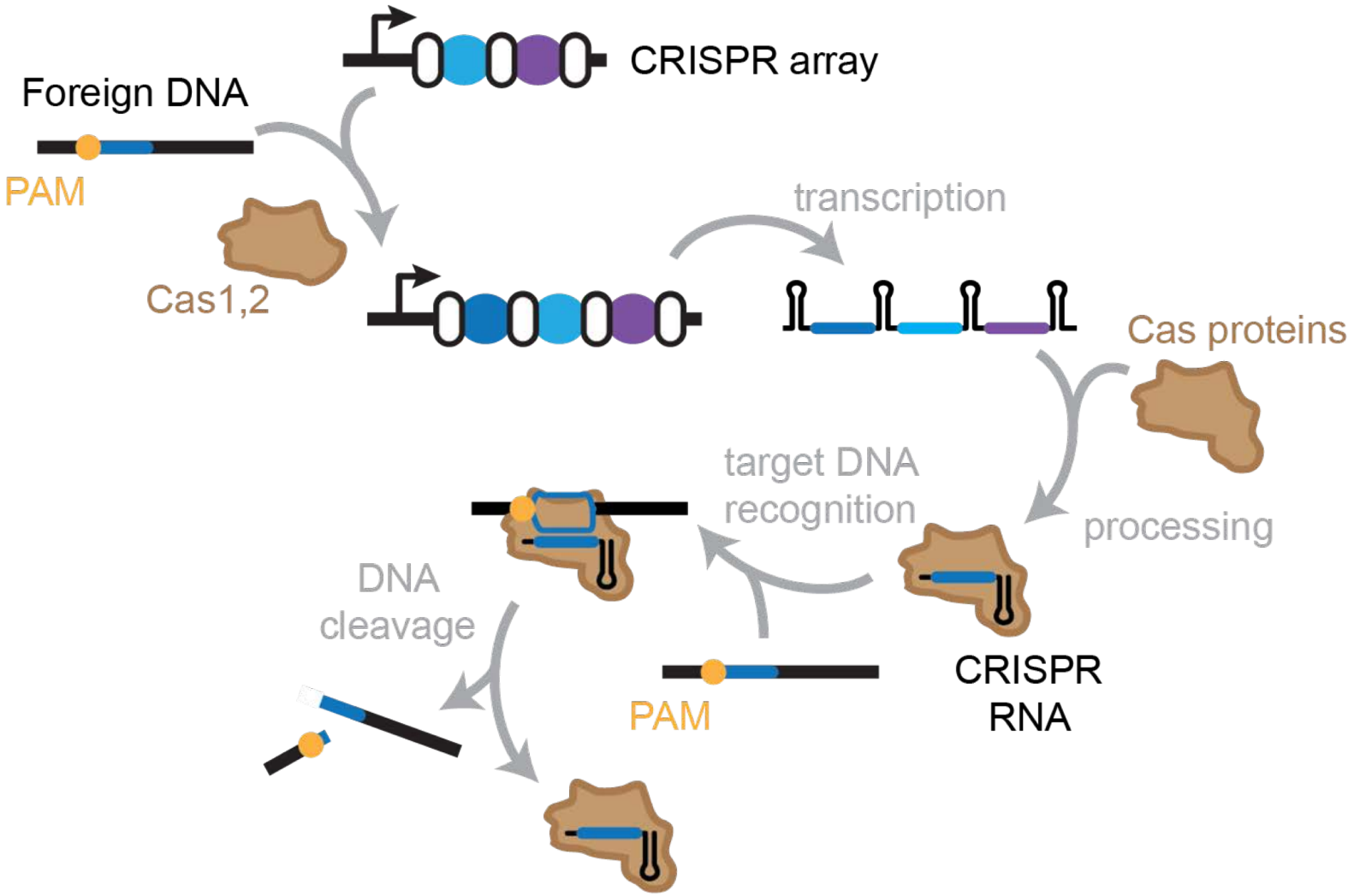
Step 1: Acquisition

CRISPR and Cas as adaptive defense systems



Step 2: Expression

CRISPR and Cas as adaptive defense systems



Step 3: Interference

CRISPR vocabulary

CRISPR array: set of alternating identical repeats and distinct spacers

CRISPR RNA (crRNA): final, processed form of a CRISPR array

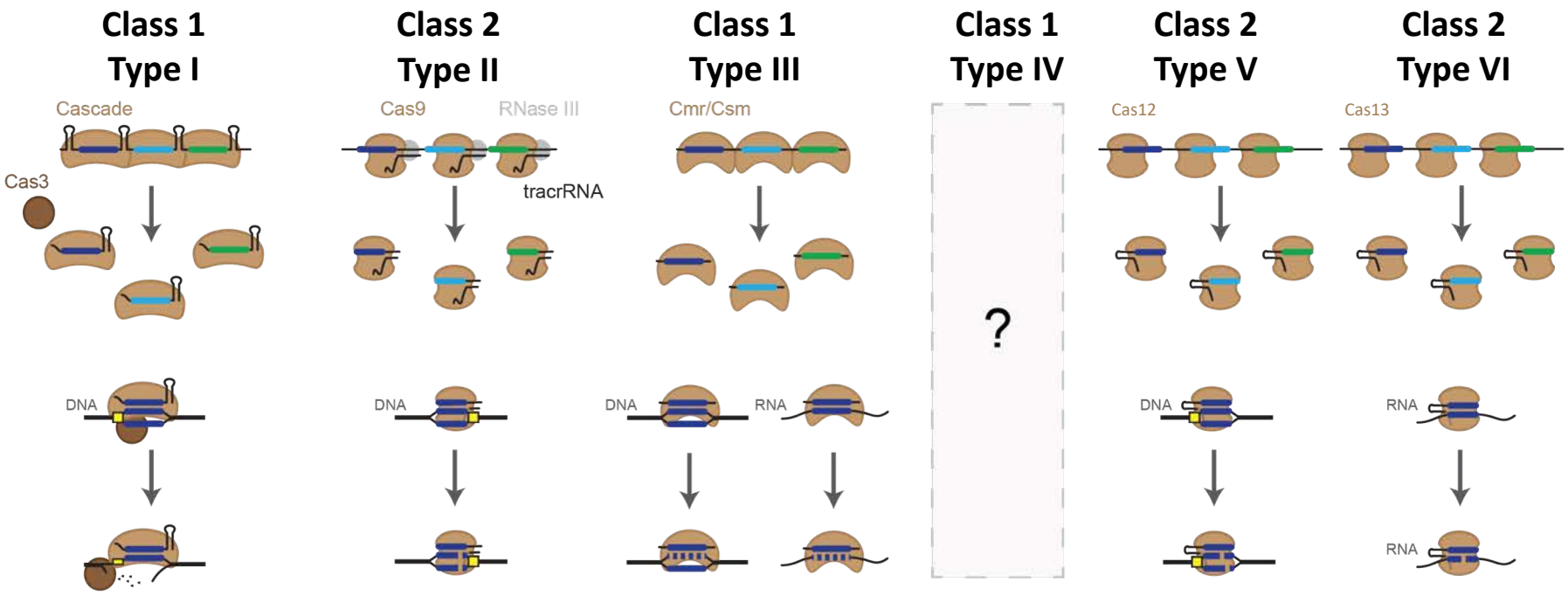
Guide sequence: sequence used for DNA targeting. Sometimes interchanged with spacer.

