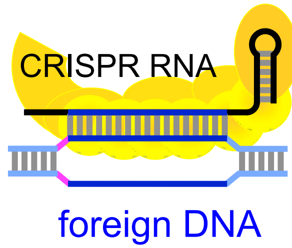
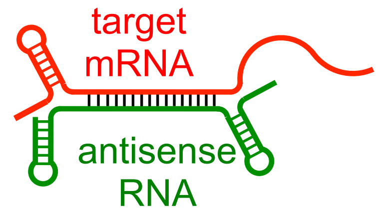




RNA-based mechanisms in the human pathogen *Clostridioides difficile:* focus on CRISPR-Cas system

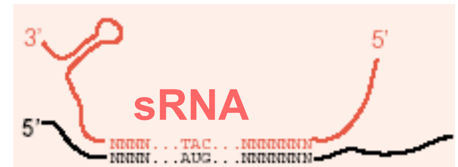
Olga Soutourina, February 7th, 2024

université PARIS-SACLAY

I2BC, Microbiology department
« Regulatory RNAs in Clostridia » Group
Professor, Paris-Saclay University

I2BC

Widespread regulatory mechanisms based on RNAs in bacteria

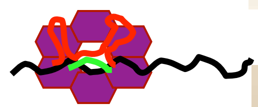


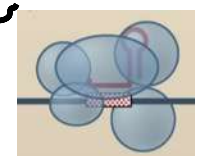
- Adaptive responses
- Metabolic, physiological processes
- Virulence in major pathogens
  Staphylococcus aureus, Listeria monocytogenes, Streptococcus pyogenes

Diversity of regulatory mechanisms

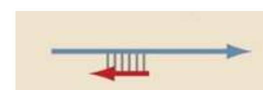
- Direct effector binding (**« riboswitch »**) (c-di-GMP)

- **Protein** binding (RNAP, CsrA, Hfq, ProQ)

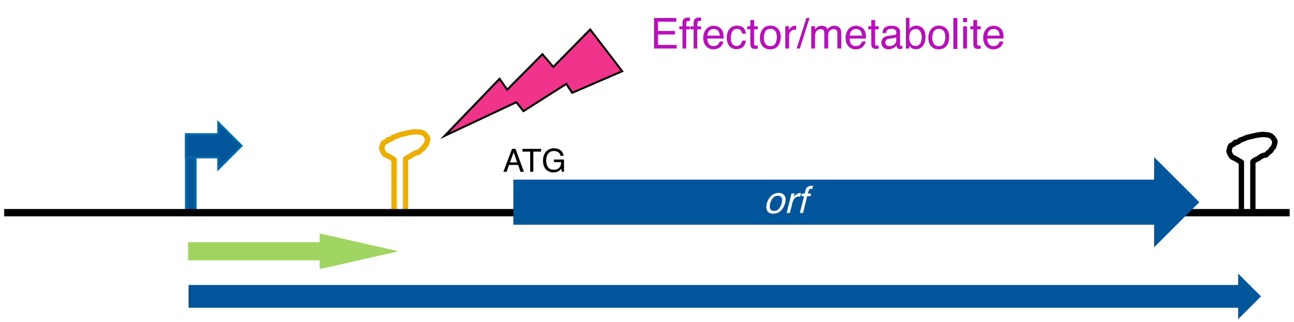


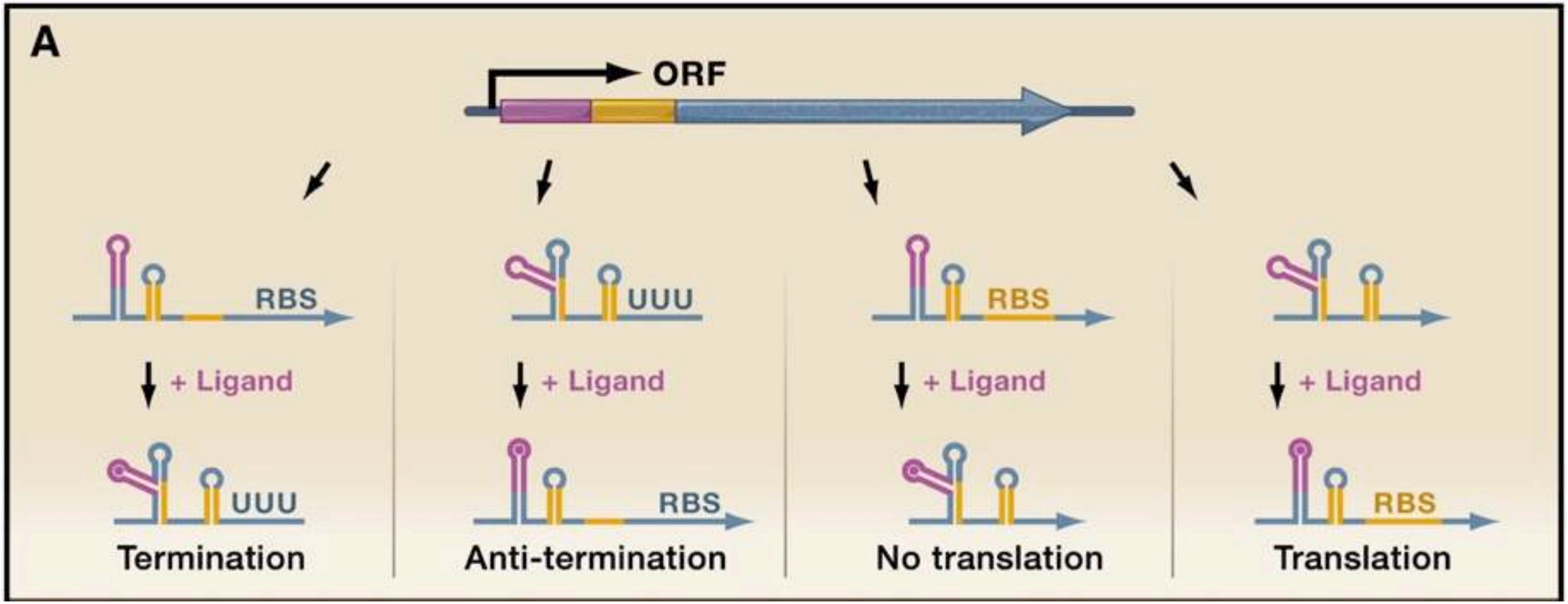


- Foreign **DNA/RNA** binding (CRISPR RNA)
- sRNA-**mRNA** duplex formation (*trans* and *cis* riboregulators)



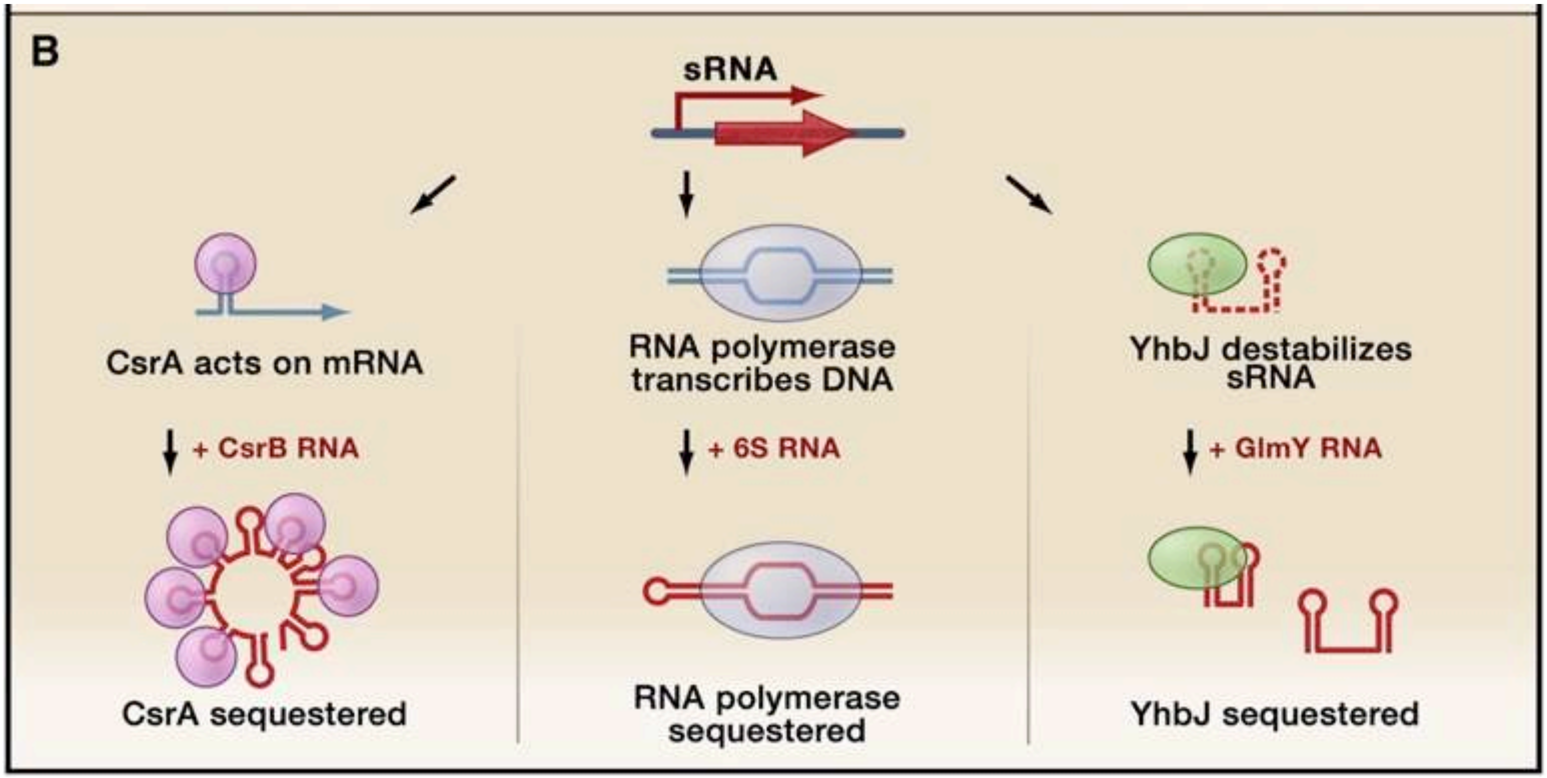
Direct effector binding by riboswitches





L. Waters and G. Storz. Cell. 2009

Regulatory RNA acting by protein binding



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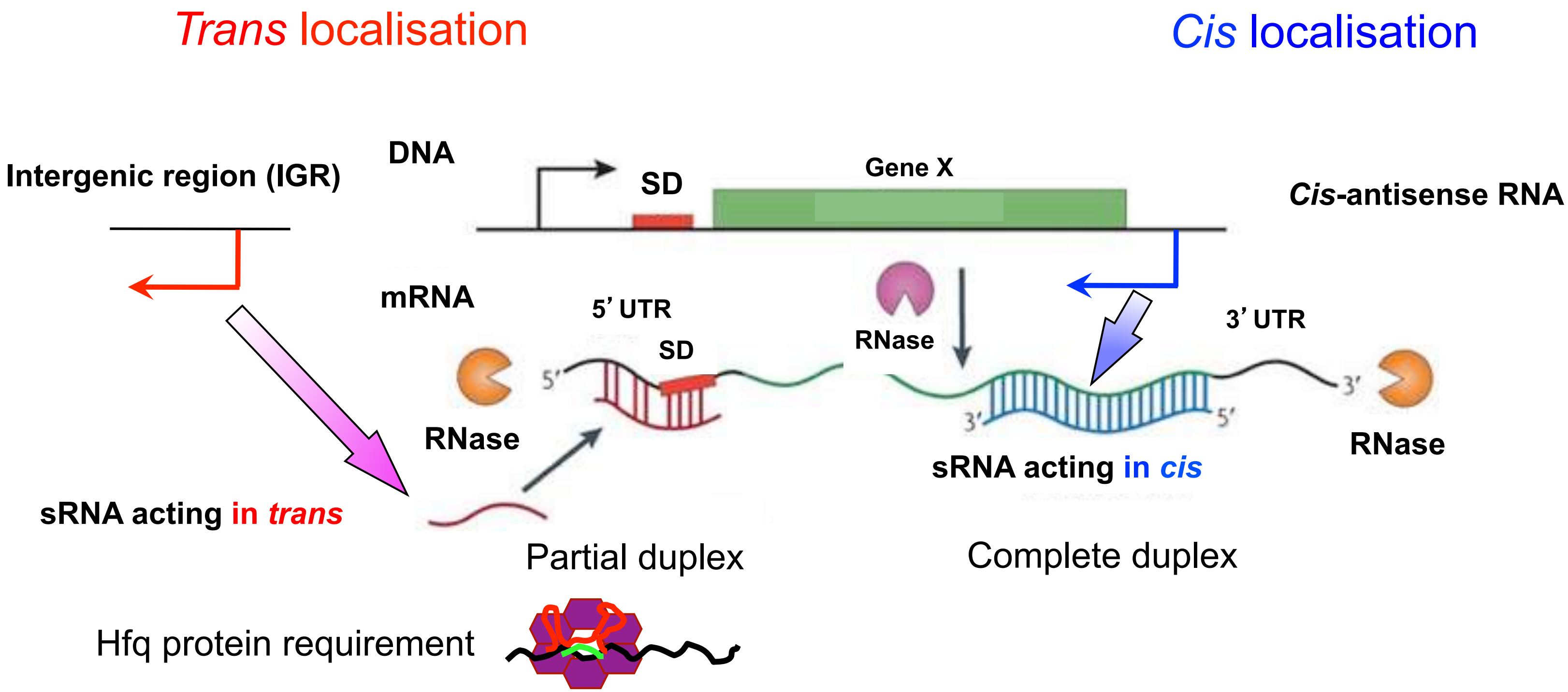
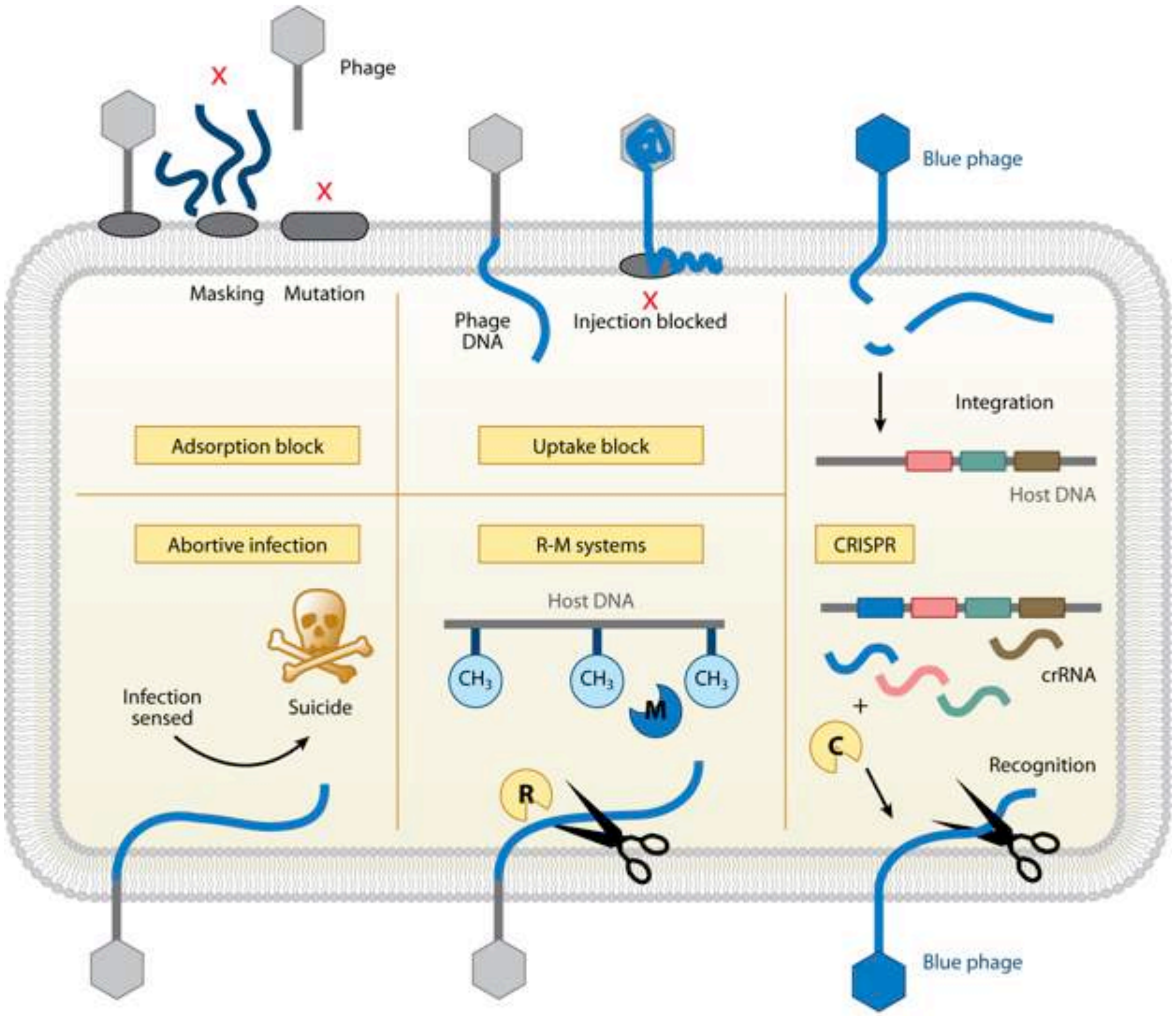


RNA duplex formation – negative or positive effect on target mRNA

J. Gripenland. et al. Nature reviews. Microbiology. 2010

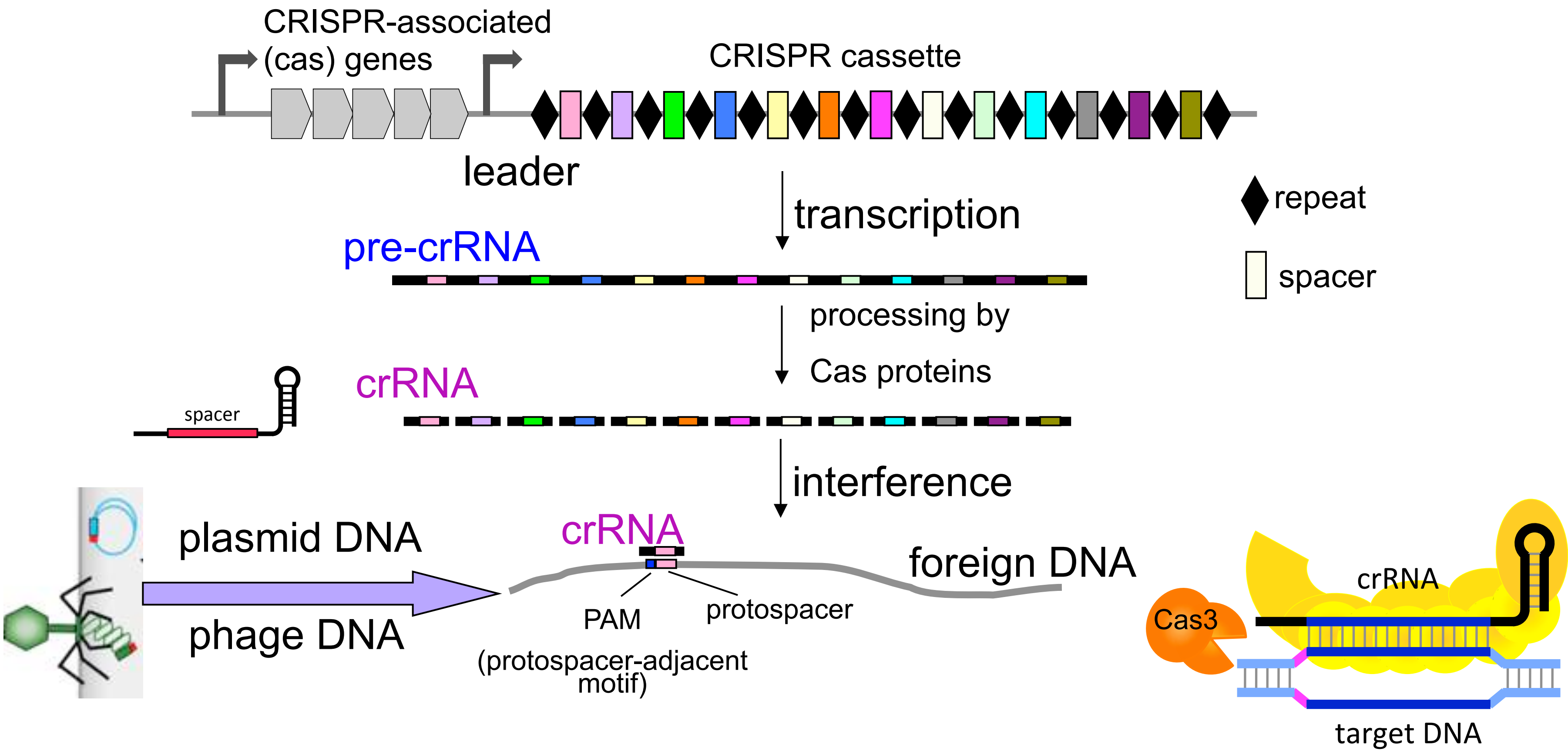
Prokaryotic defense systems



Westra ER, et al. 2012.
Annu. Rev. Genet. 46:311–39

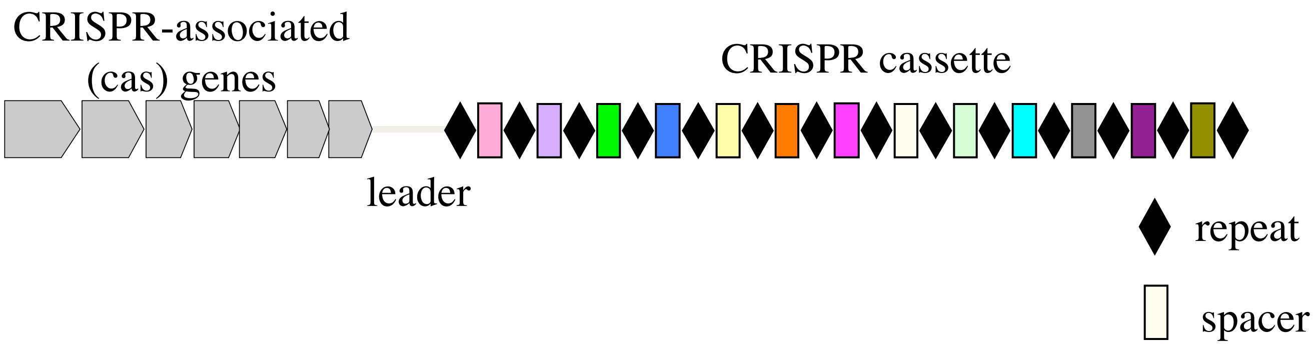
CRISPR-Cas system

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas system
found in 90% of archaeal and 40% of bacterial genomes, prokaryotic immune system



Bhaya et al. Annu. Rev. Genet. 2011

CRISPR-Cas system

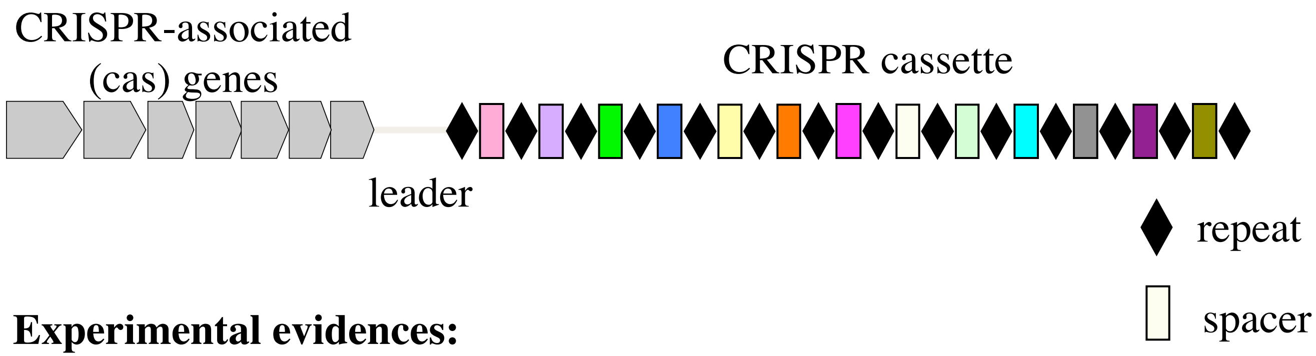


2005 - some spacer sequences match plasmid or viral DNA

⇩

CRISPR-Cas - prokaryotic immune system?

CRISPR-Cas system



Experimental evidences:

CRISPR provides acquired resistance against viruses in prokaryotes.
Barrangou *et al*., Science. 2007, 315(5819):1709-12.

Streptococcus thermophilus:
phage infection → new spacer incorporation → phage resistance

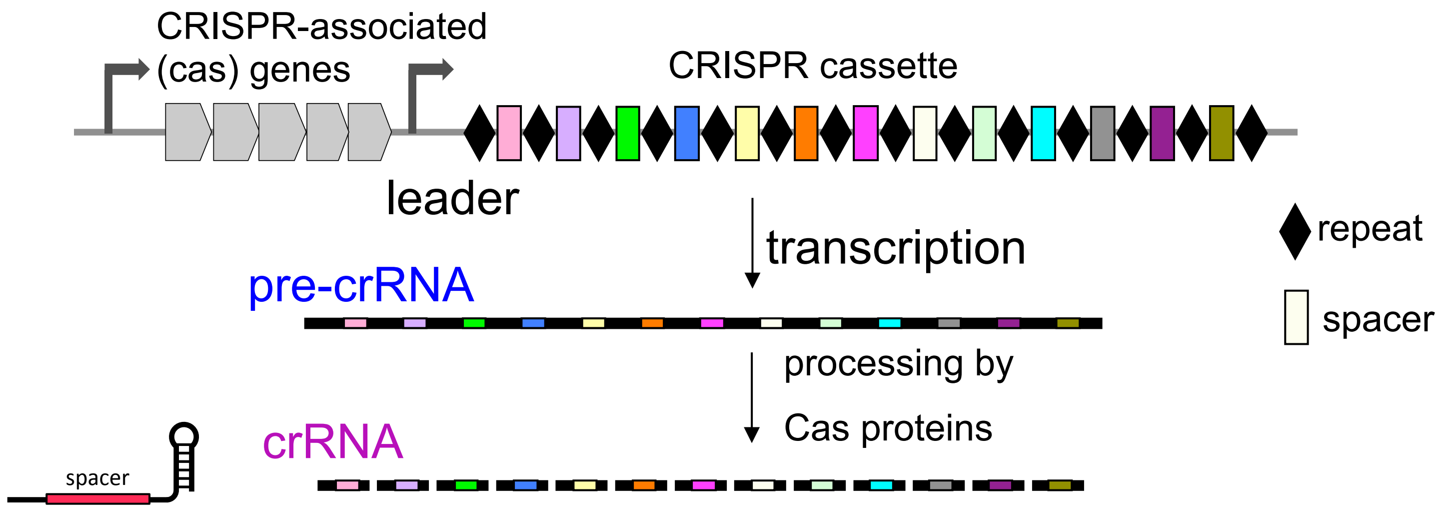
Small CRISPR RNAs guide antiviral defense in prokaryotes.
Brouns *et al*., Science. 2008, 321(5891):960-4.

Escherichia coli:
artificially engineered CRISPR locus containing λ phage DNA-matching spacer → λ phage resistance

CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA.
Marraffini & Sontheimer, Science. 2008, 322(5909):1843-5.

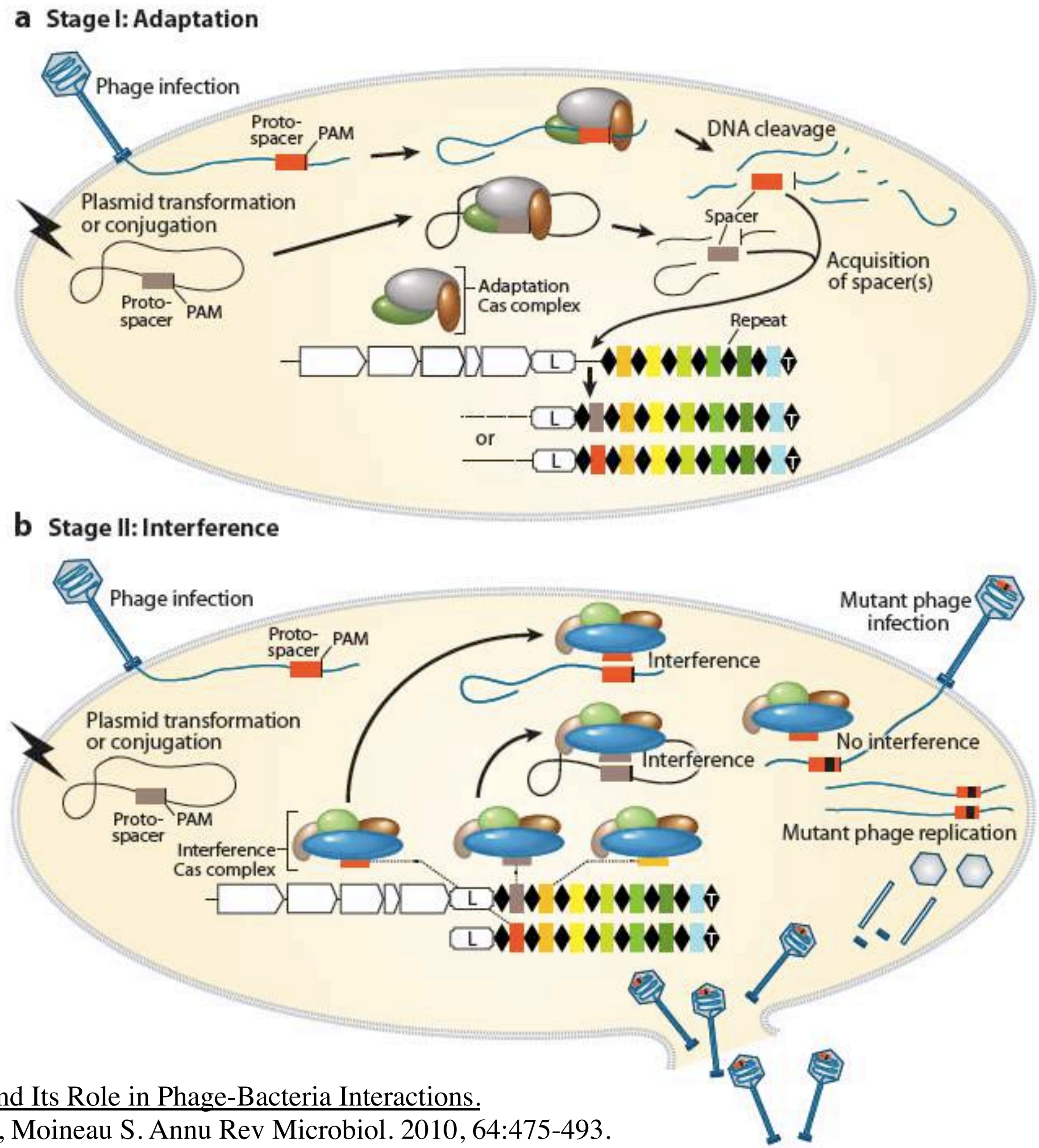
Staphylococcus epidermidis:
CRISPR-Cas system provides a barrier against plasmid conjugation/transformation

CRISPR-Cas system expression



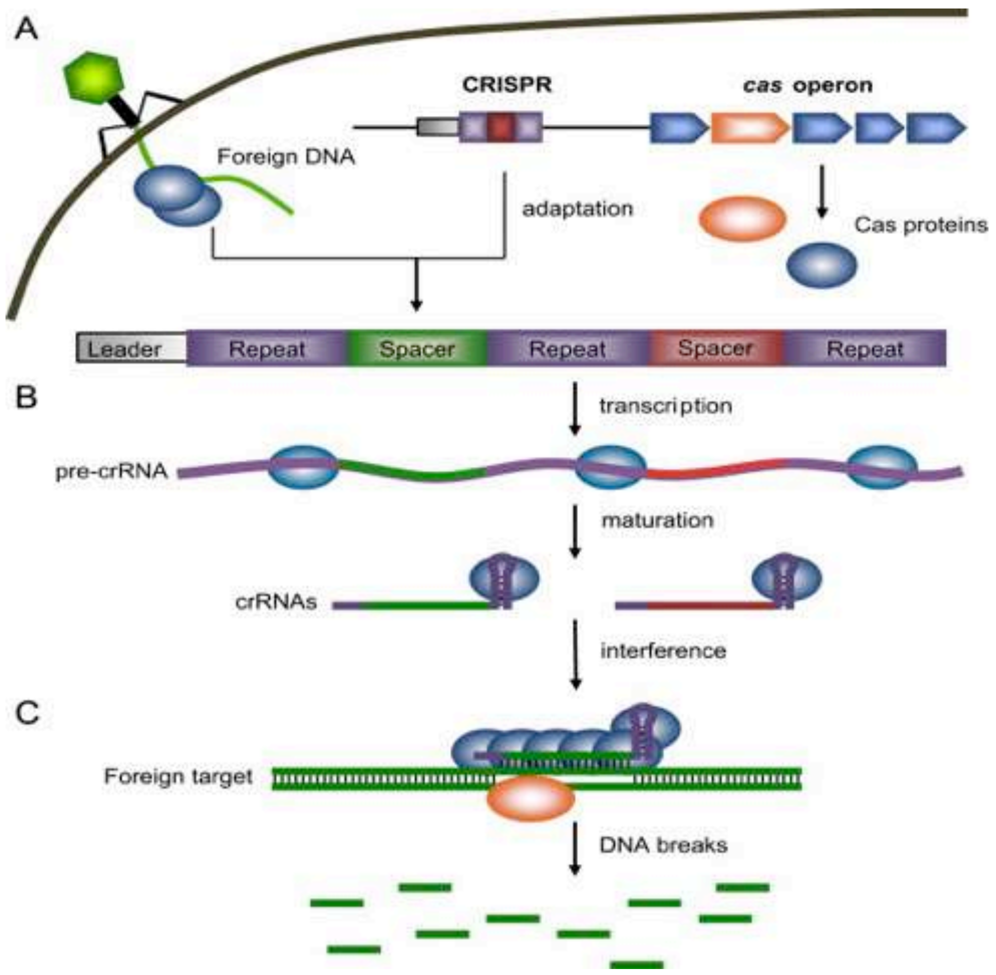
Bhaya et al. Annu. Rev. Genet. 2011

CRISPR-Cas system function



CRISPR/Cas System and Its Role in Phage-Bacteria Interactions.
Deveau H, Garneau JE, Moineau S. Annu Rev Microbiol. 2010, 64:475-493.

CRISPR-Cas system function: interference

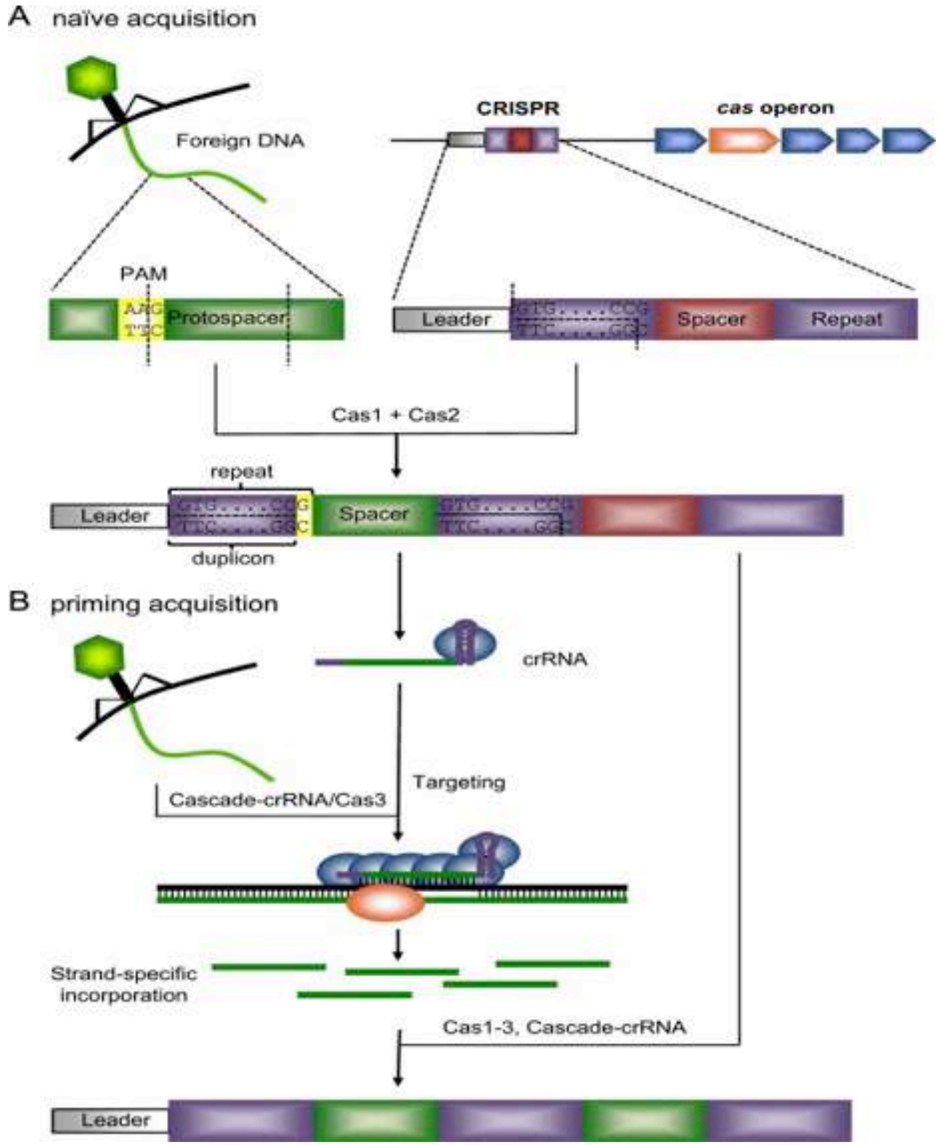


Peter C. Fineran , Emmanuelle Charpentier

Memory of viral infections by CRISPR-Cas adaptive immune systems: Acquisition of new information

Virology Volume 434, Issue 2 2012 202 - 209

CRISPR-Cas system function: adaptation



Peter C. Fineran , Emmanuelle Charpentier

Memory of viral infections by CRISPR-Cas adaptive immune systems: Acquisition of new information

Virology Volume 434, Issue 2 2012 202 - 209

Distinctive features of strict anaerobes

Anaerobic metabolism (ancient group, Clostridia)

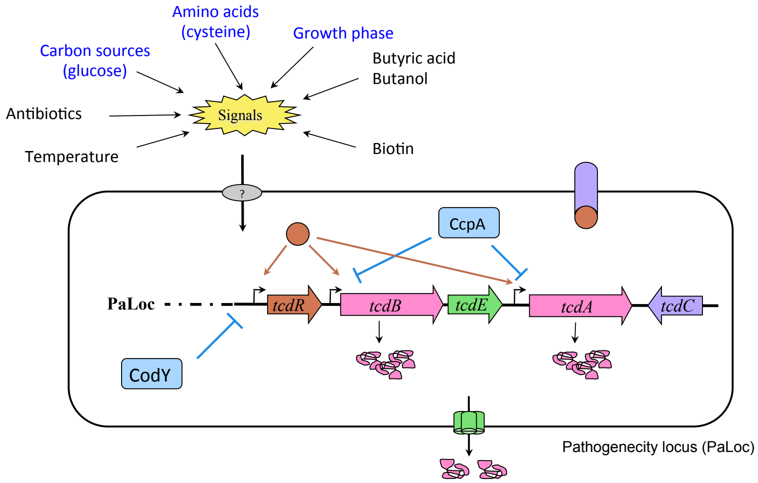
- ✓ Fe-S enzymes for energy metabolism
- ✓ Amino acid fermentations (Stickland reactions)
- ✓ Oxidative stress response mechanisms (absence of SOD, catalase)
- ✓ Establishment of sporulation
- ✓ Link between metabolism and virulence

Biotechnological applications

- ✓ Solvents production, biosynthesis of biofuels (*C. acetobutylicum, C. thermocellum*)

Regulation

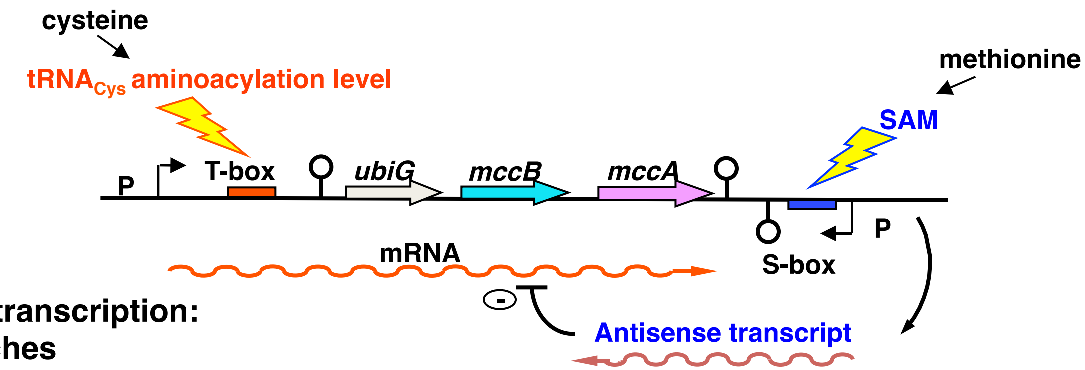
- ✓ Specific regulators, particular pathways
- ✓ Toxin production control by glucose and amino acids (cysteine)
- ✓ RNA-based mechanisms



RNA-based regulation in Clostridia

Clostridium acetobutylicum

Control of sulfur metabolic operon by an antisense RNA
(*André … Soutourina, NAR 2008*)



Premature termination of transcription:
T-box and S-box riboswitches

Clostridium perfringens

Control of toxin-encoding genes by 4 non coding RNAs
(*Okumura et al, 2008; Ohtani et al, 2010*)

Clostridium difficile

Control of virulence determinants ?

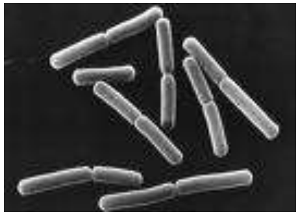
sRNAs contributing to regulatory networks governing *C. difficile* physiology and pathogenesis ?