Guide RNA: engineered or natural CRISPR RNA

Protospacer: target sequence that is complementary to spacer

PAM: protospacer-adjacent motif, required for targeting by many CRISPR effector proteins

Classes and types of CRISPR-Cas systems



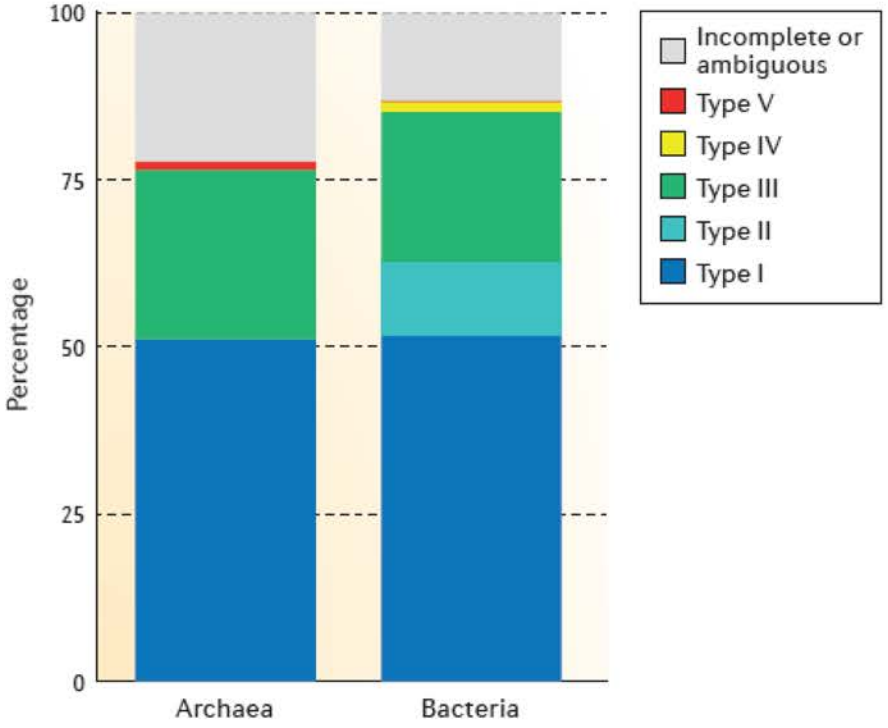
See Makarova et al. *Nat Rev Microbiol* (2020)

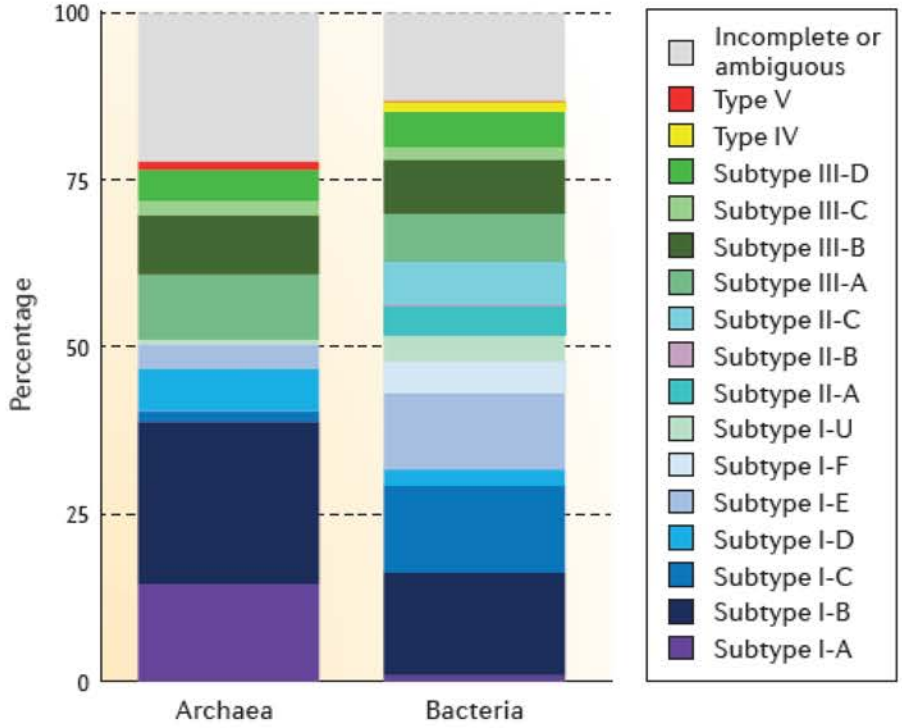
Class (2) – encompasses all systems with a multi-protein effector complex (**Class 1**) or single effector protein (**Class 2**)

Type (6) – defined by the effector complex or single-effector protein

Subtype (>30) – defined by set and configuration of accessory proteins

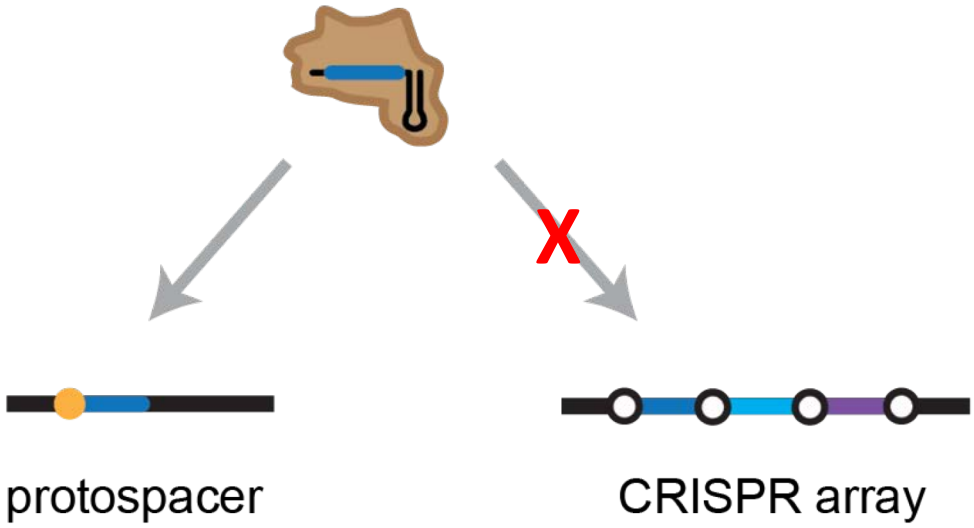
Prevalence of system types, sub-types varies widely





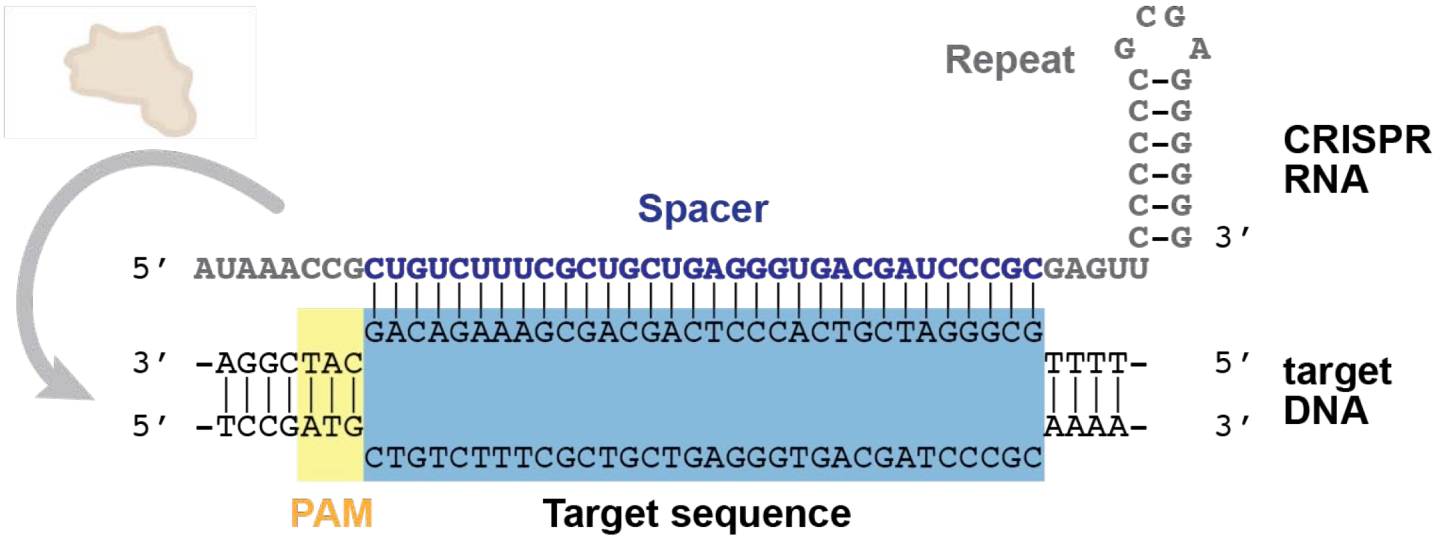
Makarova et al. *Nat Rev Microbiol* (2015)

Differentiating self from non-self



How does CRISPR recognize the target, but not its own array?

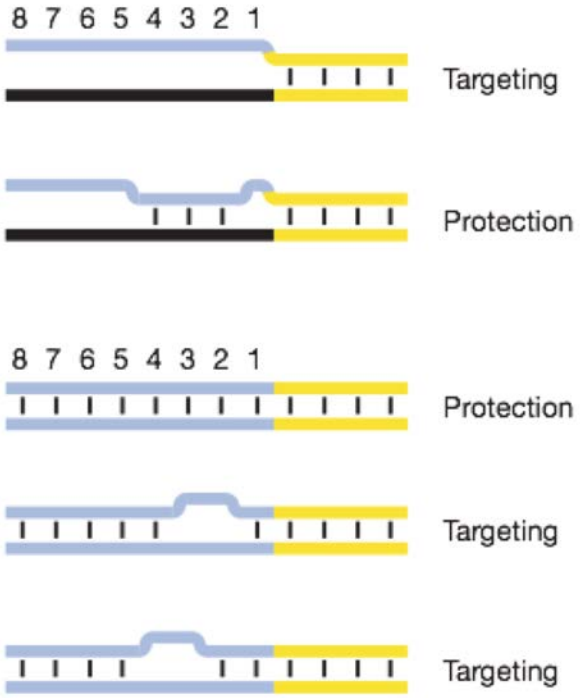
DNA target recognition for Type I, II, IV, V systems

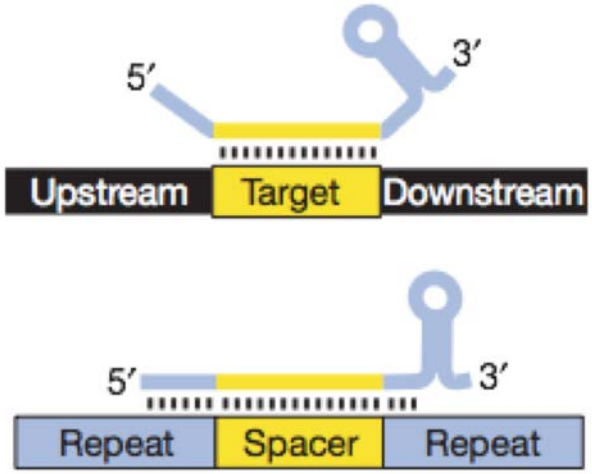


- PAM - protospacer-adjacent motif
- Sequence recognized by Cas protein to initiate DNA interrogation
- PAM sequence, size, and location depends on nuclease

S. pyogenes Cas9: **NGG PAM** on **3' end** of matching target
F. novicida Cas12a: **TTTV PAM** on **5' end** of matching target

RNA target recognition for Type III, VI systems

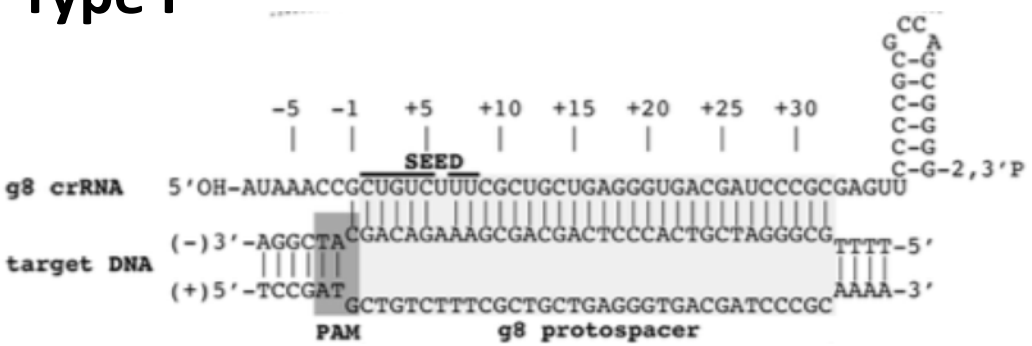


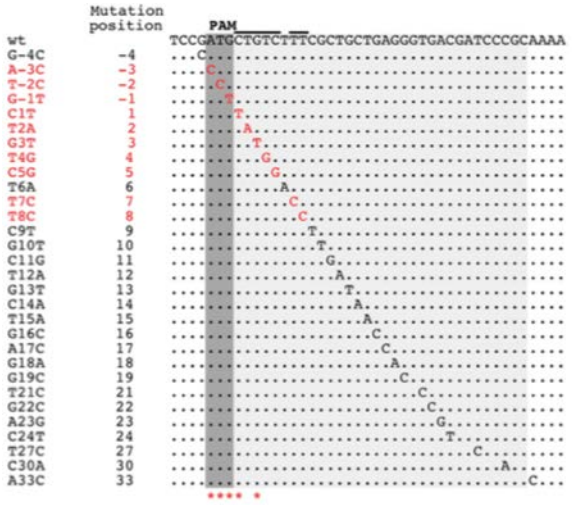


Marraffini & Sontheimer. *Nature* (2010)