Regulatory RNAs in Clostridia team
Microbiology Department

https://www.i2bc.paris-saclay.fr/

Clostridioides (Clostridium) difficile

✓Gram positive spore forming
✓strictly anaerobic bacterium
✓colonization of intestinal tract
✓**a major nosocomial enteropathogen**



C. difficile became a public health issue

✓95% cases of pseudomembranous colitis (PMC)
✓10-25% cases of antibiotic-associated diarrhoea (AAD)

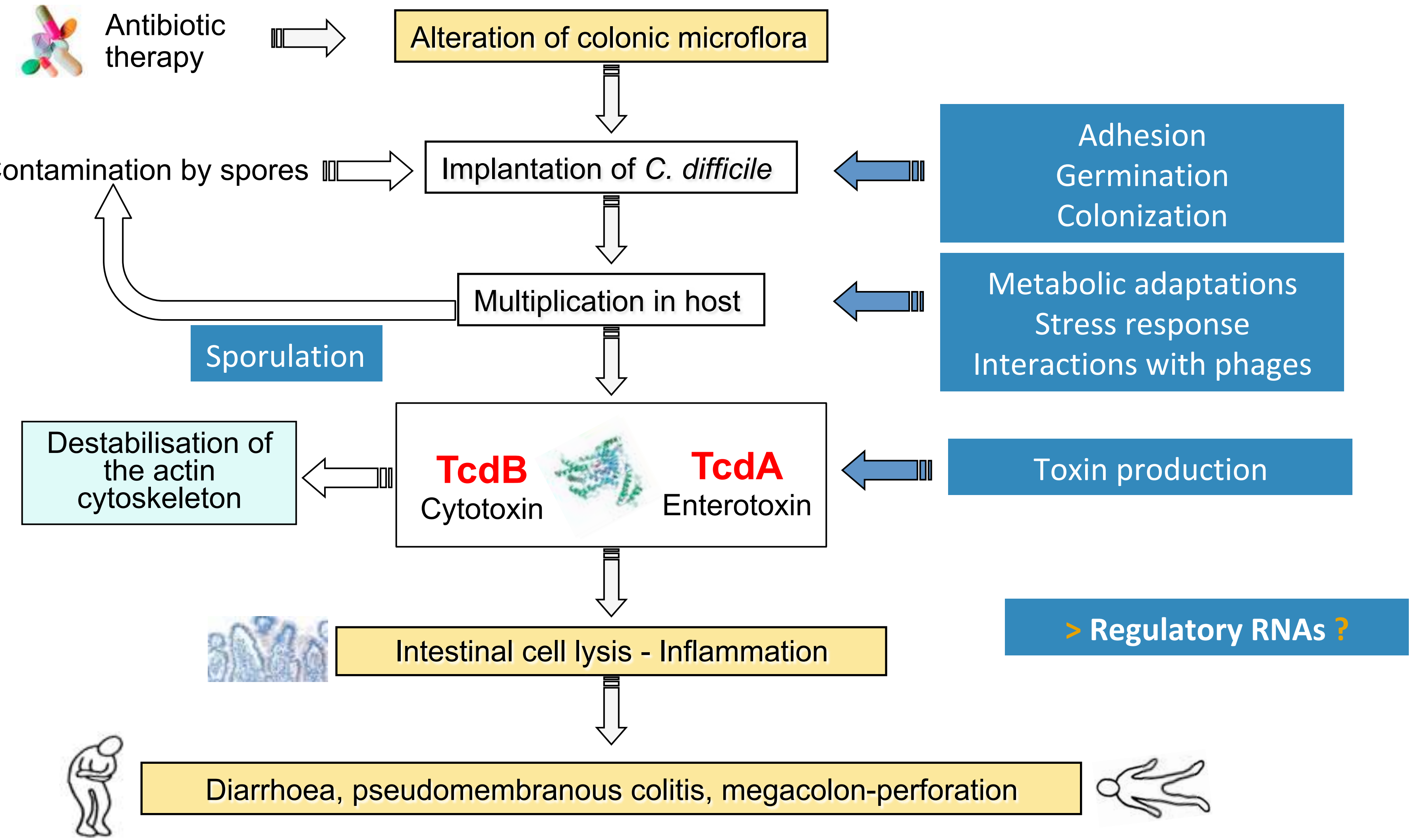
✓**Most common cause of nosocomial infectious diarrhoea in adults**

Emergence of hypervirulent and epidemic strains (as 027 strain)

Economic burden in the developed countries (3 billions € or $ of costs in US and EU)

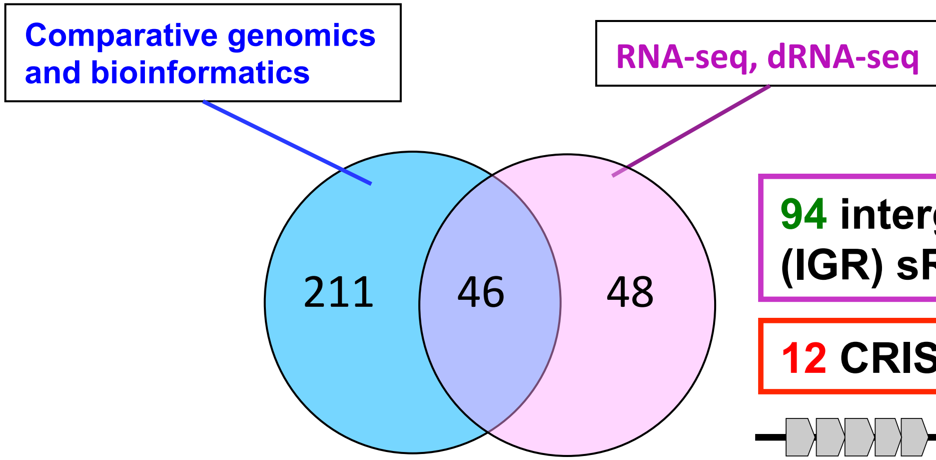
Risk factors of *C. difficile* infection (CDI): **Antibiotic exposure** and **old age** (over 65)

Infection cycle of *C. difficile*



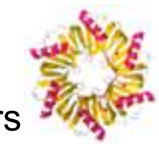
Regulatory RNAs could be involved in several stages of the infection cycle

Genome-wide identification of sRNAs in *C. difficile*

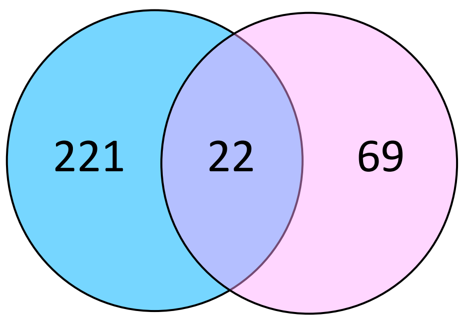


94 intergenic region (IGR) sRNAs

Potential ***trans*** riboregulators



12 CRISPR RNAs



91 Antisense sRNAs

Potential ***cis*** riboregulators

66 riboswitches
16 cyclic di-GMP riboswitches

About 185 sRNAs (35/40 detected by Northern blot)

Soutourina et al. PLoS Genetics. 2013

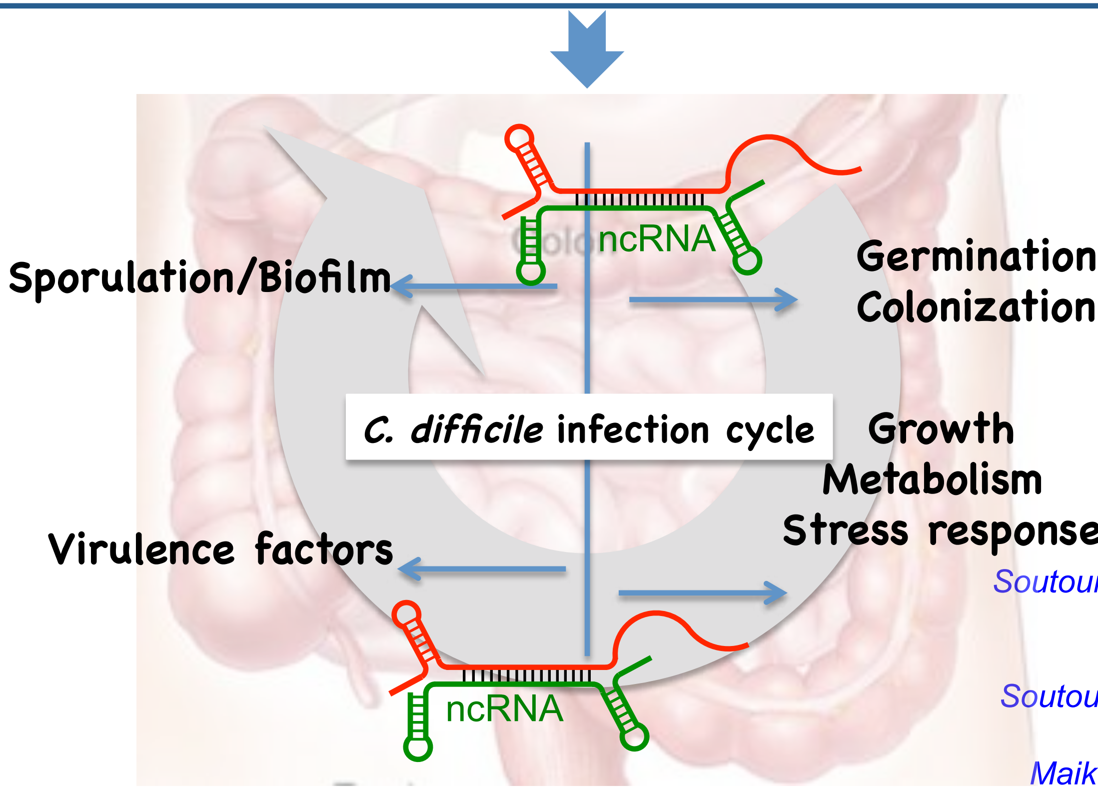
Role of the regulatory RNAs in *C. difficile* physiology

More than **250 ncRNA** & **large diversity** of mechanisms in *CD* (CRISPR RNAs, *trans* and *cis* antisense RNAs and riboswitches)

TSS-mapping

RNA-seq

In silico



Soutourina et al. PLoS Genetics. 2013
Boudry et al. J. Bacteriol. 2014
Boudry et al. mBio. 2015
Soutourina. Curr Opin Microbiol. 2017
Maikova et al. NAR. 2018
Maikova et al. Front Microbiol. 2018
Soutourina. Toxins. 2019, Maikova et al AEM. 2019
Soutourina et al. Frontiers in Microbiol. 2020
Peltier et al. Communications Biol 2020
Piattelli et al. Genes 2020, Kreis & Soutourina Current Opinion Microbiol 2022
Boudry et al. RNA Biol. 2021, Maikova et al. mBio 2021
Muzyukina et al. mSphere 2023, Muzyukina & Soutourina. Biochimie. 2023

Role of the regulatory RNAs in *C. difficile* physiology

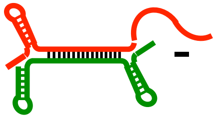
More than **250 ncRNA** & **large diversity** of mechanisms in *CD* (CRISPR RNAs, *trans* and *cis* antisense RNAs and riboswitches)

Original aspects of RNA-based control in regulatory function

- **Riboswitches** responding to **c-di-GMP** involved in cellular processes

- **RNA chaperone protein Hfq** in the ncRNA network

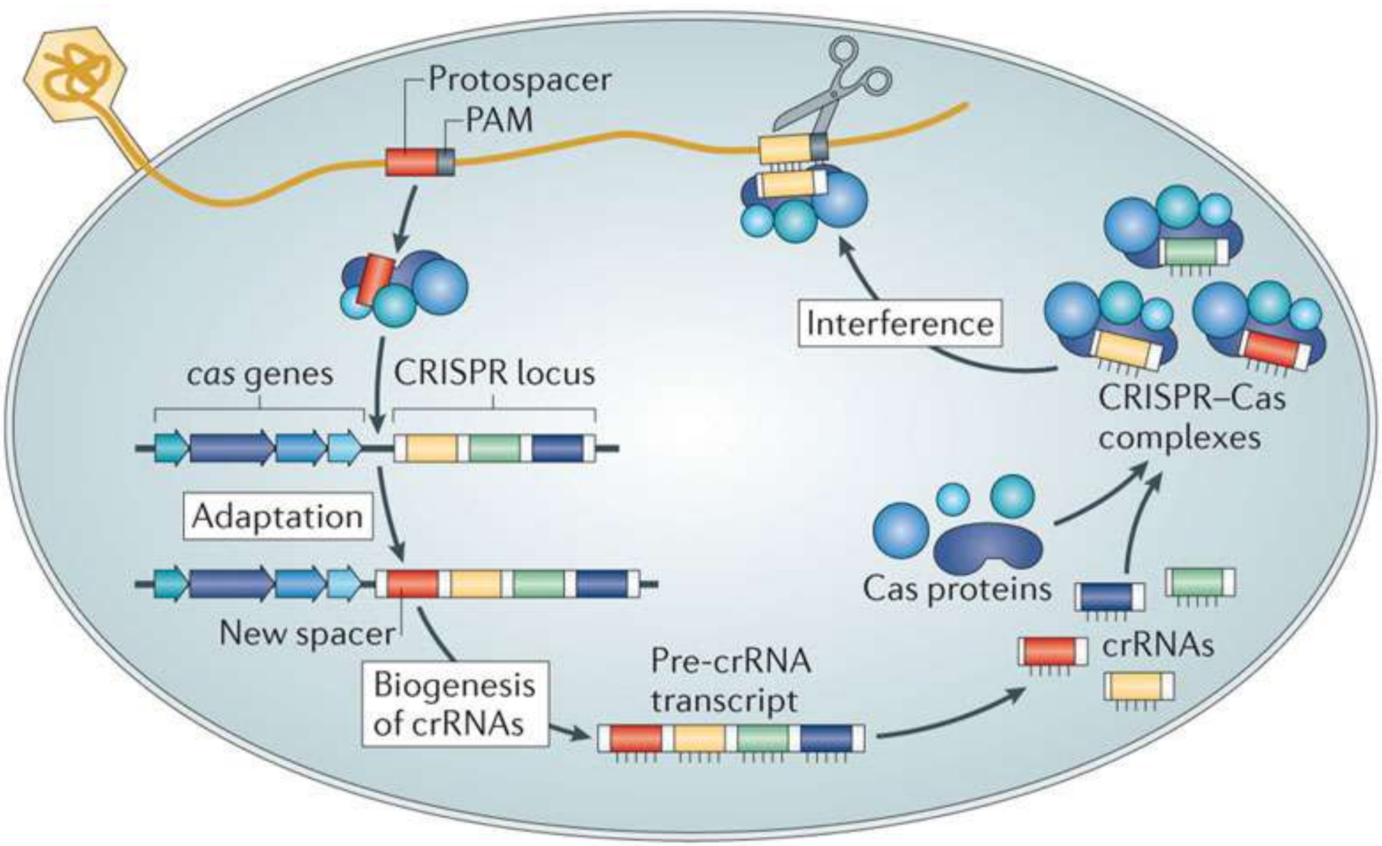
- Function and regulation of ***CD* CRISPR-Cas system**



- Antisense RNAs within **type I toxin-antitoxin systems**

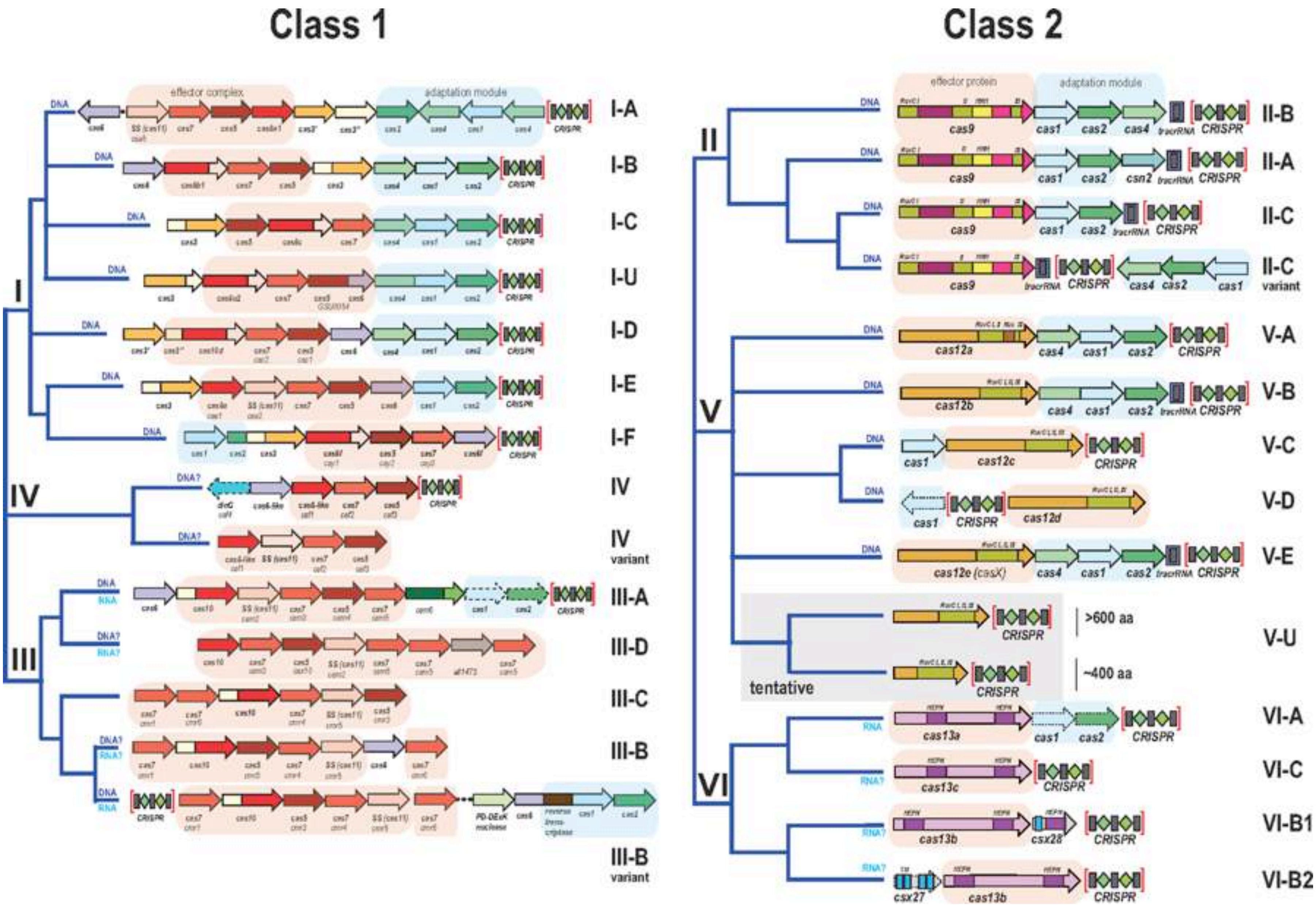
CRISPR-Cas system

clustered **r**egularly **i**nterspaced **s**hort **p**alindromic **r**epeats



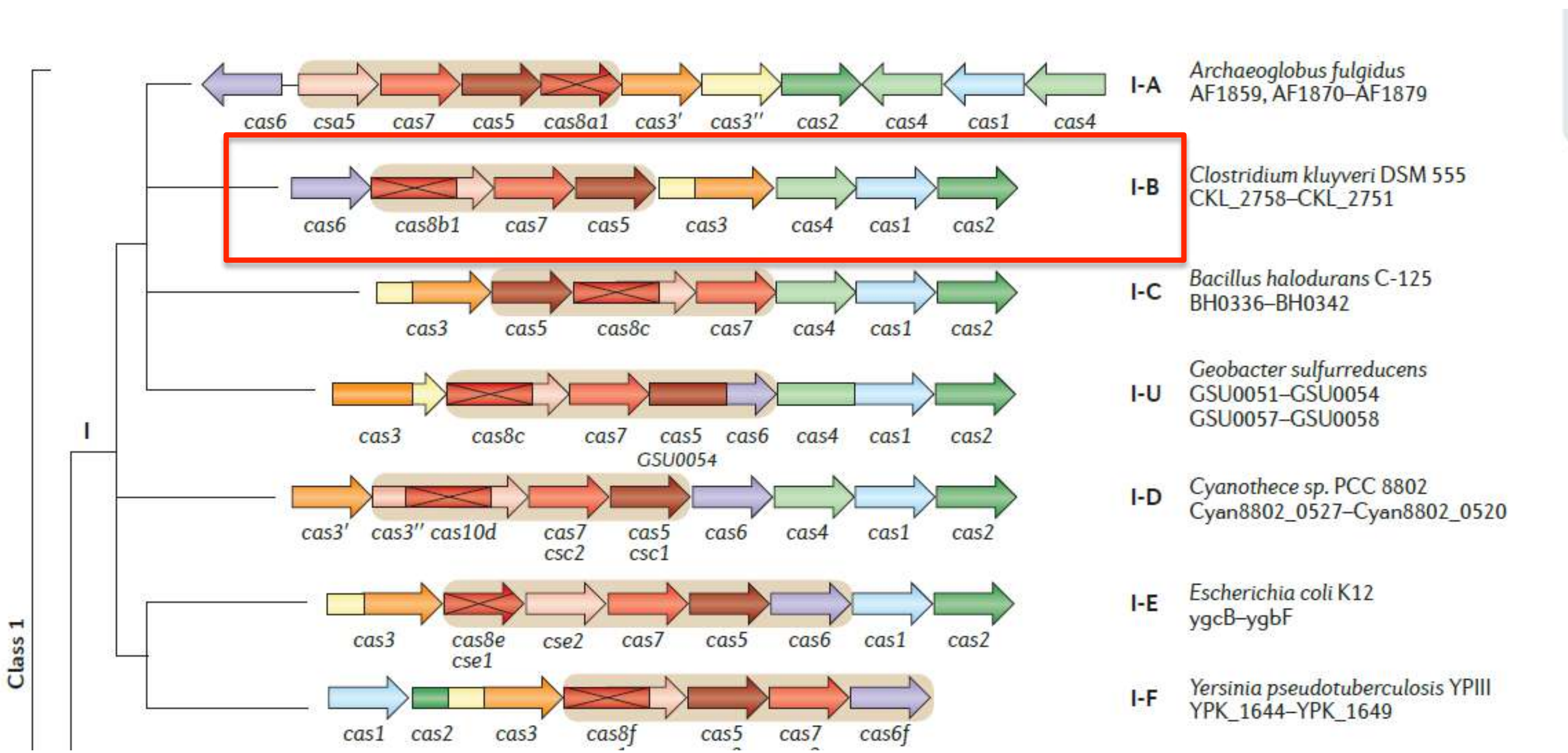
Nature Reviews | Microbiology

Current classification of CRISPR-Cas systems



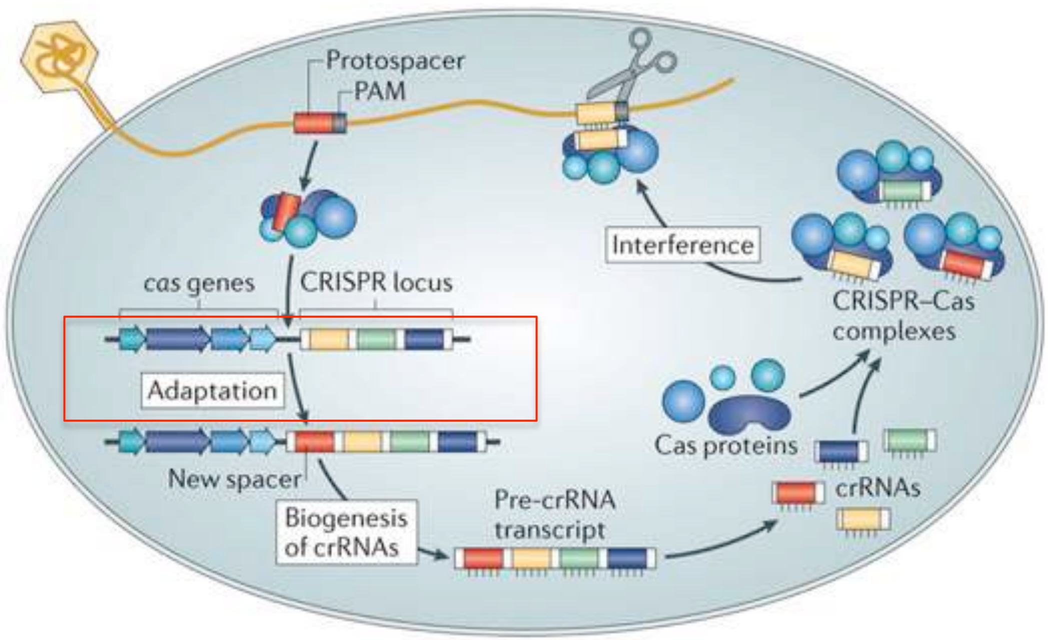
Koonin and Makarova. GBE. 2017

Class 1 type I CRISPR-Cas systems



Adapted from: Makarova *et al.* Science. 2015

Function and regulation of *C. difficile* CRISPR-Cas system



Nature Reviews | Microbiology

In *CD* strain 630 : 12 CRISPR regions exist and are expressed

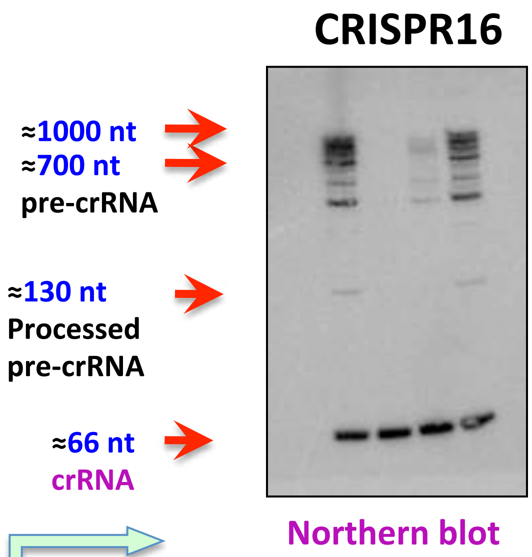
In epidemic *CD* strain 027: 9 active CRISPR arrays

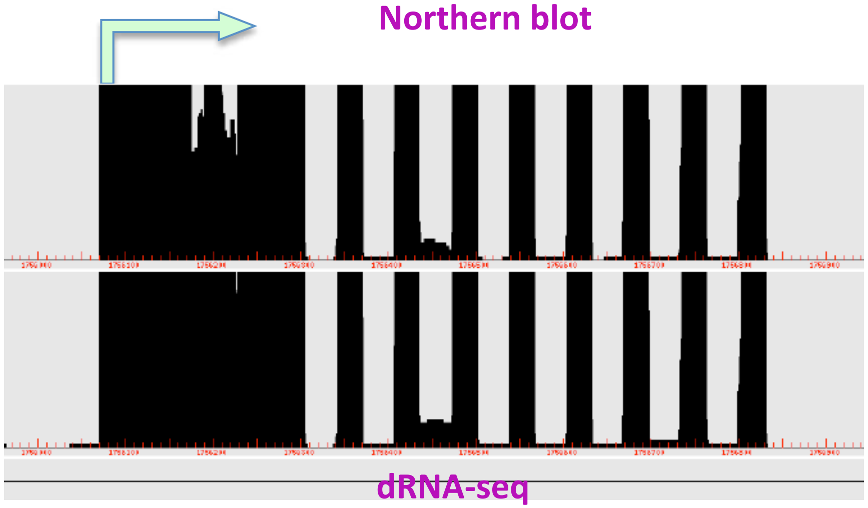
…. A total of 819 spacers from nine *CD* strains

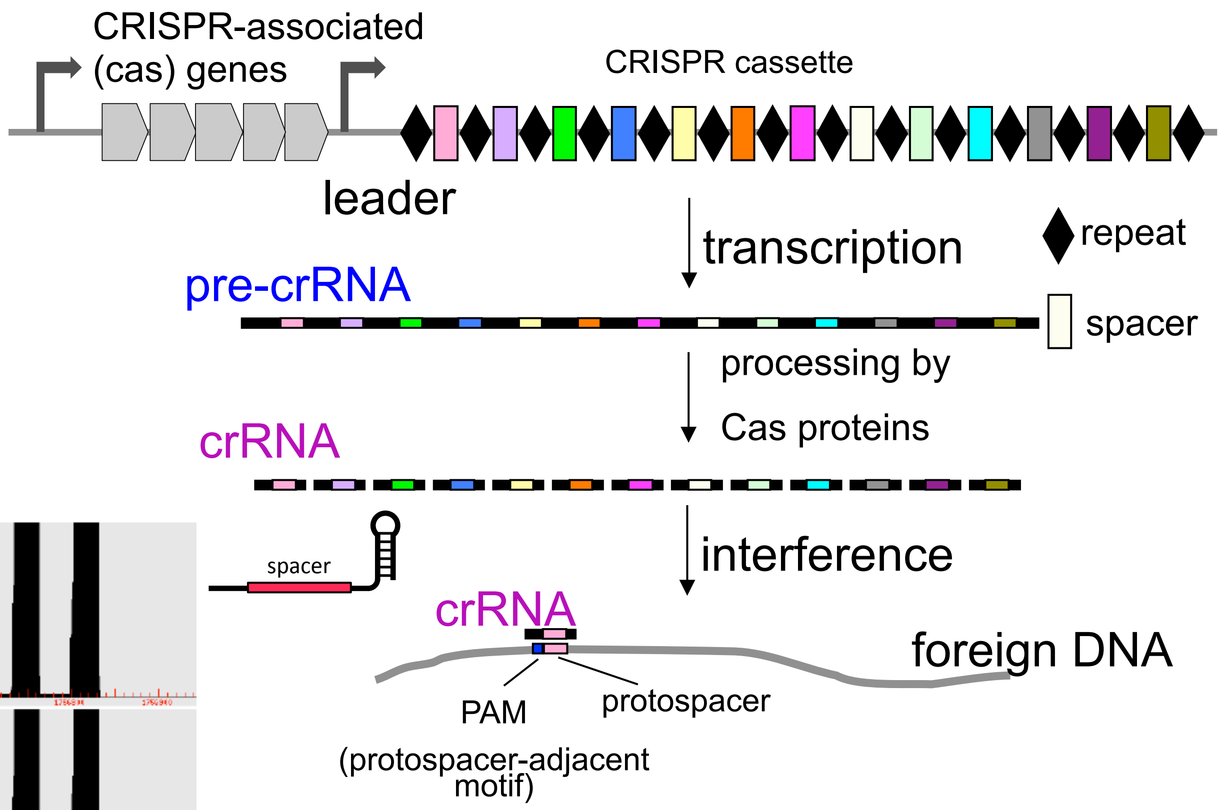
Large defence capacity within phage-rich gut communities

CRISPR RNAs identified by dRNA-seq

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas system

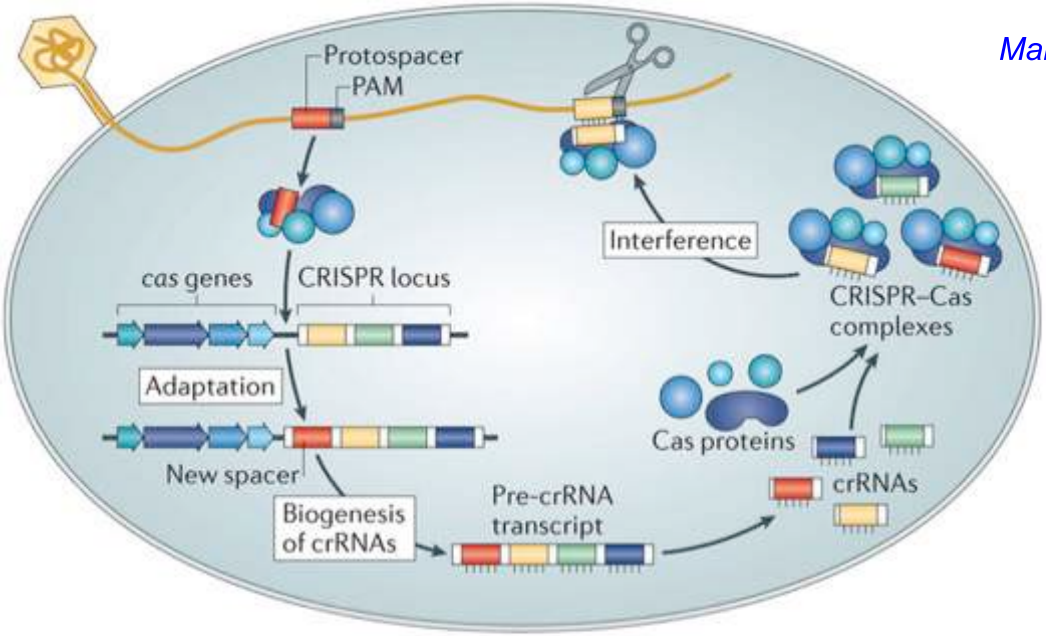


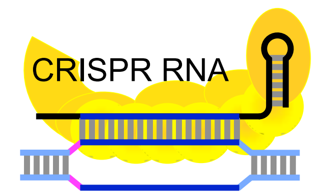




Project 1: *CD* **CRISPR-Cas** system function and regulation

Boudry et al. mBio. 2015
Maikova et al. NAR. 2018
Maikova et al. Frontiers Microbiol. 2018
Maikova et al. AEM. 2019
Maikova et al. PhD thesis





Endogenous system for *CD* genome editing

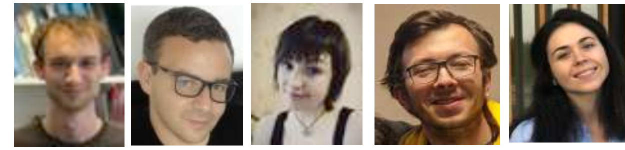
Sensitive high-resolution CRISPR-based typing for epidemiology & *CD* microevolution survey

Self-targeting, autoimmunity

Great potential for genome editing and therapeutic applications

Project 1: *CD* **CRISPR-Cas** system function and regulation

ARNCLO



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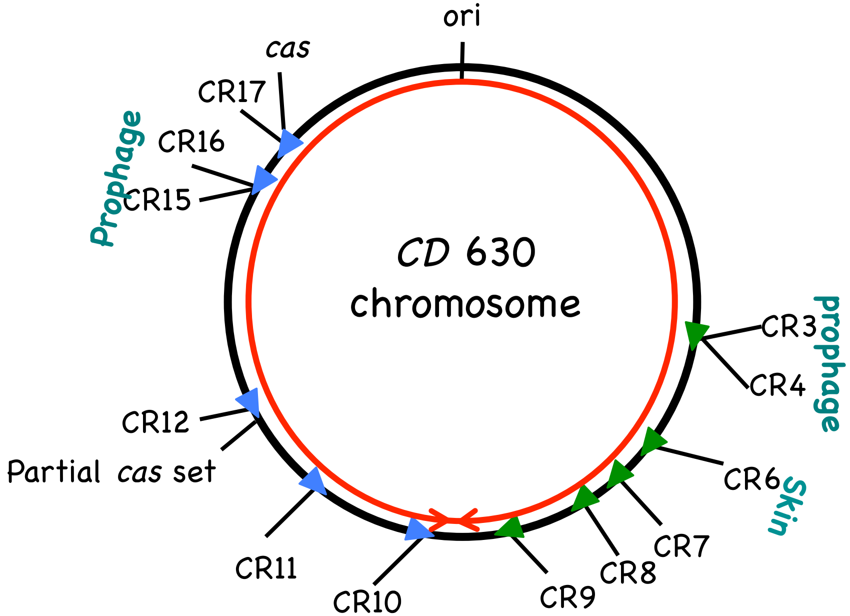

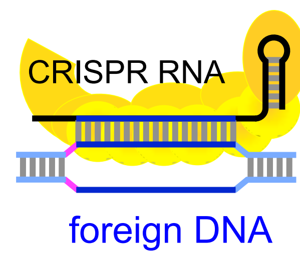

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In epidemic *CD* strain 027: 9 active CRISPR arrays

…. A total of 819 spacers from nine *CD* strains
Large defense capacity within phage-rich gut communities

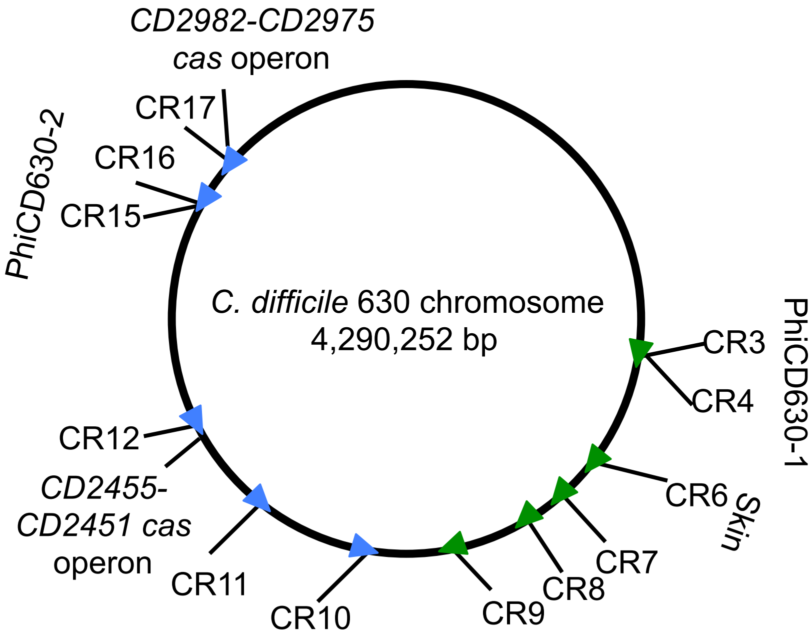
CRISPR analysis in *C. difficile*

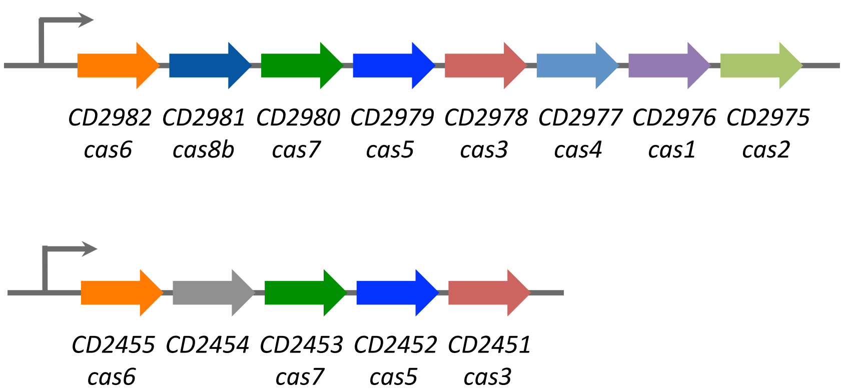
dRNA-seq/ RNA-seq: expression of all 12 CRISPR RNAs:

2 associated with ***cas* genes (type I-B)**

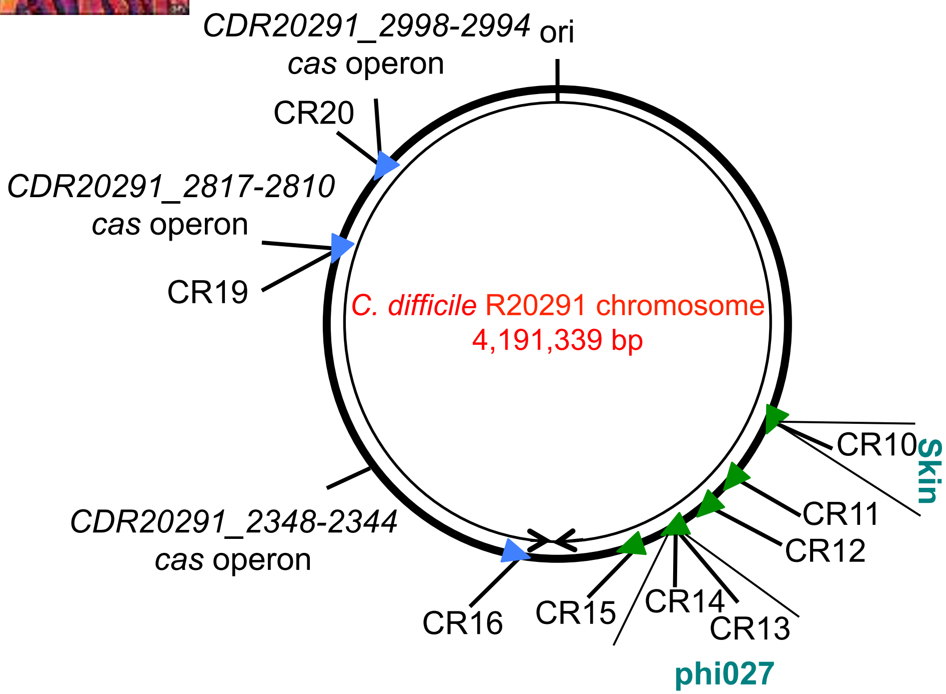
5 in **phage** regions (**3** highly expressed), strategy for limiting competing phages ?

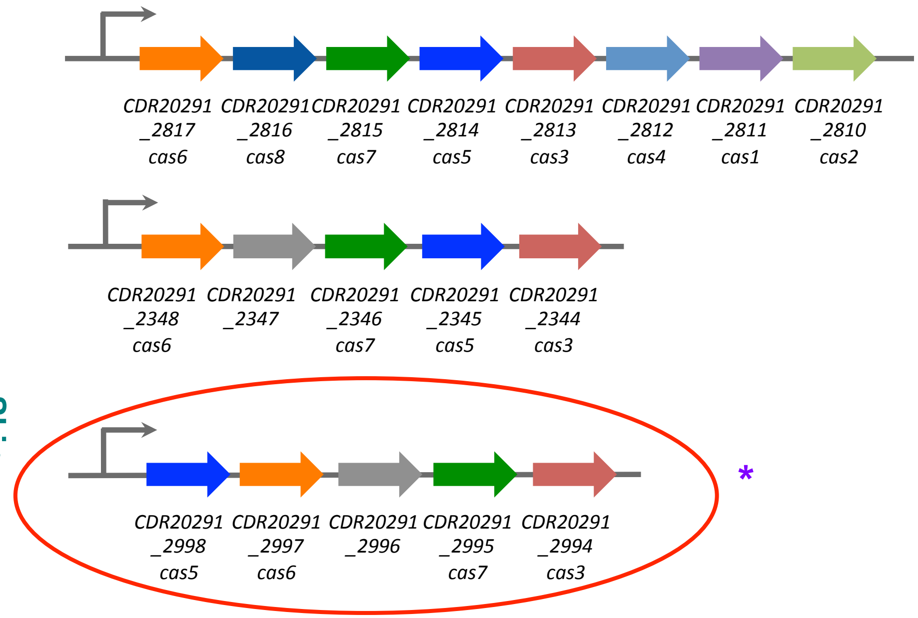
complex **regulation** by stress- and biofilm-related stimuli, link with TA systems





CRISPR analysis in epidemic *C. difficile* strain of 027 ribotype





RNA-seq: expression of all 9 CRISPR RNAs:

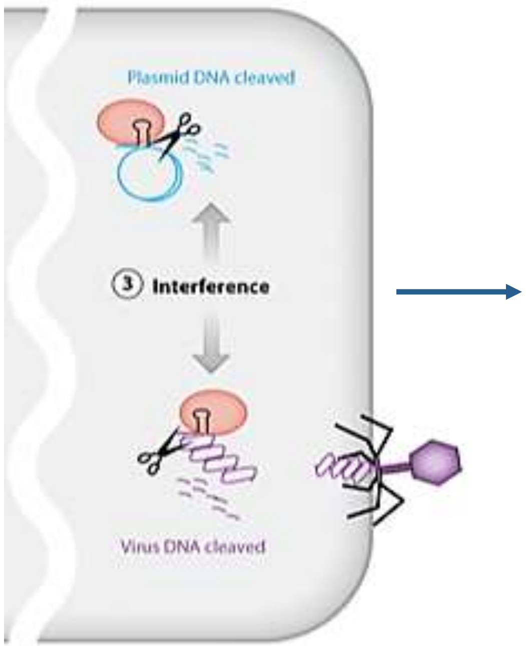
- transcriptional orientation in the direction of replication
- 2 associated with ***cas* genes (type I-B)**
- **additional *cas* operon** specific to 027 ribotype strains*
- **3** in **phage** regions

Boudry et al. mBio. 2015

Results:

I. **Functionality** of CRISPR-Cas system in *C. difficile*

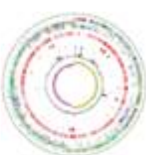
Function of *C. difficile* CRISPR-Cas system



Functionality of CRISPR system

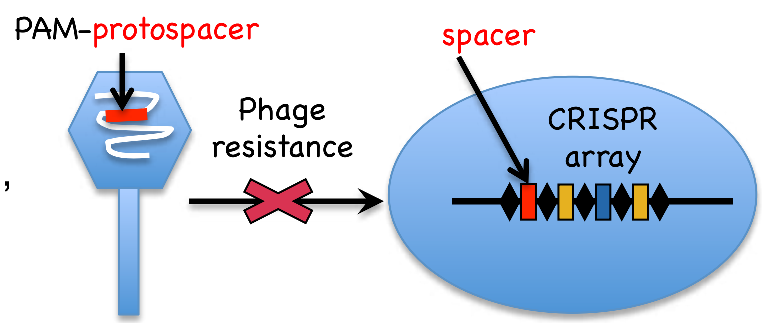
Phage	Strain	CRISPR Spacer match	Phage sensitivity
PhiCD27	630	+	R
	027	+	R
PhiCD38-2	630	+	R
	027	-	S

Correlation between **phage resistance** phenotype and CRISPR spacer match



Large *in silico* CRISPR spacer homology analysis (**9** strains/**22** phages, 11 new phage genome sequencing):

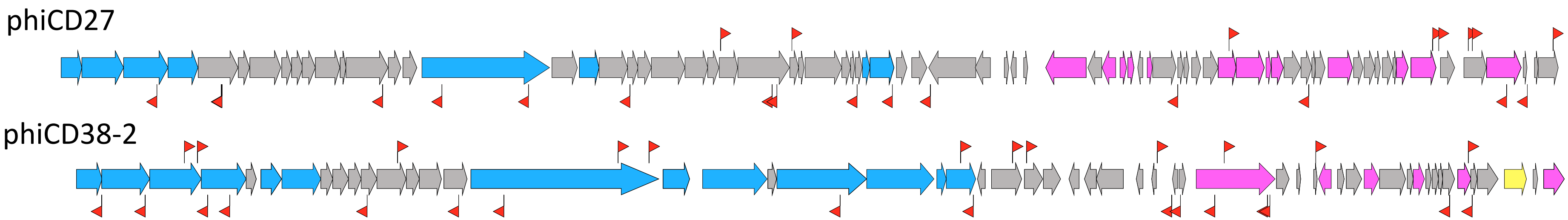
- extensive targeting of clostridial phages;
- multiple targeting of conserved homologous genes within related phages

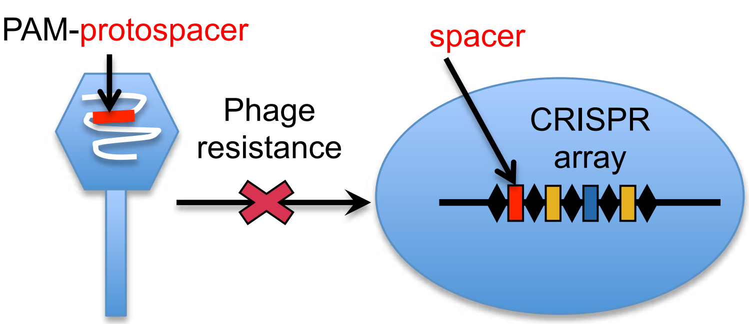


Boudry et al. mBio. 2015

Functionality of CRISPR system in *C. difficile:* interactions with phages

Example of CRISPR spacer targeting of *C. difficile* phages

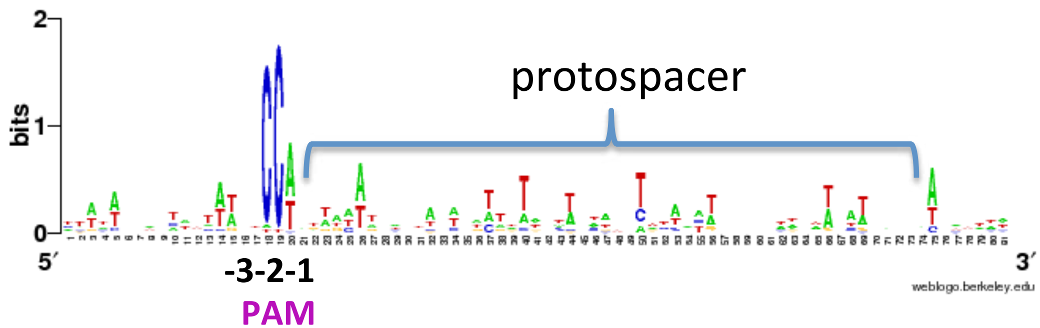




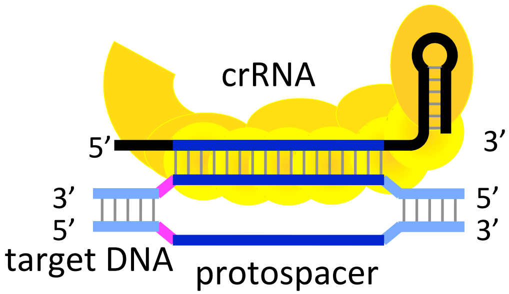
Boudry *et al*. mBio. 2015.