A spacer “seed” sequence is sensitive to mismatches

Type I





Semenova et al. *PNAS* (2011)

- Helps helps ensure sequence-specificity of targeting
- Some mismatches can be accommodated, particularly outside of the seed
- Exact length, location of seed depends on the nuclease

Why might it be advantageous for CRISPR nucleases to accept some mismatches?

How to identify CRISPR-Cas systems

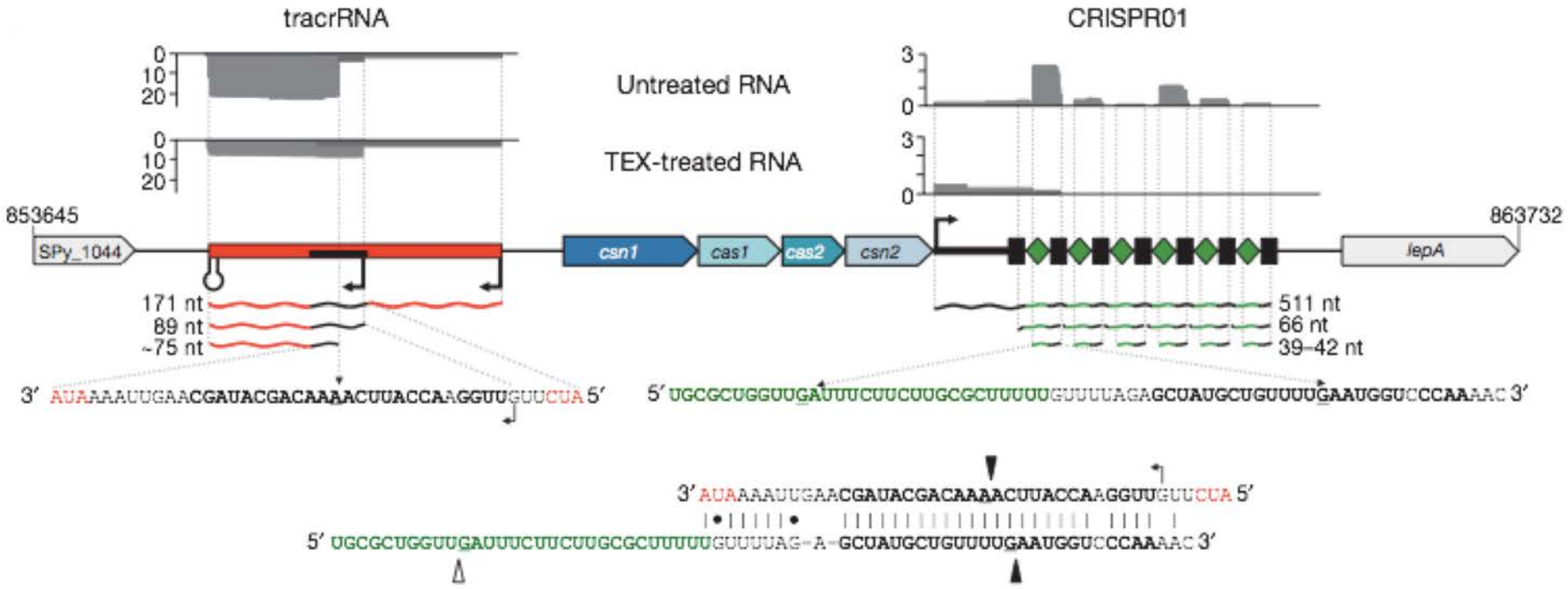
The CRISPR database for identifying CRISPR loci and *cas* genes in sequenced prokaryotic genomes

http://crispr.u-psud.fr

Harnessing CRISPR

- Original studies of CRISPR focused on Type I CRISPR-Cas systems
- But Type I systems require 4 – 7 proteins in defined stoichiometries
- Type II systems require only one protein (Cas9)
- How do you generate the CRISPR RNA for Cas9?

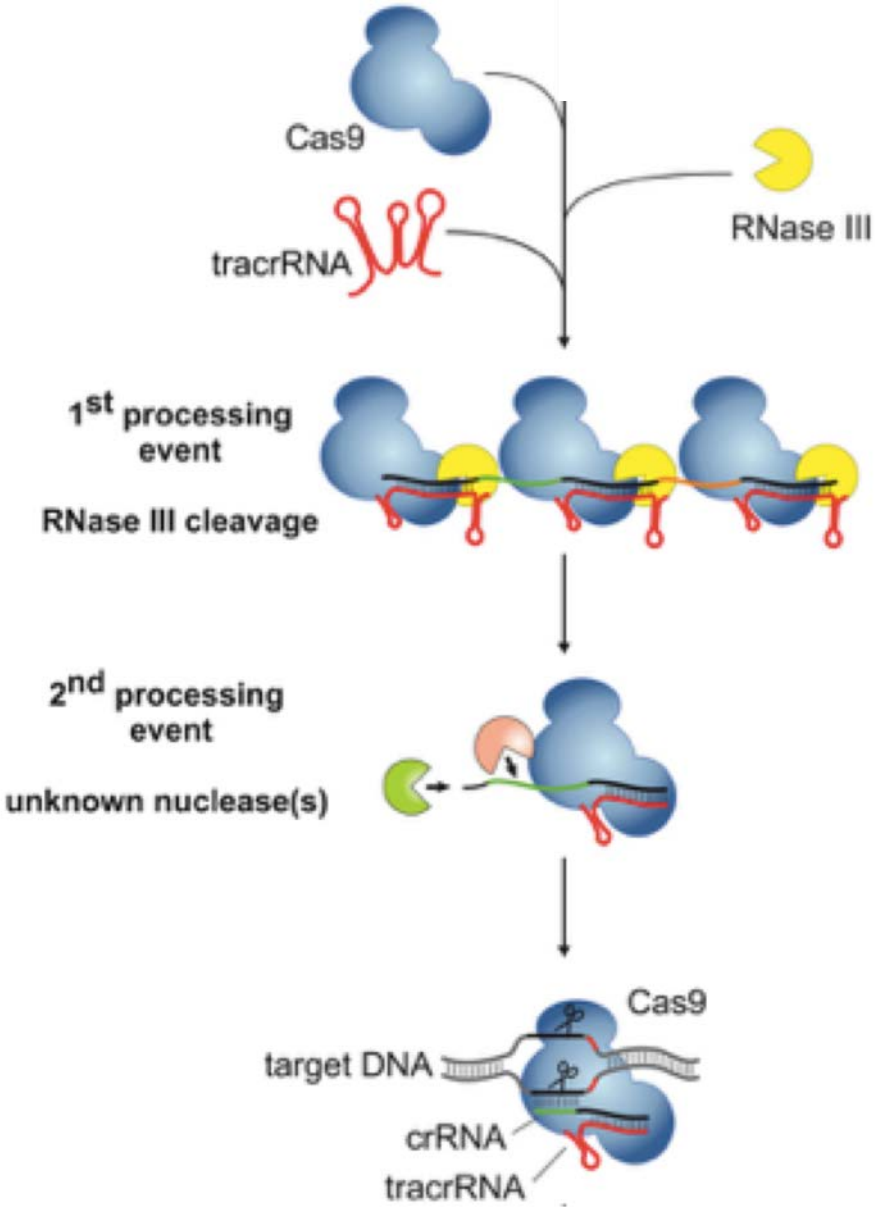
Discovery of the tracrRNA in *S. pyogenes*