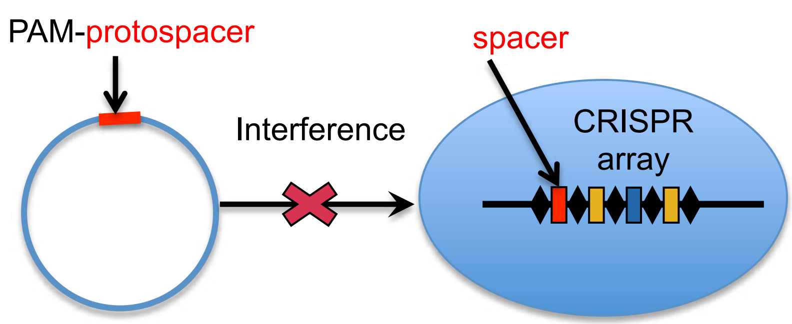
Functionality of CRISPR system in *C. difficile:* plasmid interference

PAM identification by protospacer alignment

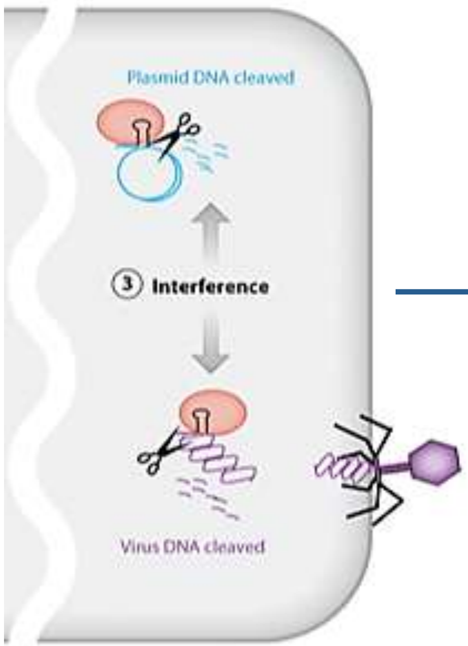


Identification of 5’ CCA/CCT protospacer-adjacent motif





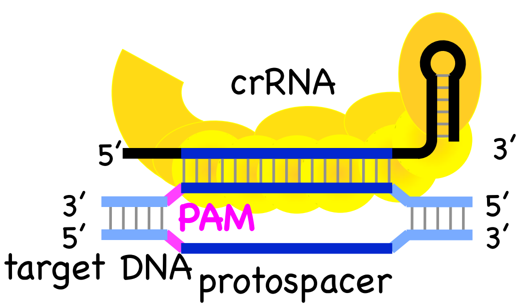
Boudry *et al*. mBio. 2015.

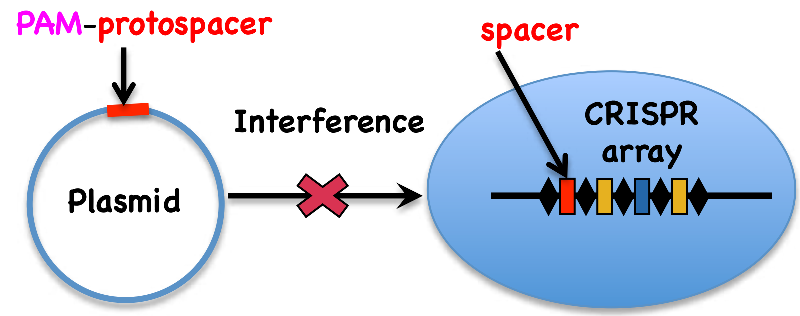


Functionality of CRISPR system

Active interference process in **plasmid conjugation** efficiency assays in *CD* and in heterologous host *E. coli*

Boudry et al. mBio. 2015

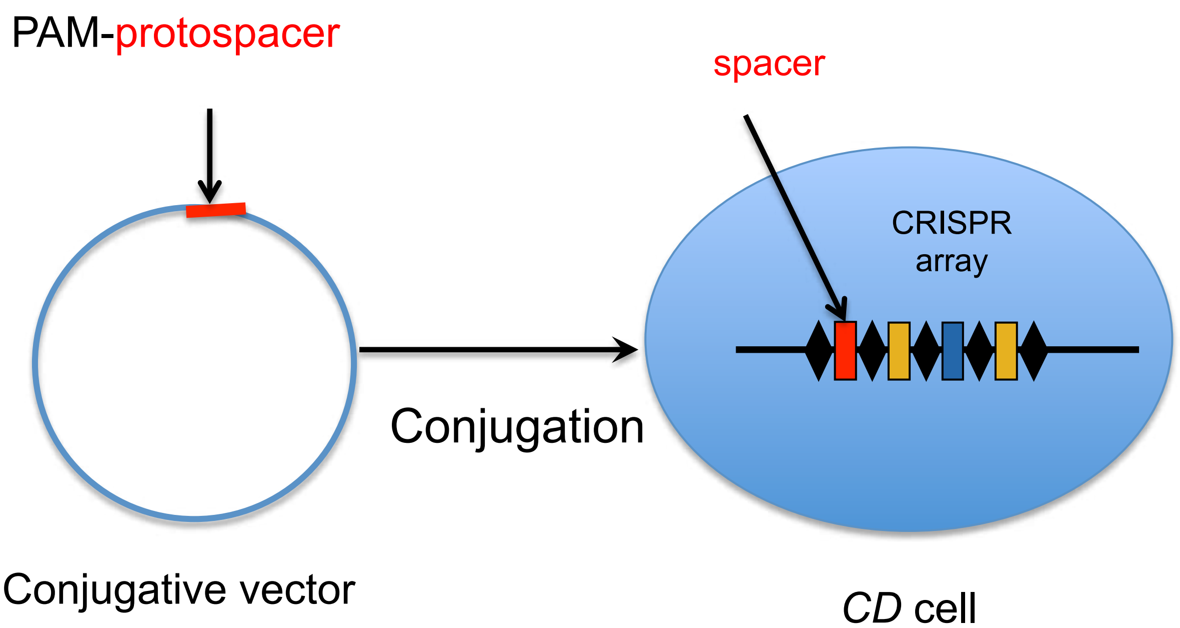




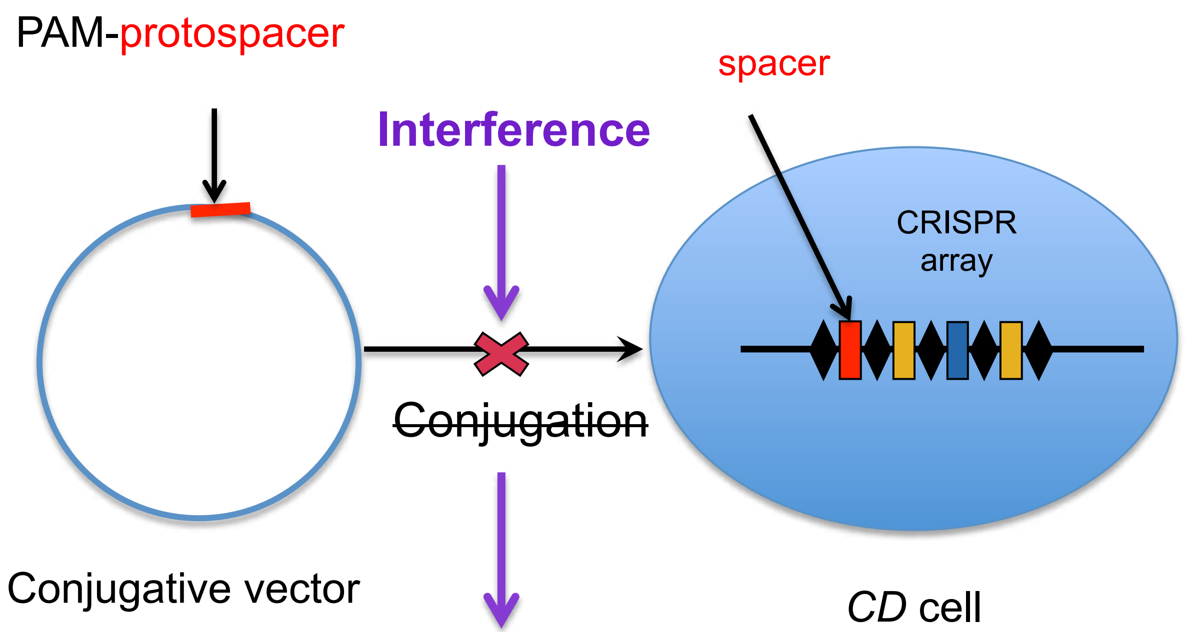
Almost all CRISPR arrays are active for interference !

Maikova. PhD thesis; Maikova et al. mBio 2021

Functionality of CRISPR-Cas system in *C. difficile:* plasmid interference

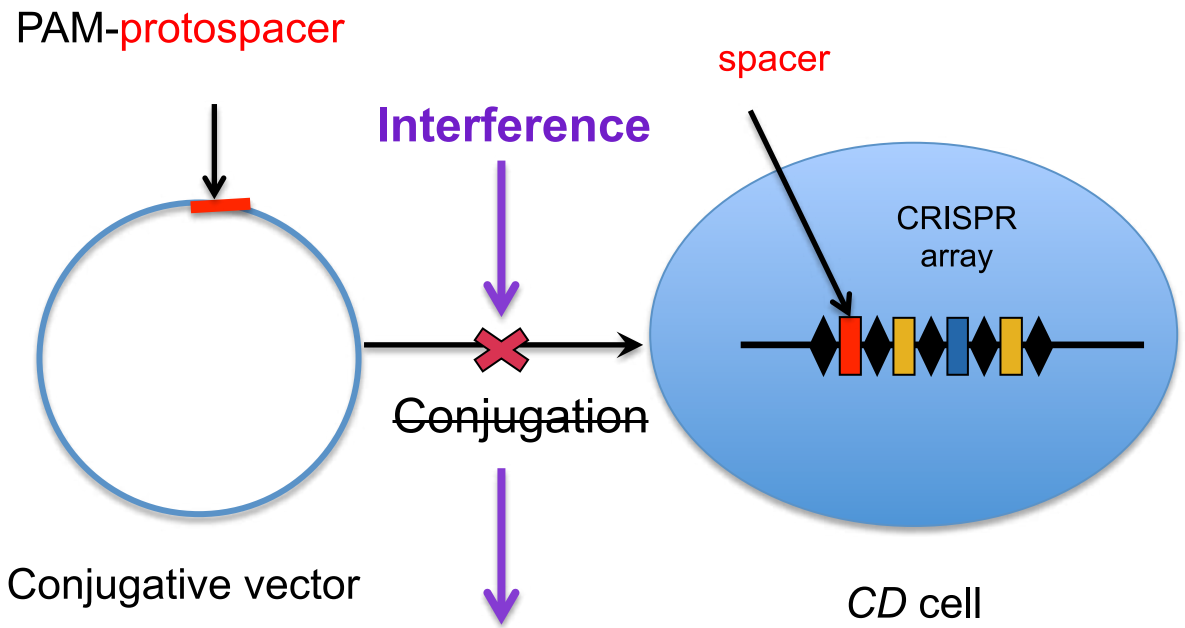


Functionality of CRISPR-Cas system in *C. difficile:* plasmid interference



No or less transconjugants

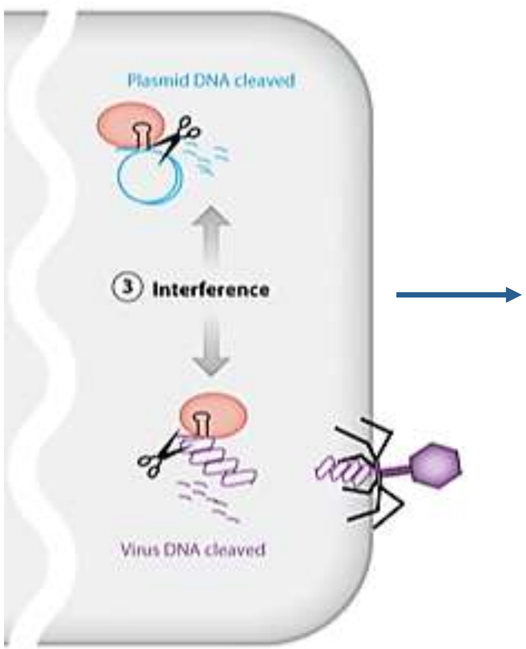
Functionality of CRISPR-Cas system in *C. difficile:* plasmid interference



No or less transconjugants

Strategy for interference experiments

Function of *C. difficile* CRISPR-Cas system

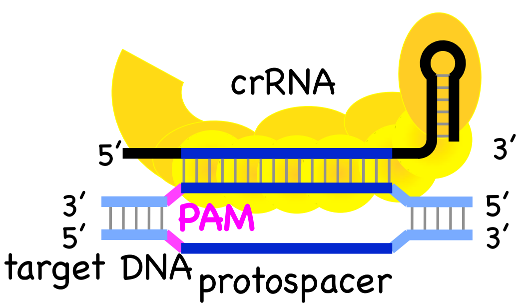


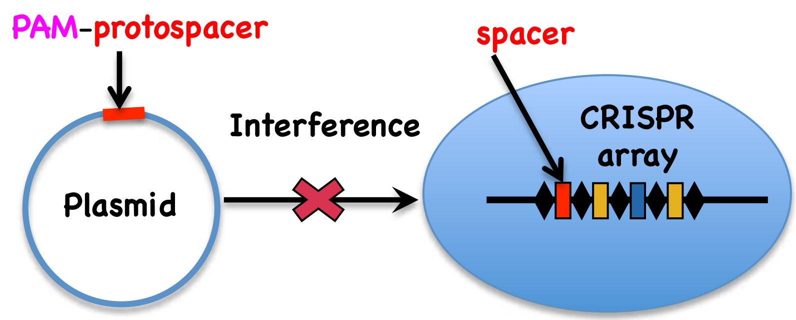
Functionality of CRISPR system

Experimental evidence for **PAM 5'CCW**:
plasmid conjugation efficiency

PAM	Number of transconjugants	Conjugation efficiency
CCA PAM	0	less than $1x10^{-9}$
CCT PAM	0-1	$1x10^{-9}$
GAG	138	$5x10^{-7}$
AAT	178	$6x10^{-7}$

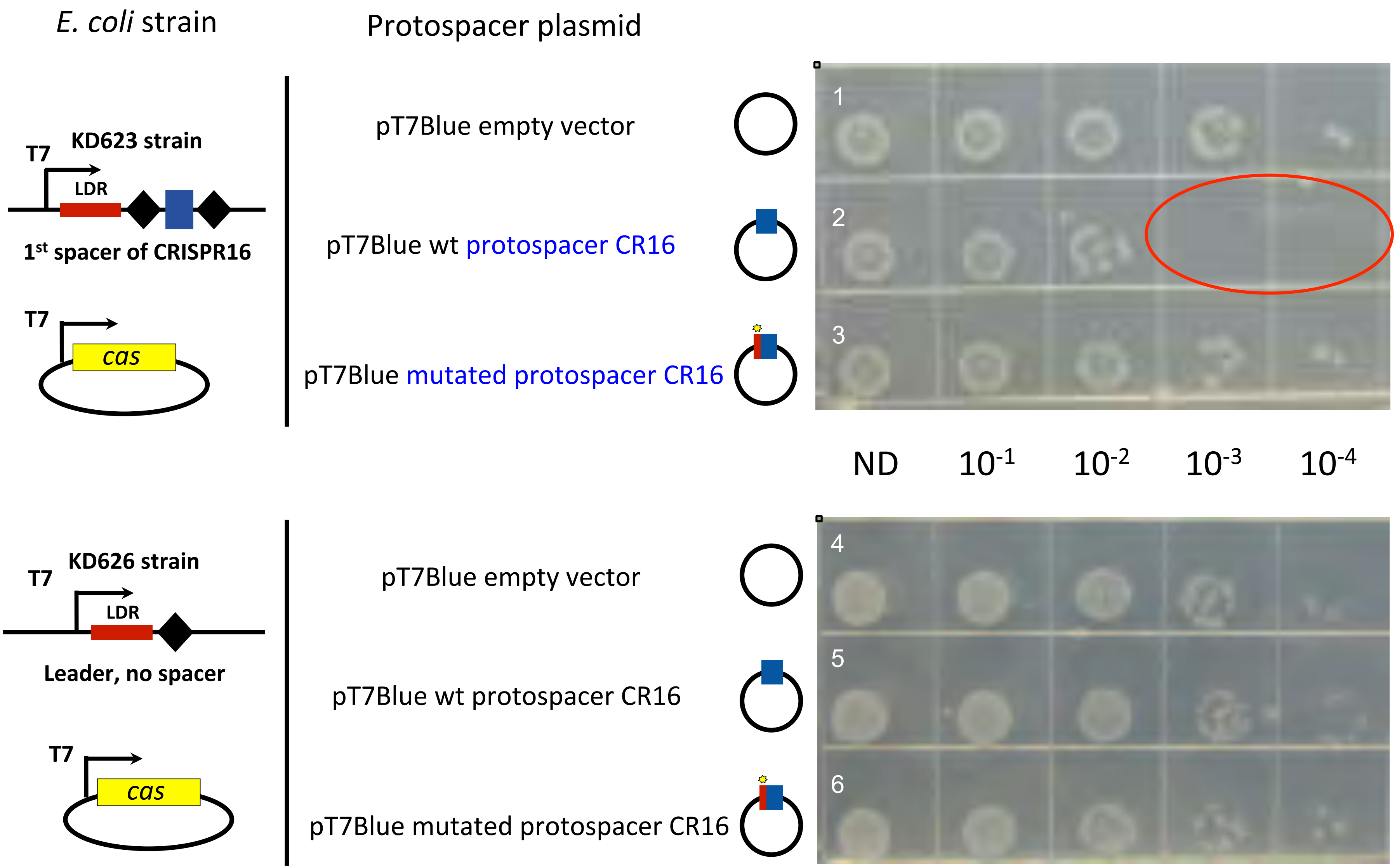
1st protospacer of CRISPR12





Boudry et al. mBio. 2015

Functionality of *C. difficile cas* genes for plasmid interference in *E. coli*



Boudry et al. mBio. 2015

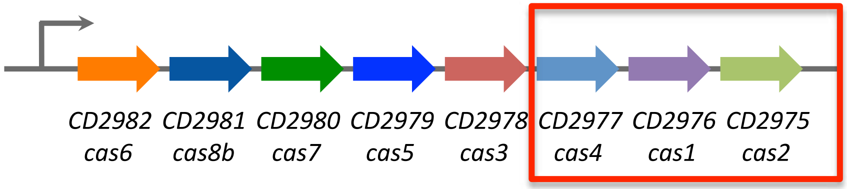
Functionality of CRISPR-Cas system in *C. difficile:* adaptation assays