Deltcheva et al. *Nature* (2011)

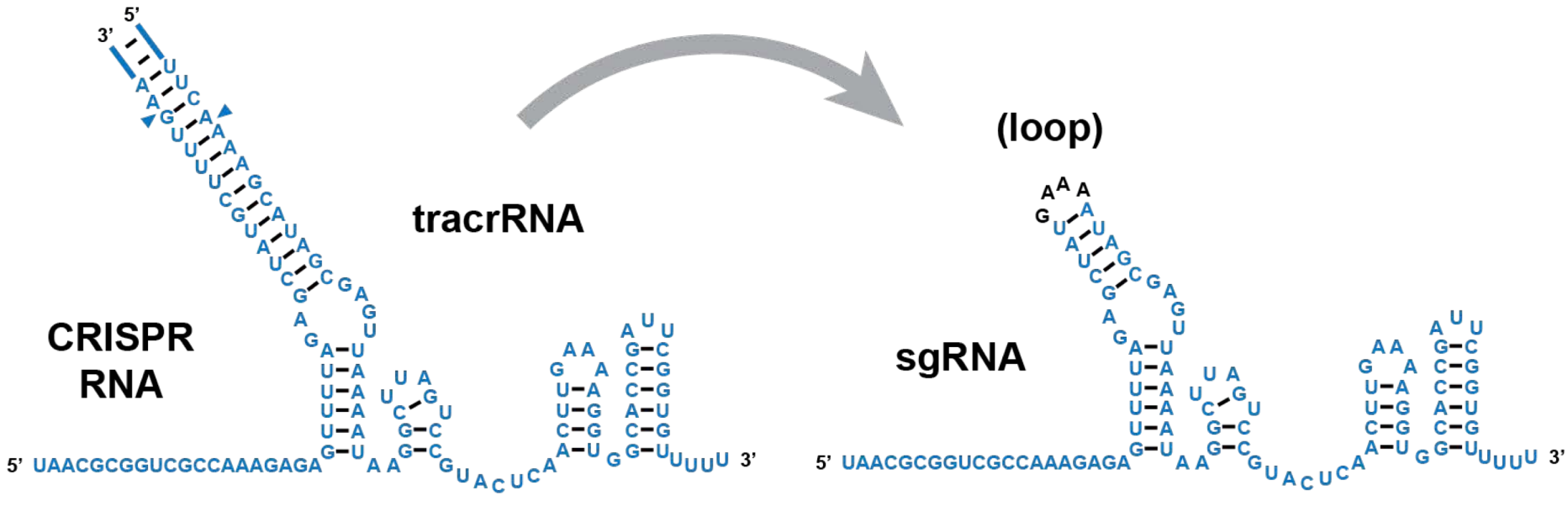
Type II Cas9 as single-effector protein with dual RNA guide

- Hybrid of crRNA and tracrRNA processed by RNase III
- 5’ end of crRNA processed by unknown nucleases
- Allows for processing of array into individual crRNAs



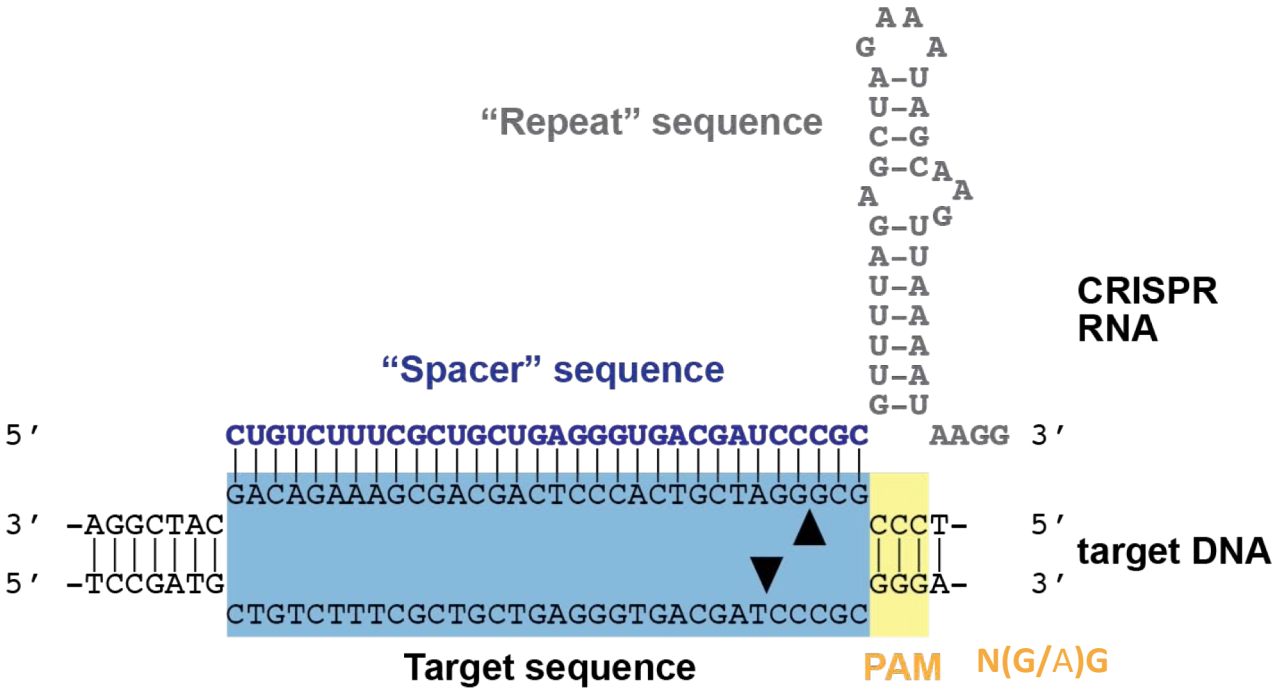
Chylinski et al. *Nucleic Acids Res* (2014)

Creating single-guide RNAs (sgRNAs)

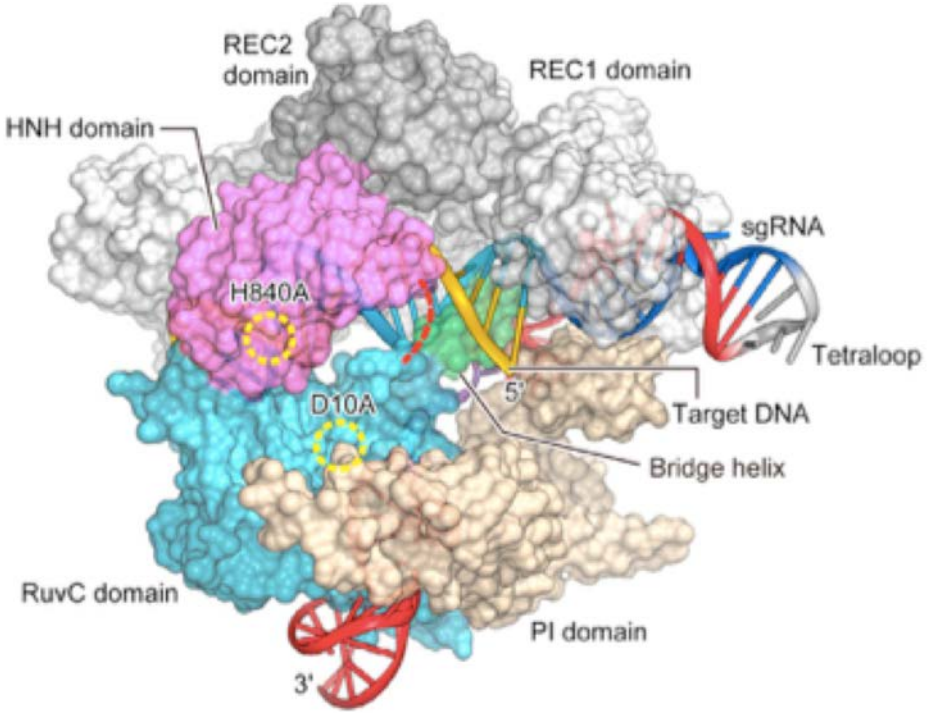


S. pyogenes Cas9 as the standard

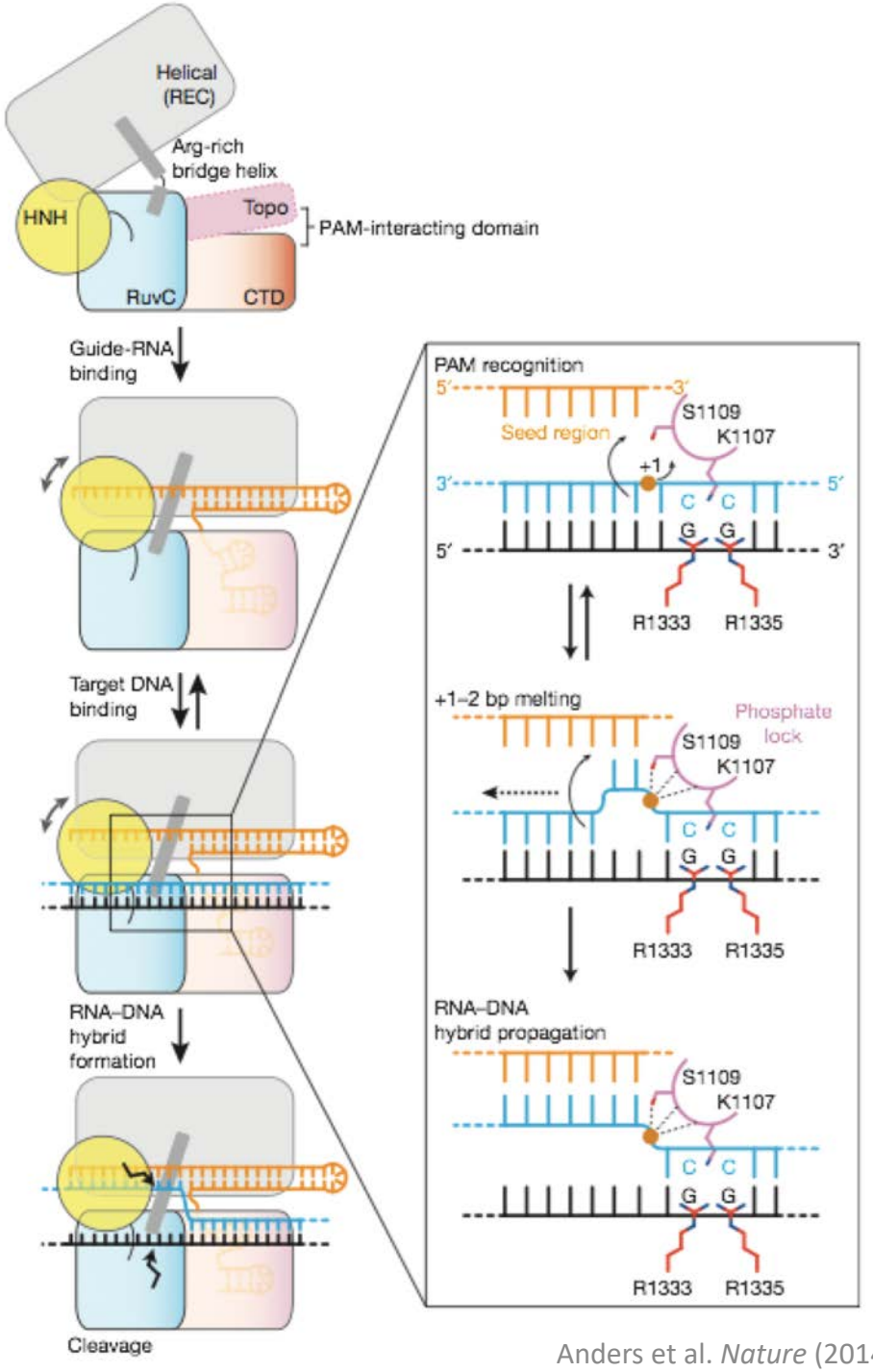
- Historical (see tracrRNA paper)
- Convenient (NGG consensus PAM)
- Numerous constructs (see Addgene)