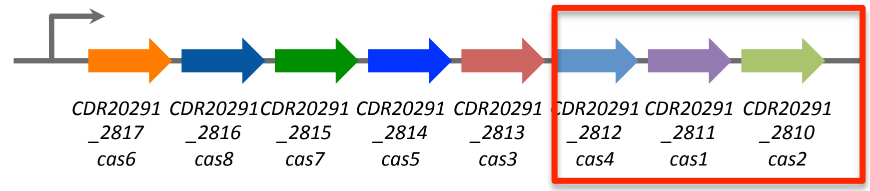
C. difficile **630E**

C. difficile

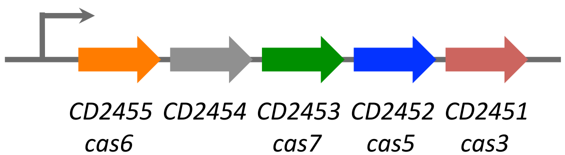
R20291

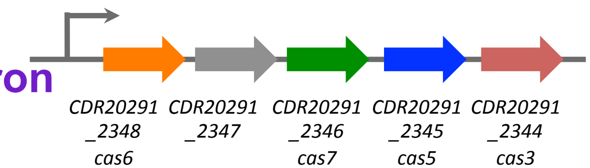
Full operon

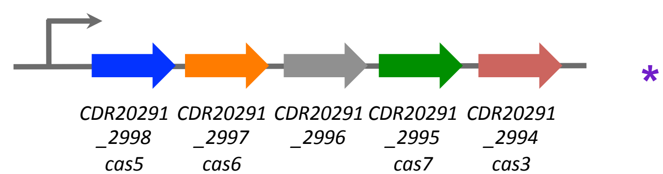




Partial operon







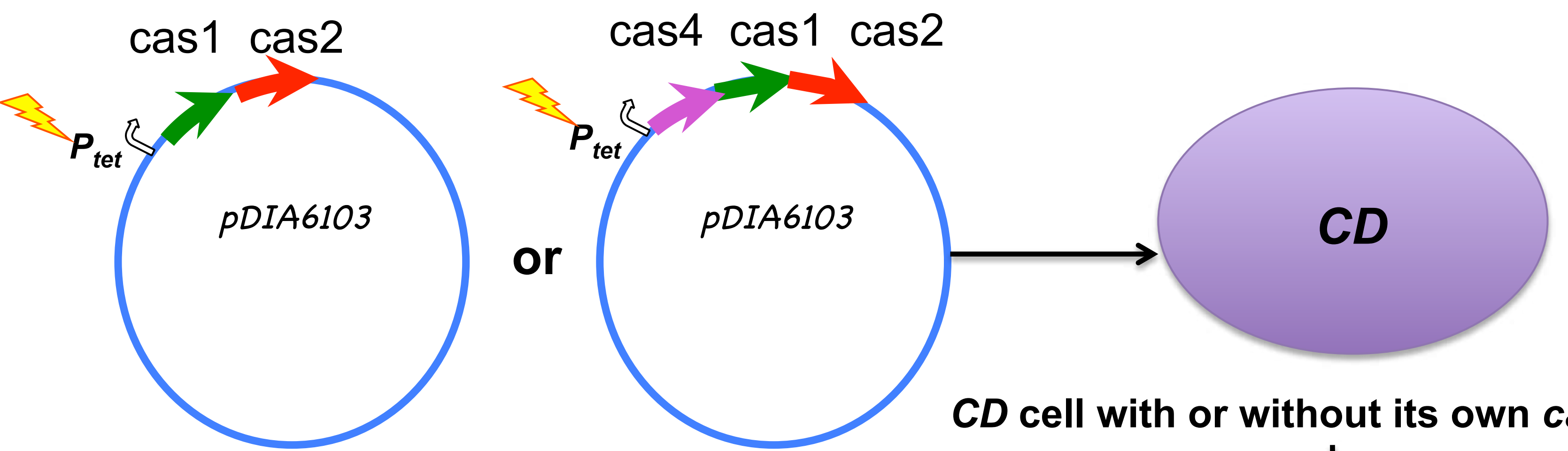
cas* operon specific to 027 ribotype strains

- **Cas1 and Cas2** are essential for new spacer acquisition on **all** CRISPR-Cas system types
- Potential role of **Cas4** in CRISPR-adaptation is actively discussed

Functionality of CRISPR-Cas system in *C. difficile:*

adaptation assays

Strategy

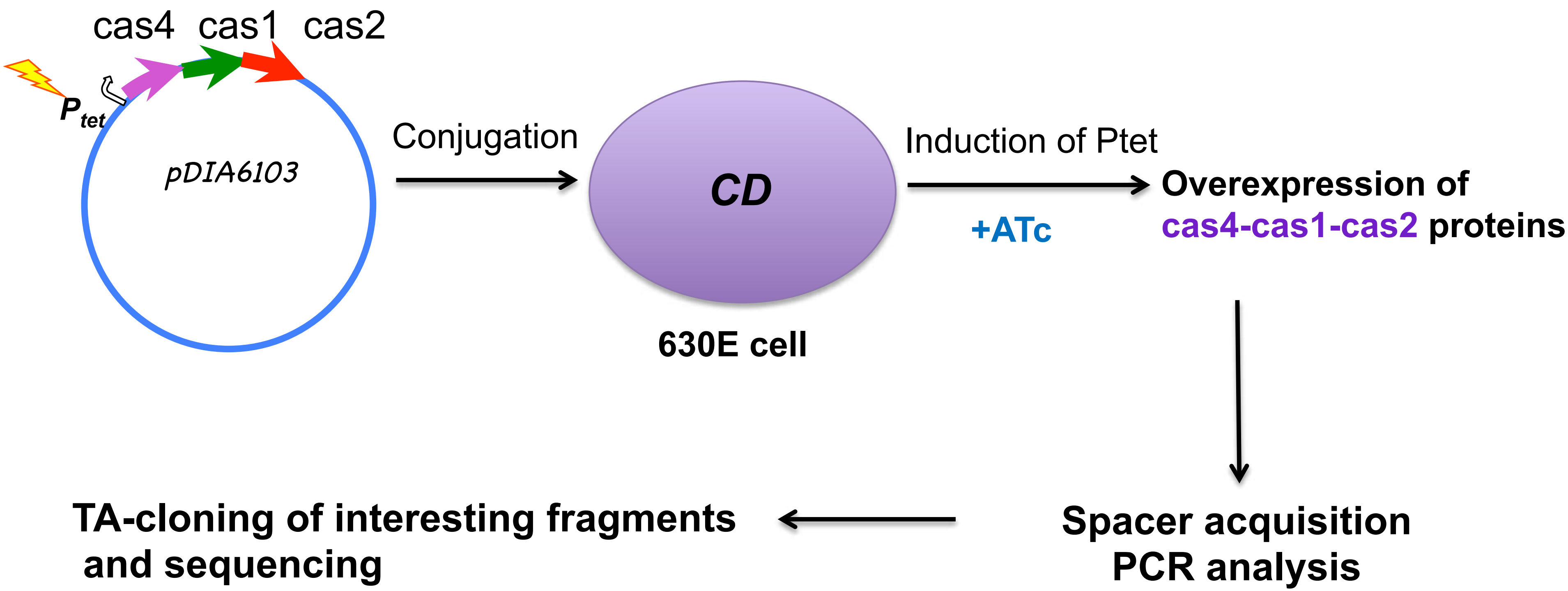


***CD* cell with or without its own *cas* operons**

+ same vectors
with mutated protospacers
for primed adaptation

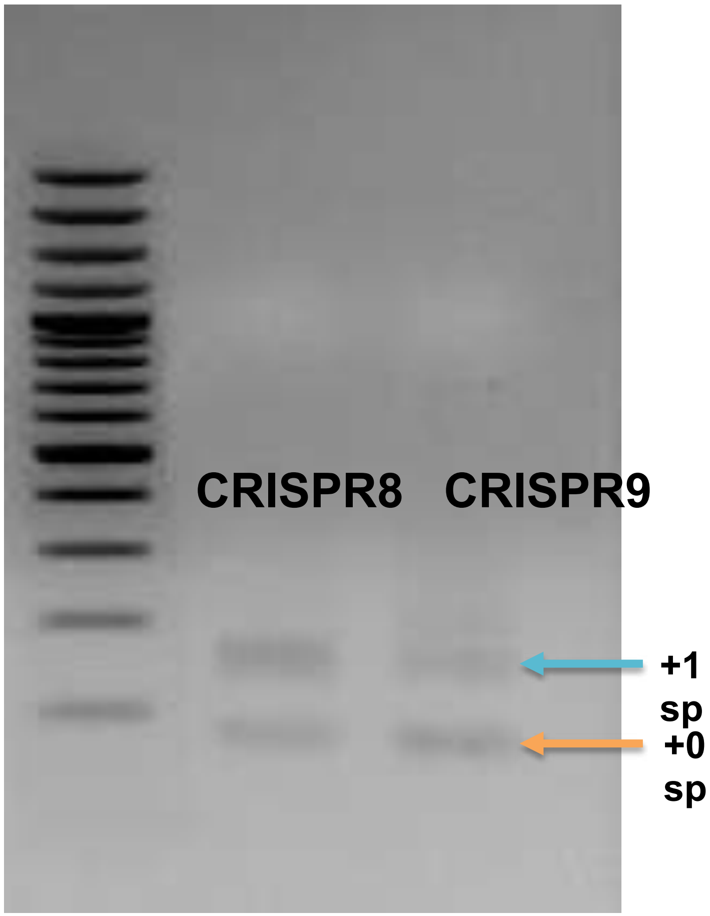
Spacer acquisition PCR analysis

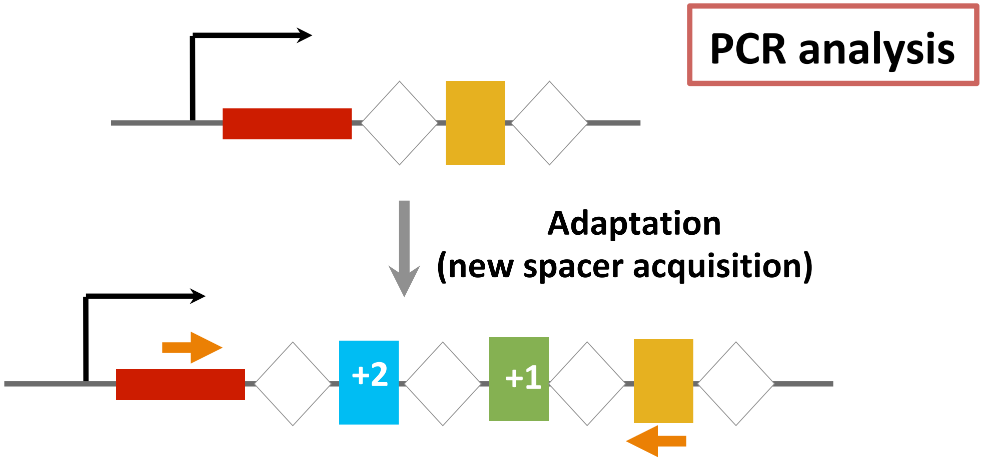
Functionality of CRISPR-Cas system in *C. difficile:* naïve adaptation assays



Functionality of CRISPR-Cas system in *C. difficile:*

naïve adaptation assays



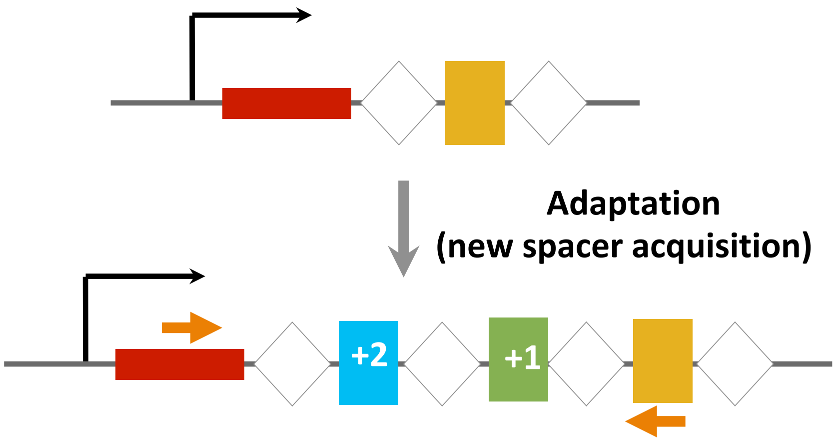


- CRISPR-Cas system in *CD* 630E **is functional for adaptation**
- New spacer acquisition was detected in **CR8** and **CR9** arrays
- These arrays are also **active for interference**
- All new acquired spacers were from the **plasmid**

Functionality of CRISPR-Cas system in *C. difficile:*

naïve adaptation assays

Maikova. PhD thesis
Maikova et al. mBio 2021

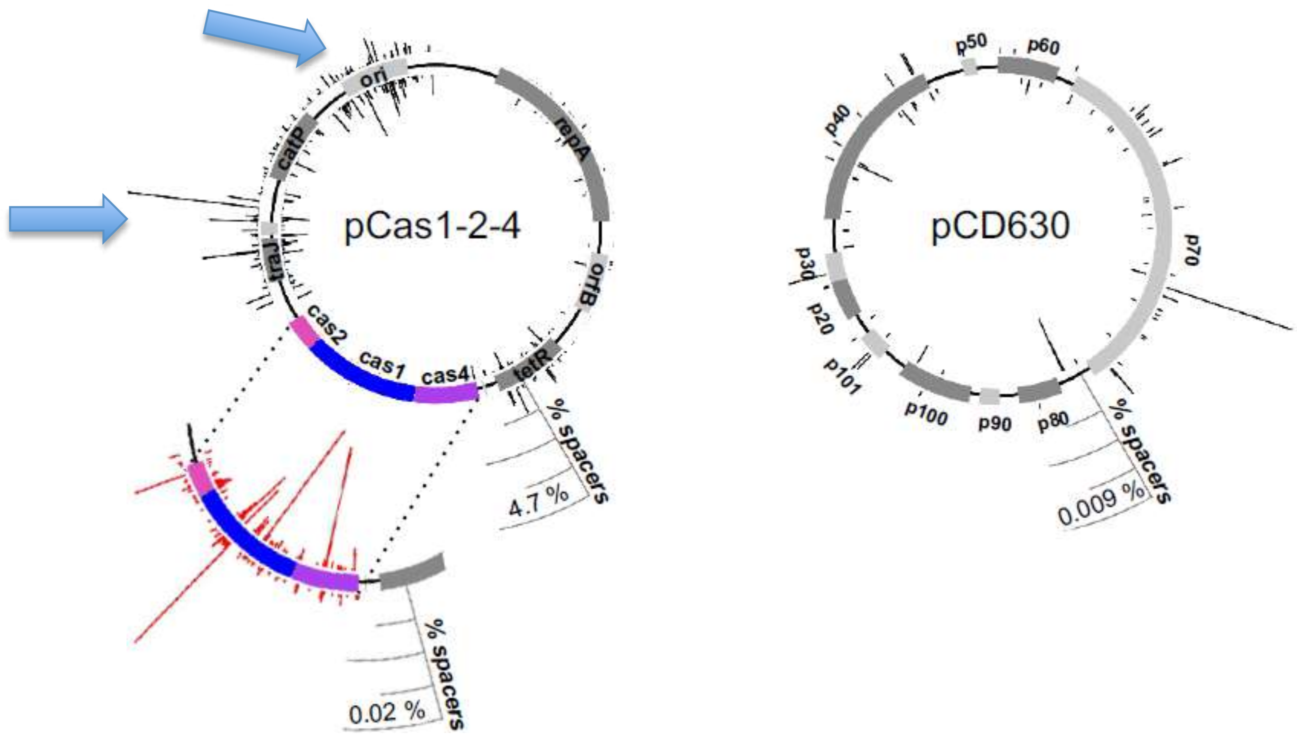


PCR analysis and NGS

Uniquely aligned spacers CRISPR8

Genome		**Plasmid**	
dir strand	rev strand	dir strand	rev strand
2474	1884	119274	111496
1.1%	0.8%	**50.7%**	**47.4%**

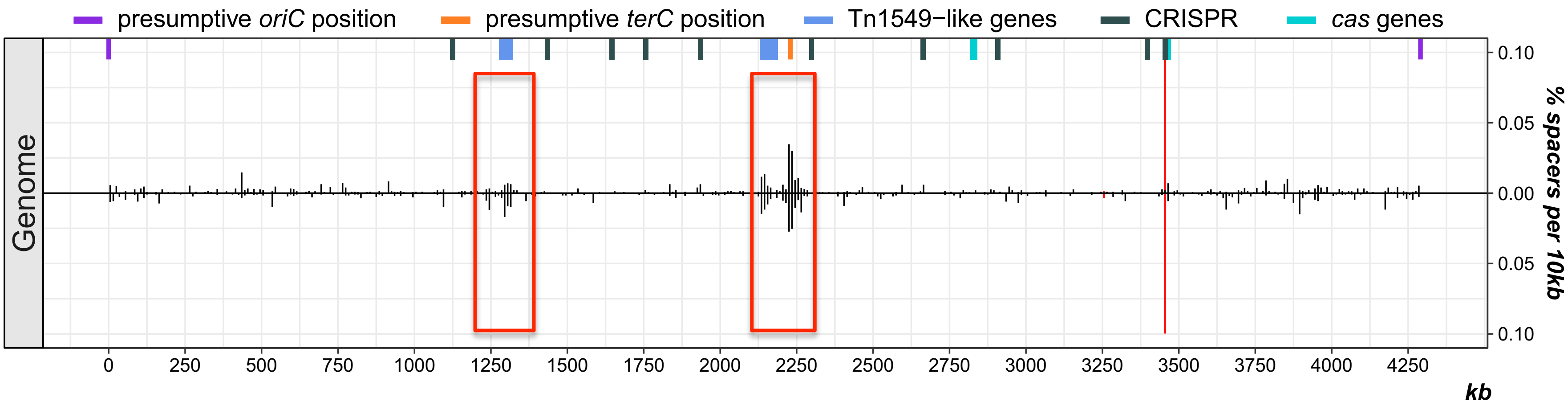
Spacer acquisition hot spots in *C. difficile*



- Adaptation hot spots on **pCas1-2-4 plasmid** are located in ***traJ*** and **ori** regions

Maikova. PhD thesis
Maikova et al. mBio 2021

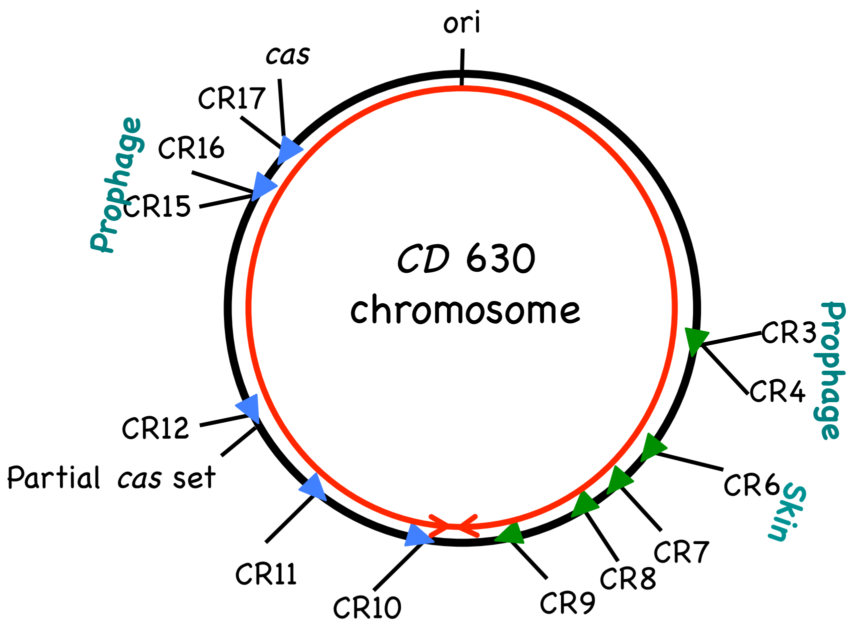
Spacer acquisition hot spots in *C. difficile*

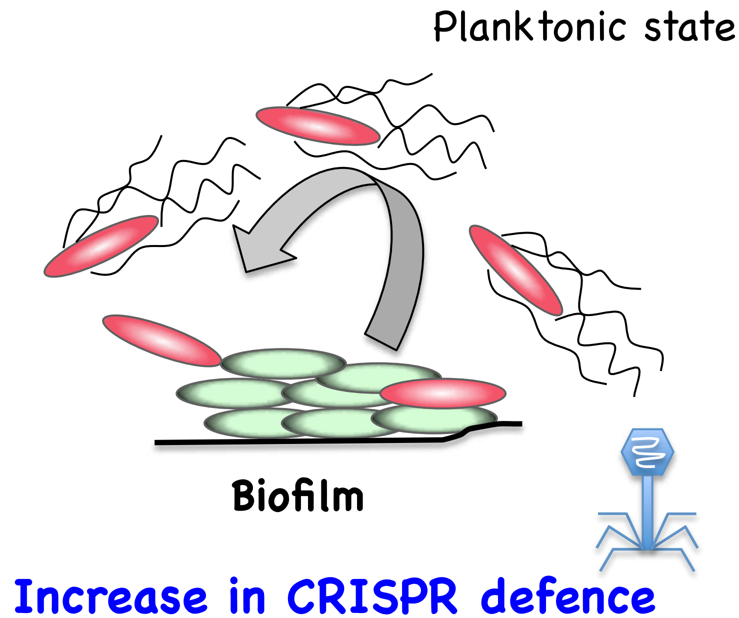


- Adaptation hot spots on **genome** are located in ***terC*** and **Tn1549-like gene** regions

Maikova. PhD thesis
Maikova et al. mBio 2021

Function and regulation of *C. difficile* CRISPR-Cas system



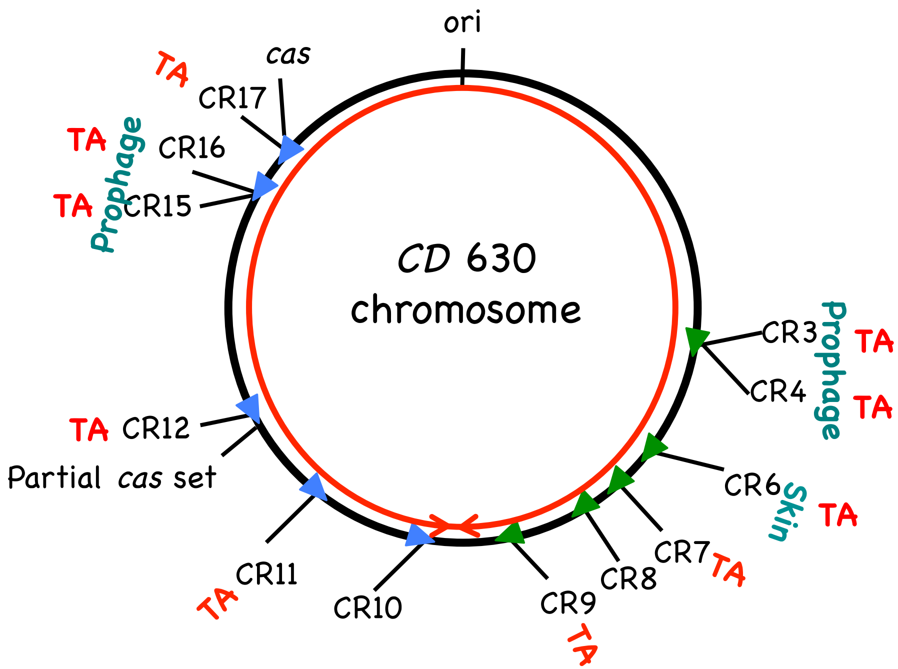


Perspectives: Role of the CRISPR-Cas during infection

- Regulation of **CRISPR-Cas expression** in response to stress-related factors (phages-biofilm)