Cas9 as a sophisticated molecular machine

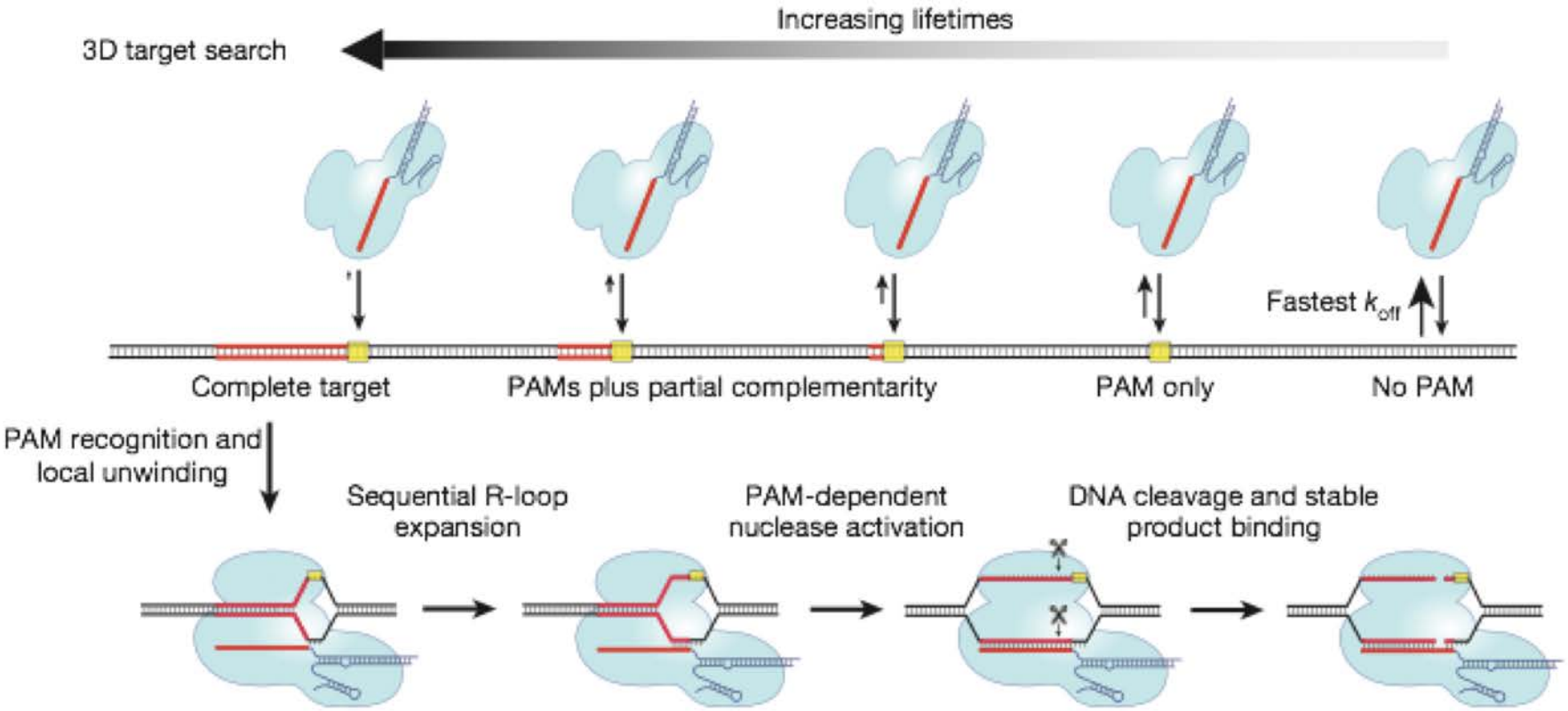


Nishimasu et al. *Cell* (2014)



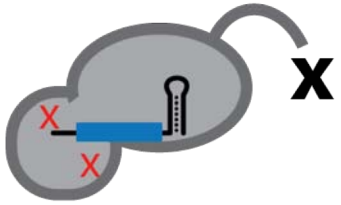
Anders et al. *Nature* (2014)

Cas9 as a single-turnover enzyme

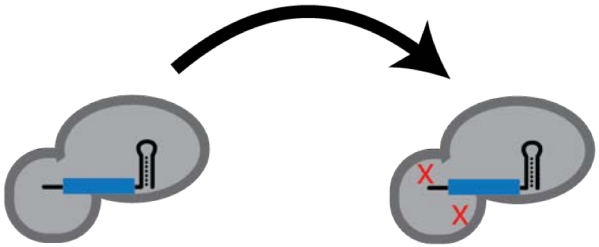


Sternberg et al. *Nature* (2014)

Programmable binding, effector recruiting with dCas9



Introduce disruptive mutations in HNH, RuvC domains



WT Cas9

Cleaves DNA

dCas9

Binds DNA

X =

- Nothing
- KRAB
- VP64
- GFP
- FokI
- APOBEC

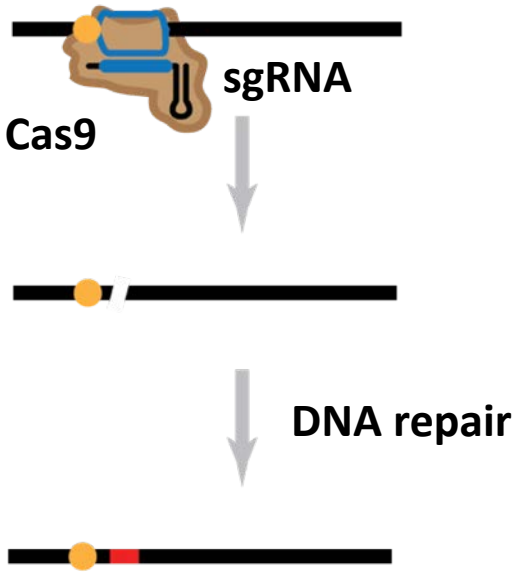
Gene regulation

Imaging

Editing

Cas9 synonymous with CRISPR technologies

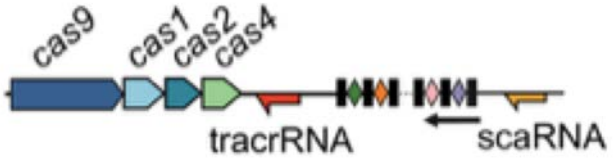
- Strain typing
- Phage resistance
- Plasmid clearance
- Genome editing
- Gene drives
- Gene regulation
- Antimicrobials
- Imaging
- In vitro diagnostics
- Biological recording



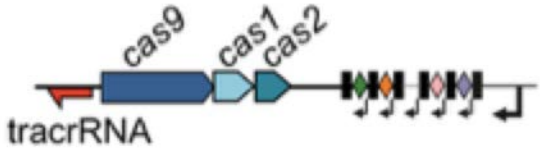
Many other Cas9 nucleases available

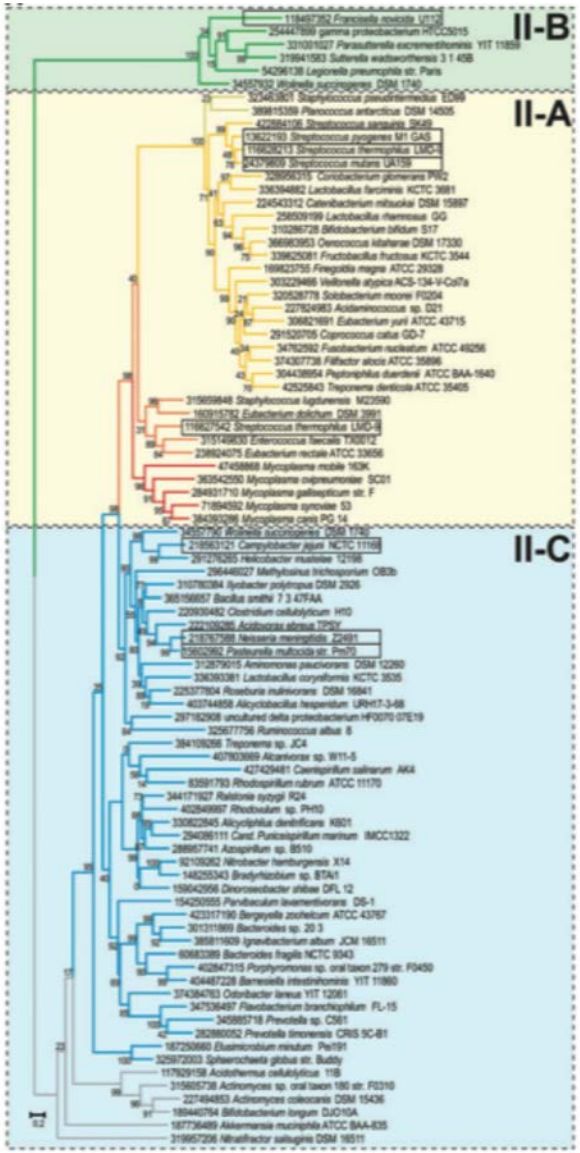
type II-A
S. pyogenes

type II-B
F. novicida



type II-C
N. meningitidis



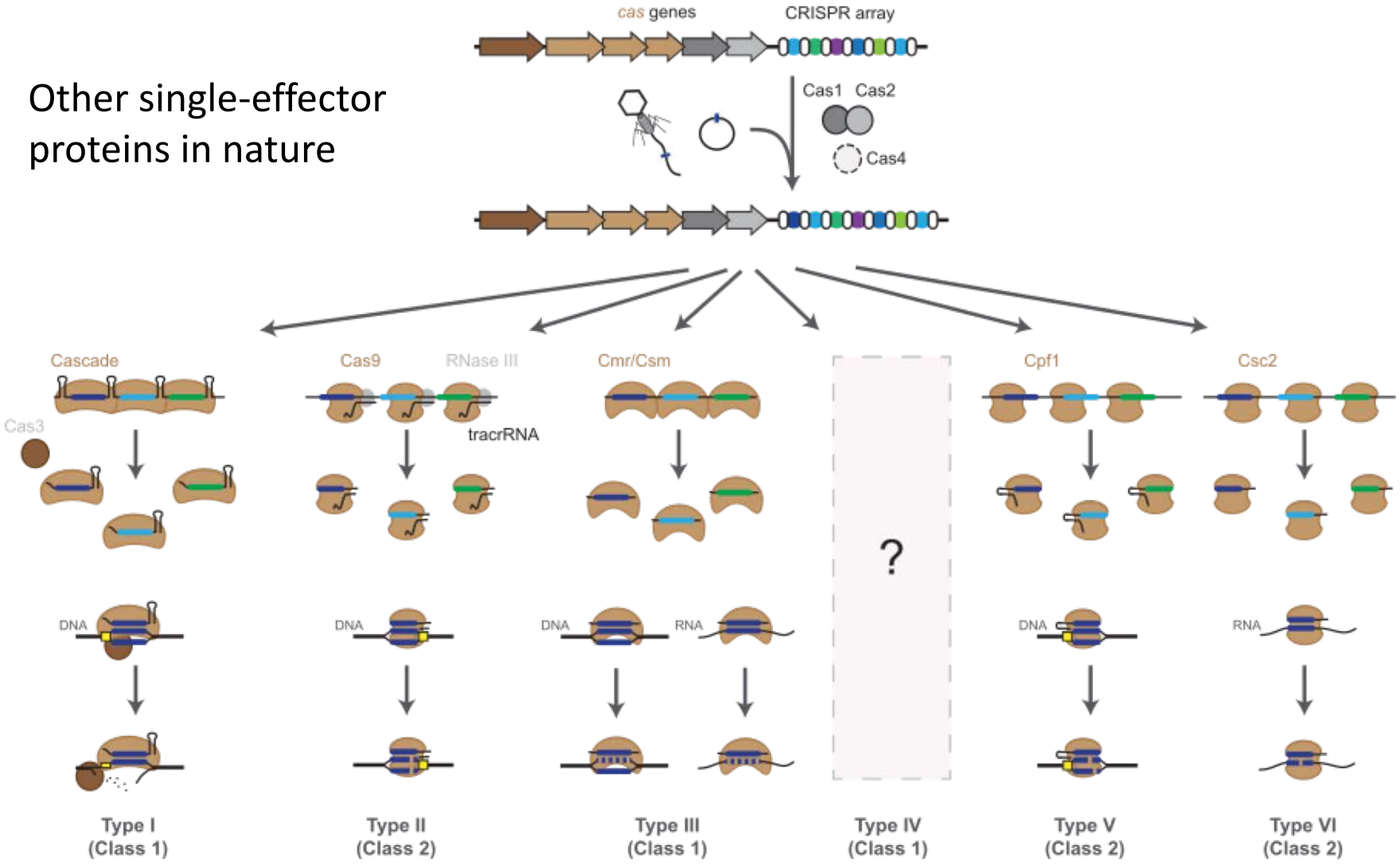


- Different sizes
- Different PAMs
- Different functions
- Different immunogenicities

Chylinski et al. *Nucleic Acids Res* (2014)

Is Cas9 the best we can hope for?

Other single-effector proteins in nature



More recently explored aspects of CRISPR

- CRISPR evolution
- Natural diversity, functions of CRISPR-Cas system
- Mechanism, application of spacer acquisition
- CRISPR transposons
- Engineering Cas proteins

In this lecture…

- Discovery of CRISPR-Cas systems
- Types and mechanisms
- Cas9 and the sgRNA

Learning objectives

- Define CRISPR, Cas, and other basic terms
- Identify the three steps of adaptive immunity by CRISPR-Cas systems
- Explain how a CRISPR nuclease selects its target
- Explain why CRISPR-Cas systems were readily co-opted as genome-editing technologies

Next lecture…

CRISPR technologies