Maikova A. PhD thesis

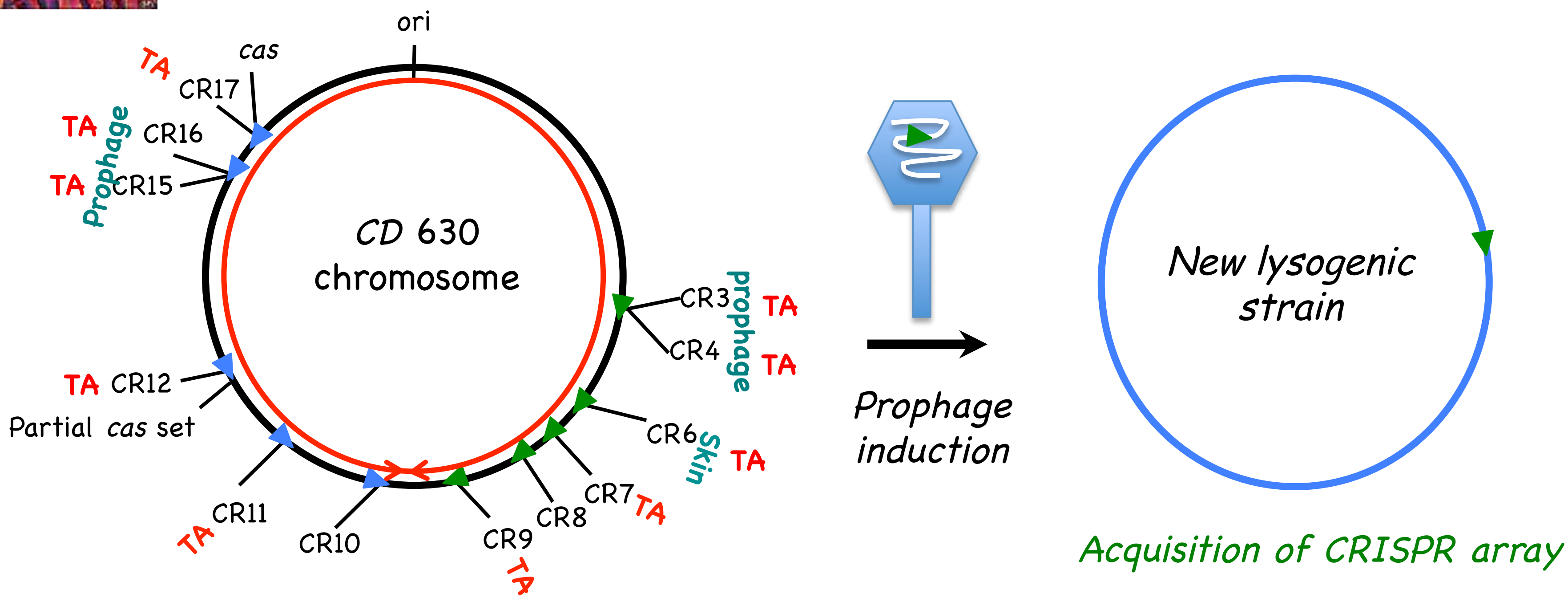
Function and regulation of *C. difficile* CRISPR-Cas system



Perspectives: Role of the CRISPR-Cas during infection

- Regulation of **CRISPR-Cas expression** in response to stress-related factors (phages-biofilm)
- Link with **Toxin/antitoxin (TA) systems of type I** (**defence islands**)
- Mechanism of **CRISPR array transfer** **(adaptation)**

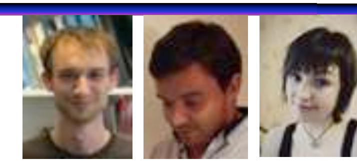
Evolution of CRISPR-Cas system in *C. difficile*

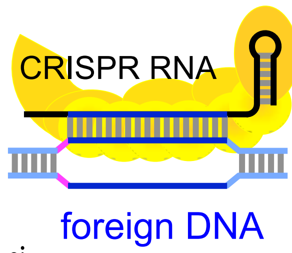


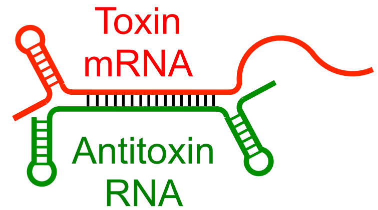
Genomic analysis of 3,000 *C. difficile* strains

- **Conservation of *cas* operons,** identification of 027-specific operon
- **Co-localisation of CRISPR and TA** (**defence islands**)
- Prophage localisation of **CRISPR arrays** **(evolution)**

Project 1: *CD* **CRISPR-Cas** system function and regulation

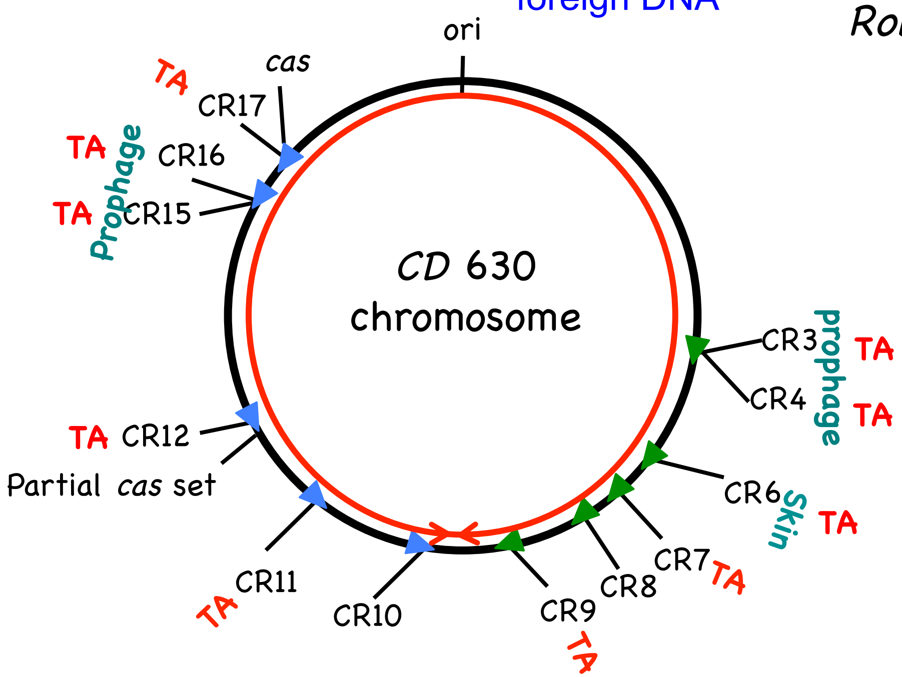


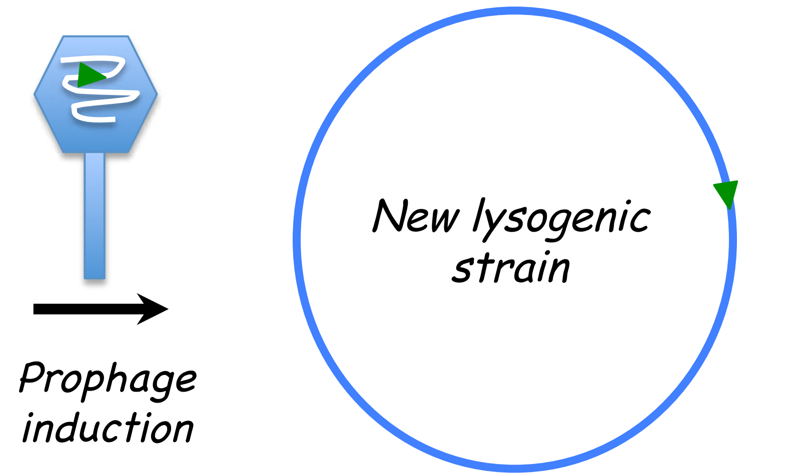




Role of TA in prophage stabilization

Boudry et al. mBio. 2015
Maikova et al. NAR. 2018
Maikova et al. PhD thesis
Peltier et al. Commun Biol 2020





Acquisition of CRISPR array

In *CD* strain 630 : 12 CRISPR regions exist and are expressed

In epidemic *CD* strain 027: 9 active CRISPR arrays

…. A total of 819 spacers from nine *CD* strains

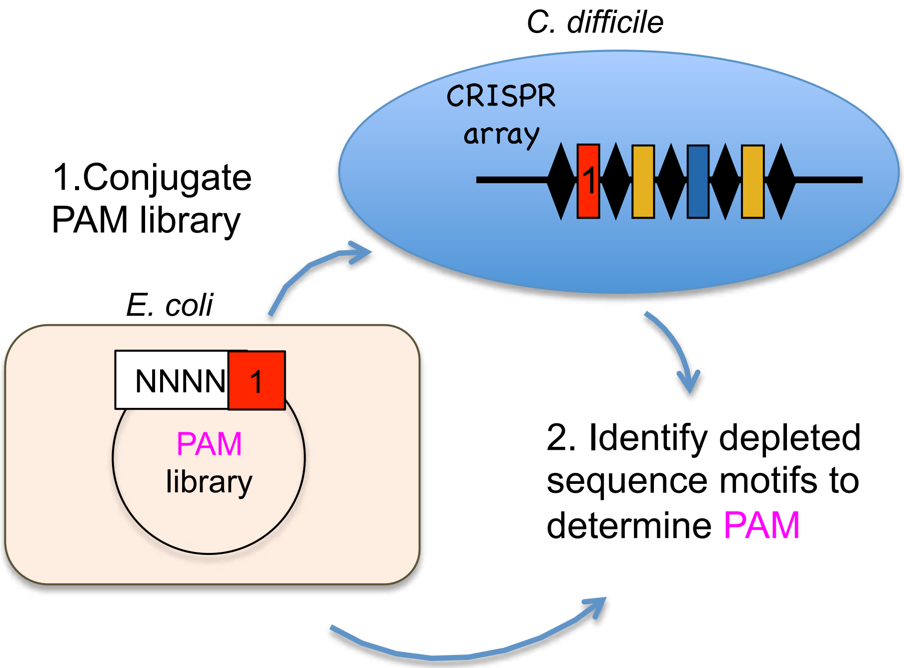
Large defense capacity within phage-rich gut communities

Results:

II. **Using** of CRISPR-Cas system in *C. difficile* for genome editing

PAM sequences in *C. difficile* 630E and R20291 strains

Maikova. PhD thesis
Maikova et al. mBio 2021

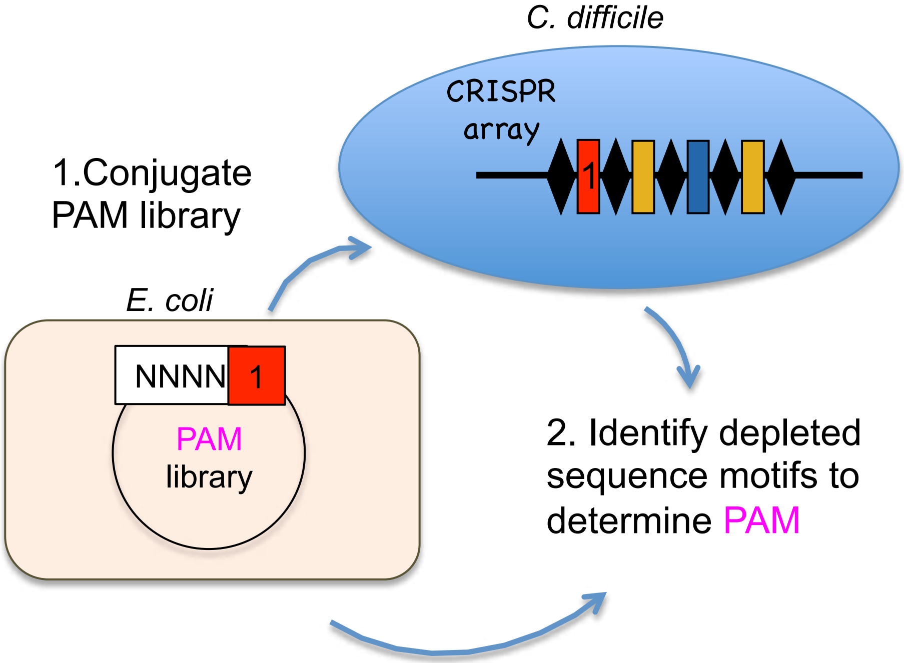


Two PAM plasmid libraries:

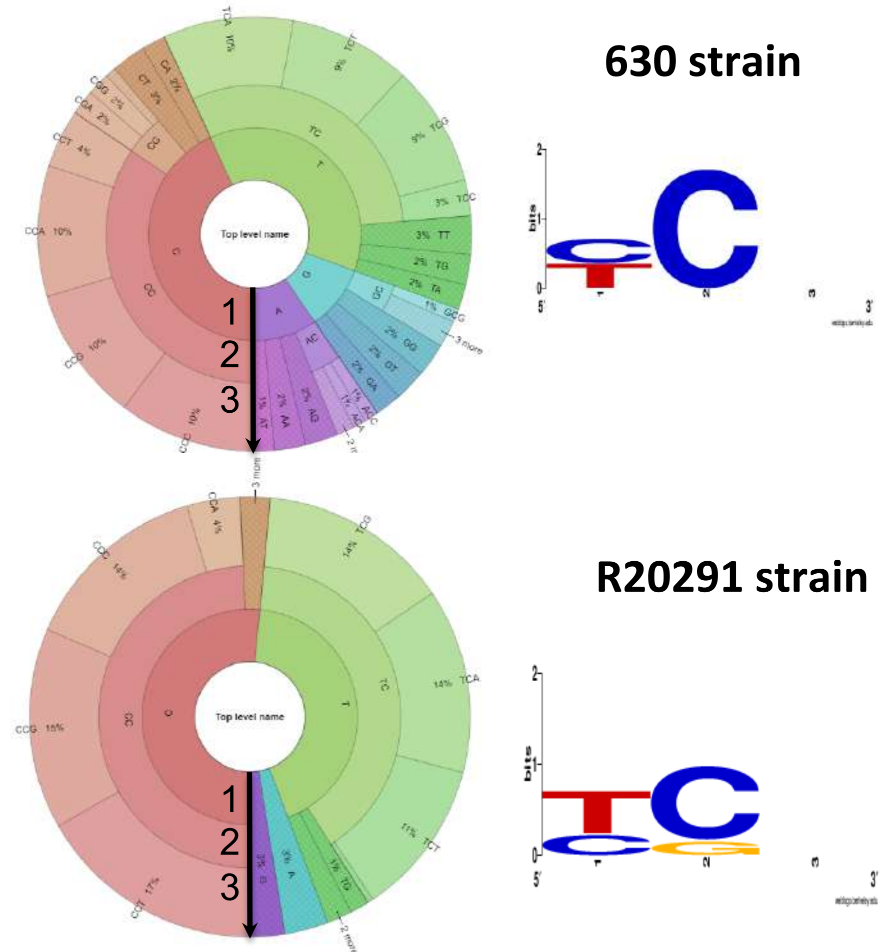
1. Vector-NNNN (PAM)-protospacer 1 CRISPR16 CD 630
2. Vector-NNNN (PAM)-protospacer 1 CRISPR13 CD R20291

PAM sequences in *C. difficile* 630E and R20291 strains

Maikova. PhD thesis
Maikova et al. mBio 2021

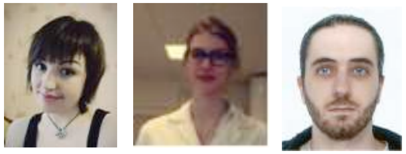


HTS analysis

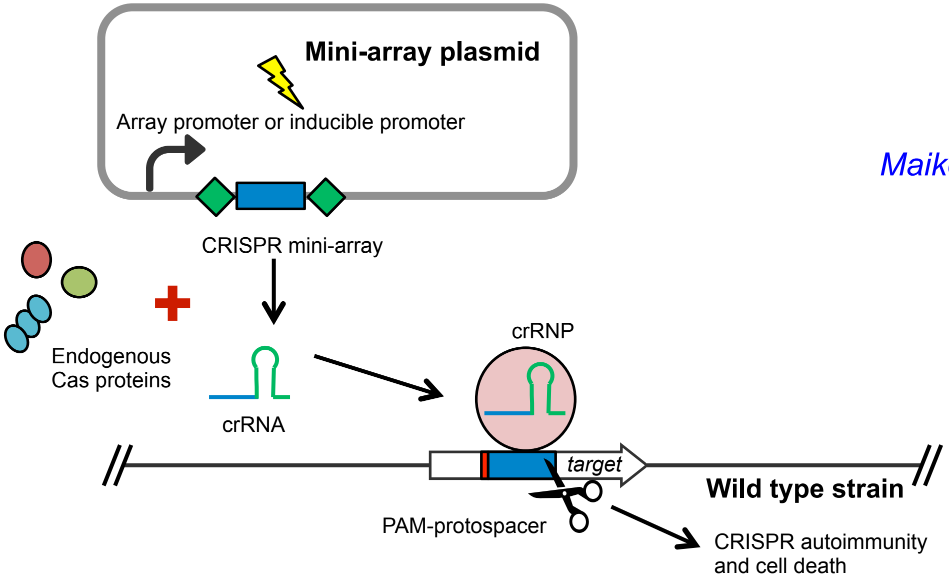


Functional PAMs **CCN**/**TCN** in accordance with interference efficiency experiments

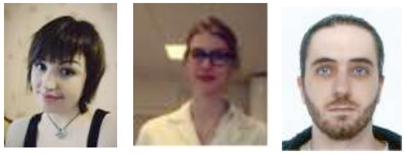
Genome editing in *C. difficile* using the endogenous CRISPR-Cas system



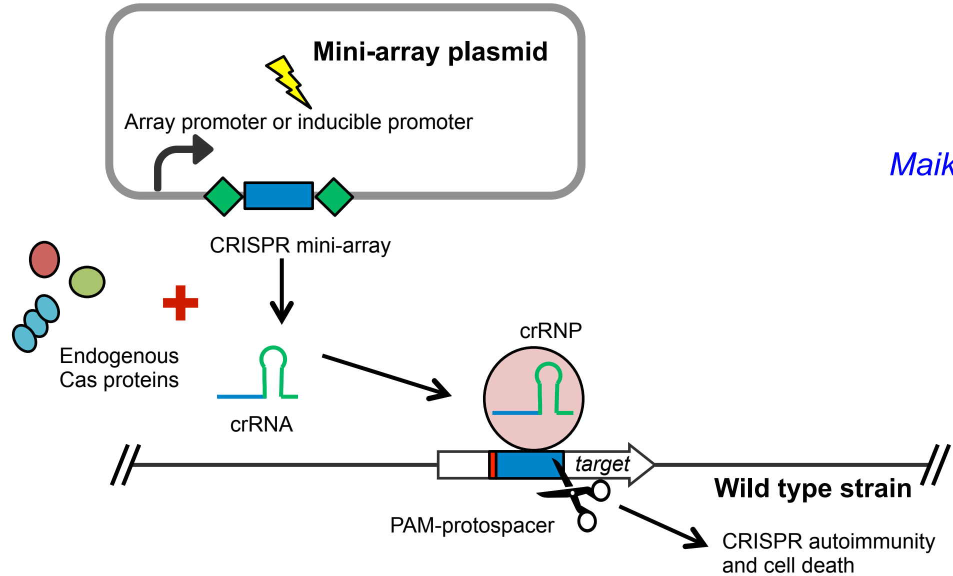
Maikova et al. Frontiers Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019.

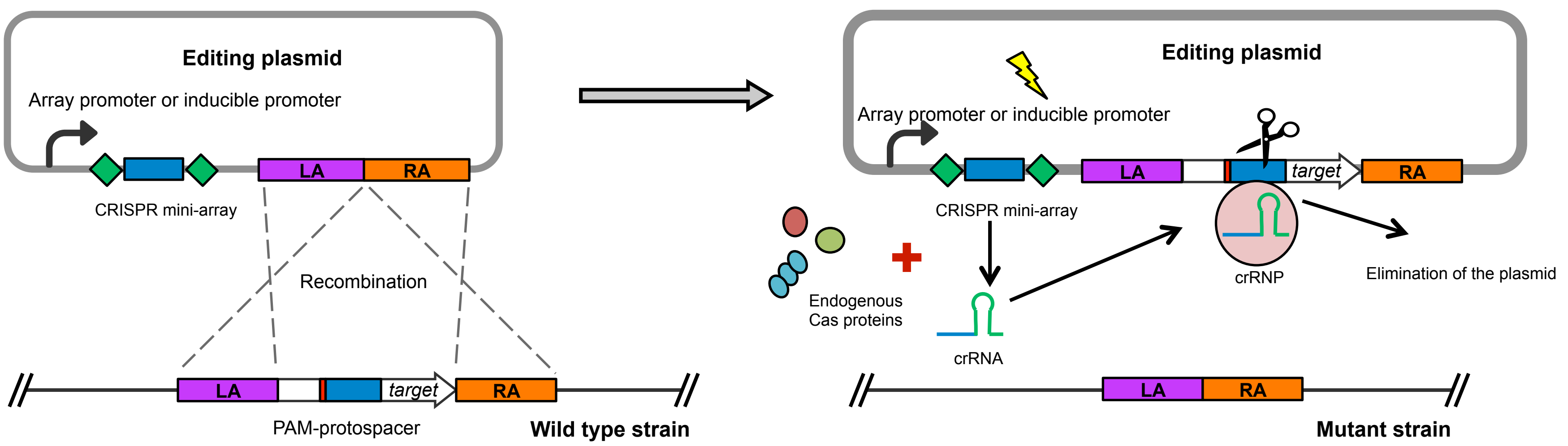


Genome editing in *C. difficile* using the endogenous CRISPR-Cas system



Maikova et al. Frontiers Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019.

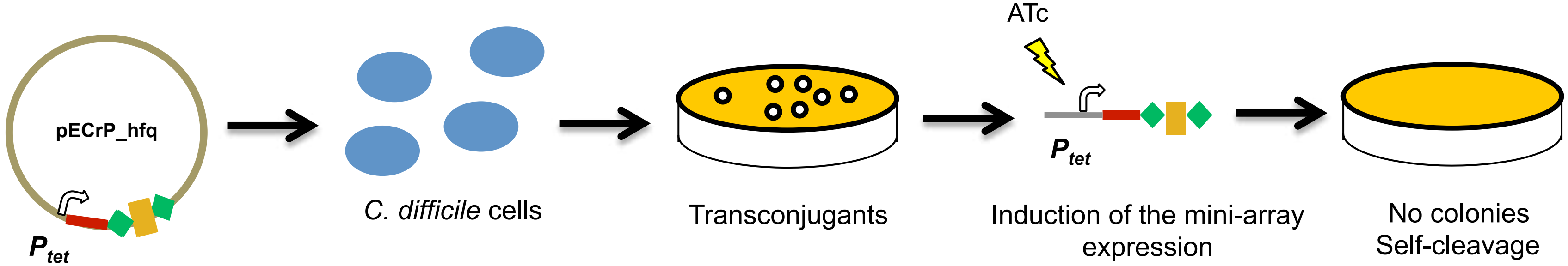




Genome editing in *C. difficile* using the endogenous CRISPR-Cas system

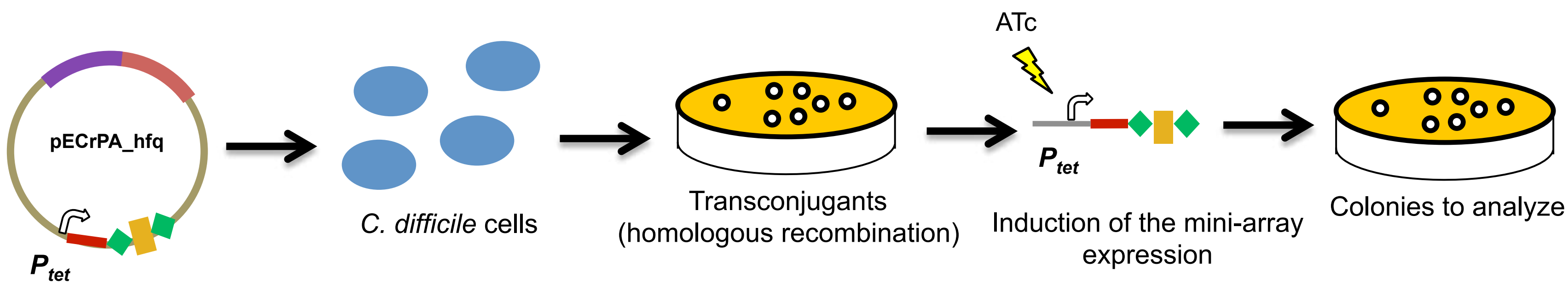
Maikova et al. Frontiers Microbiol. 2018
Maikova. PhD thesis
Maikova et al. AEM. 2019.

Induced endogenous **CRISPR-Cas autoimmunity**

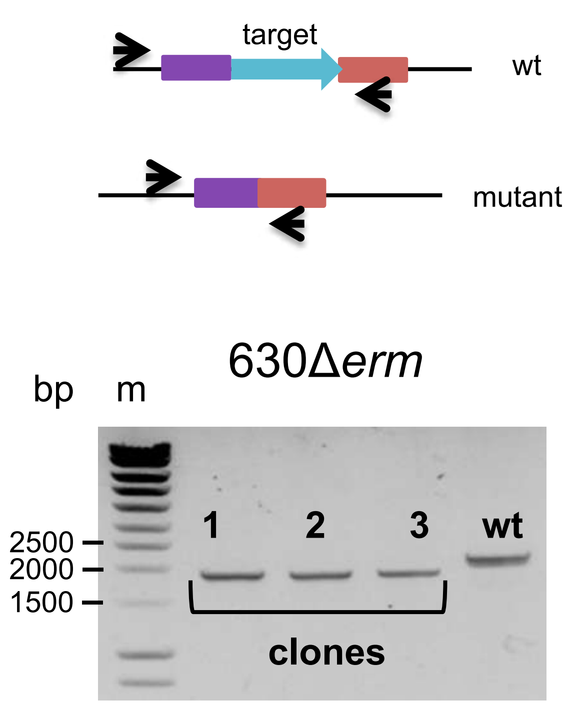


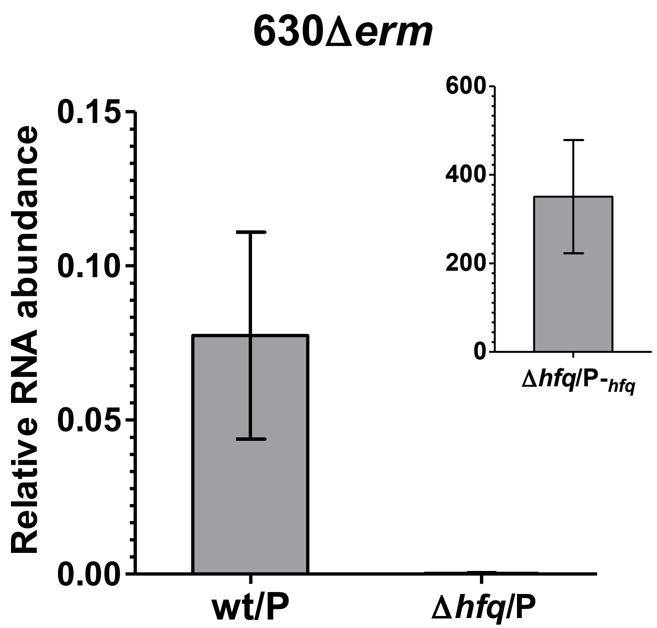
new type of drugs against *CD* ?

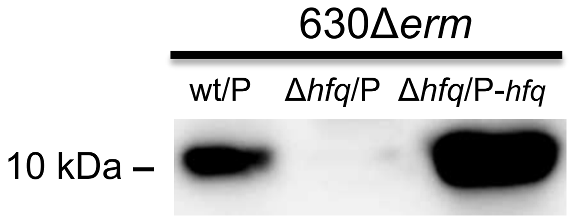
Mutant construction using plasmid with homologous arms



Genome editing in *C. difficile* using the endogenous CRISPR-Cas system







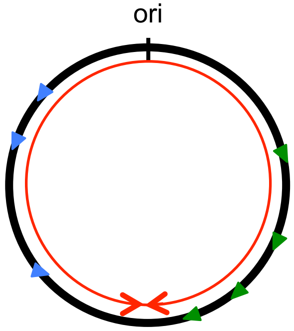
- ***Δhfq* mutants** in 630 and R20291 strains
- **Could not be obtained** using **other** genome editing methods (*codA* allele exchange and ClosTron)
- **Efficient plasmid** loss

Maikova. PhD thesis
Maikova et al. AEM. 2019.

Results:

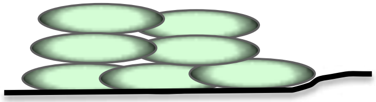
III. **Regulation** of CRISPR-Cas system in *C. difficile*

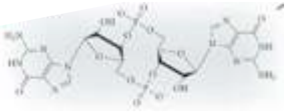
Complex regulation of CRISPR system in *C. difficile*



- Optimization of CRISPR loci transcription (conserved orientation in direction of replication)

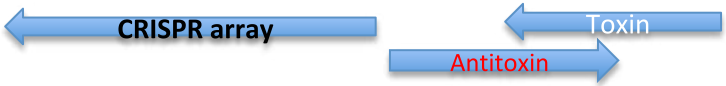
- Induction within **biofilm** communities for efficient defence against foreign DNA, role of biofilm-related signalling molecules (c-di-GMP)



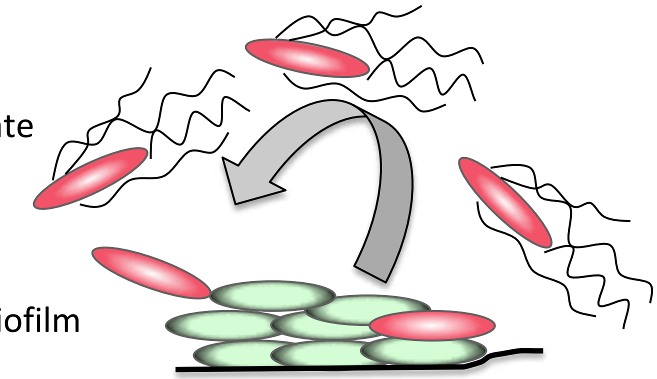


- Control in response to **stress-related factors** relevant for *C. difficile* infection cycle (sigma B control, interactions with **phages**, stress conditions inside the host)

- Link with Toxin/antitoxin (TA) systems of type I, co-localization and co-regulation of CRISPR and TA systems within “defence islands” in *C. difficile* chromosome



Regulation of *C. difficile* CRISPR system within biofilms



High Low

Motility ← **C-di-GMP** → **Biofilm formation**

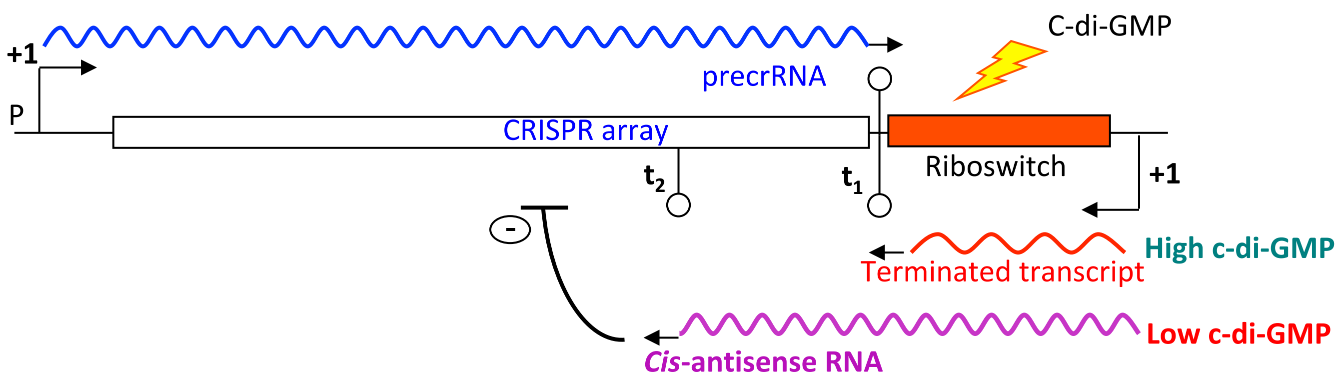
High Low

Riboswitch I
Negative control of target genes

Riboswitch II
Positive control of target genes

Phage resistance mediated by CRISPR system

Riboswitch I associated with ***cis*-antisense RNA**
Positive control of crRNA expression

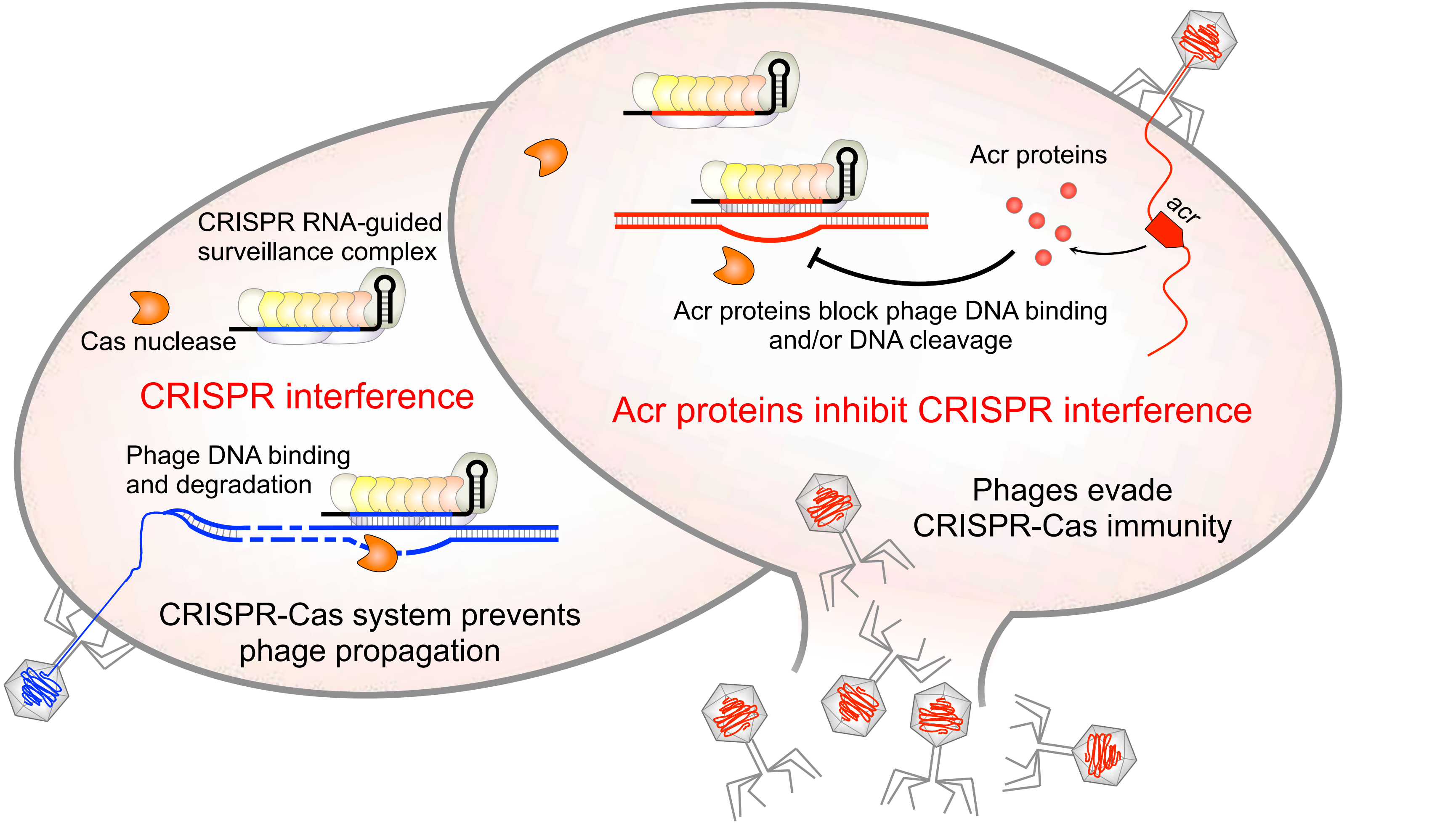


Results:

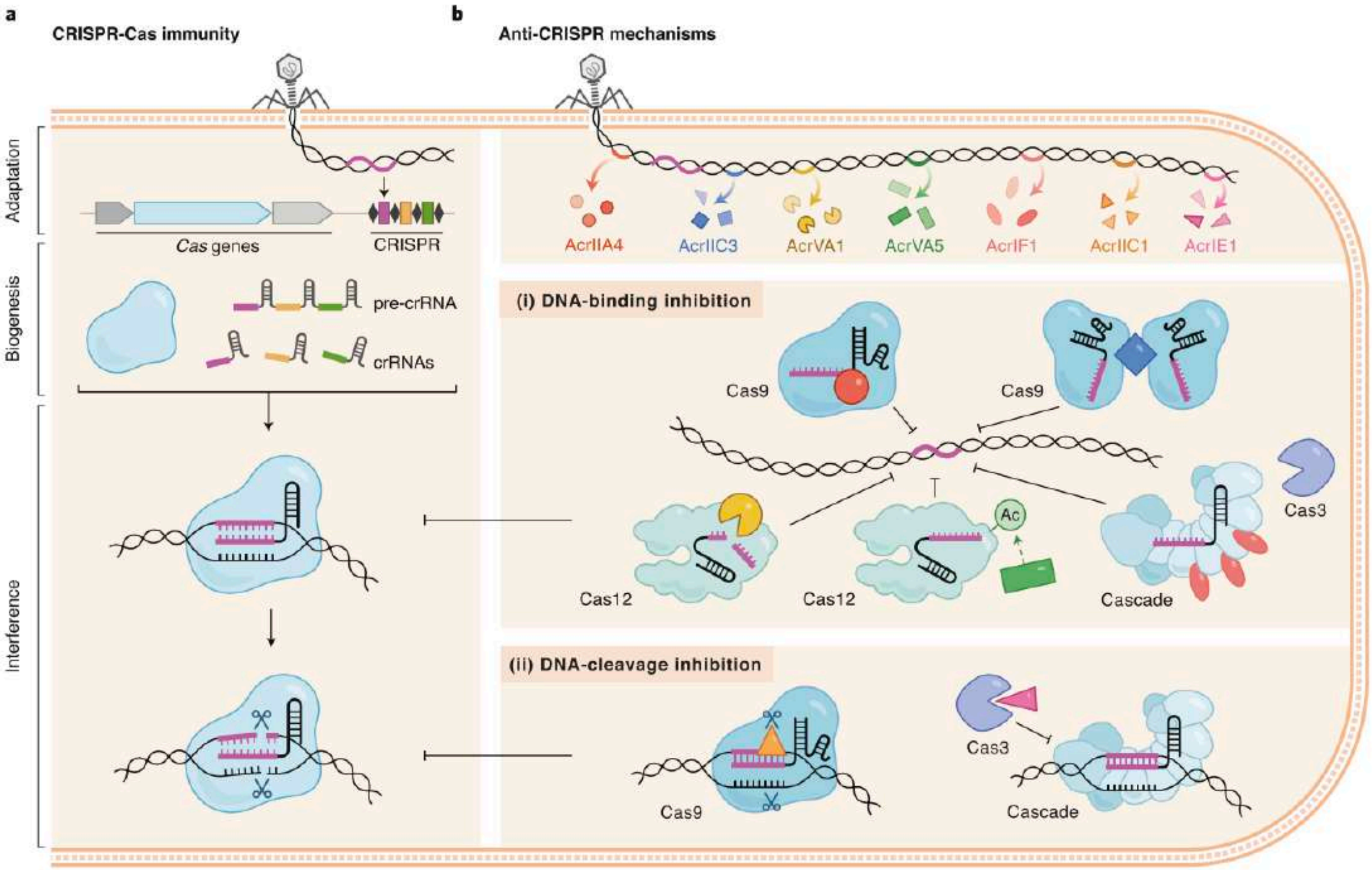
IV. **Inhibitors** of CRISPR-Cas system in *C. difficile*

Potential inhibitors of CRISPR-Cas system in *C. difficile*

Acr proteins in suppression of CRISPR-mediated immunity



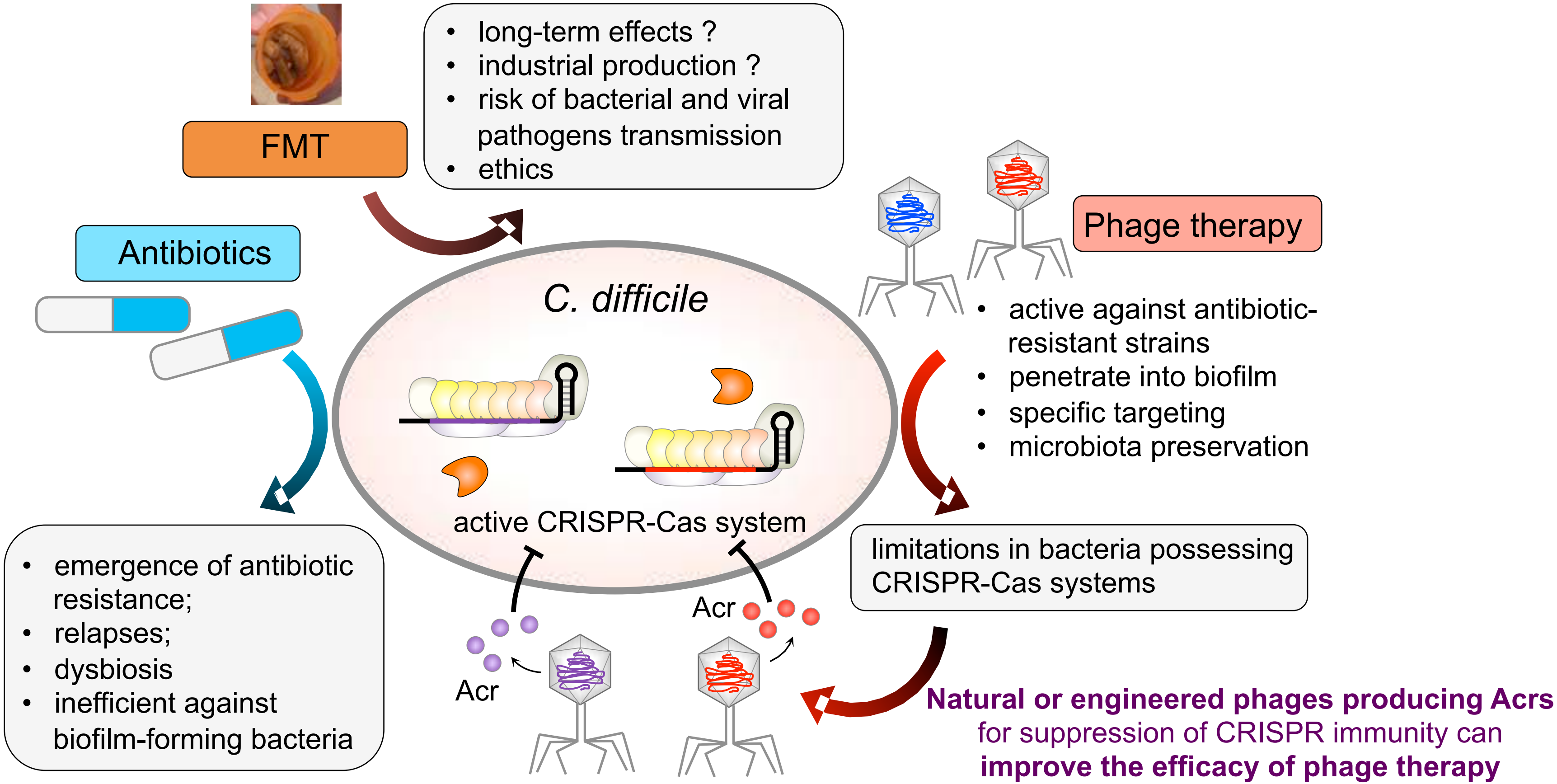
Mechanisms of anti-CRISPR protein activity



Stanley and Maxwell, 2018

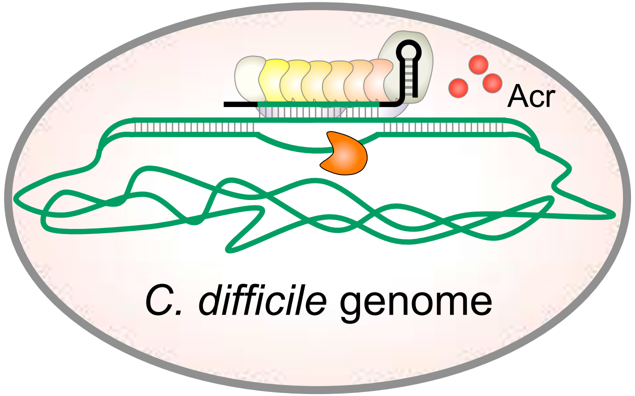
Potential applications of Acr proteins in *C. difficile* for phage therapy

Treatment of *C. difficile* infection



Potential applications of Acr proteins in *C. difficile* for genome editing

Genome editing in *C. difficile* using endogenous CRISPR-Cas system



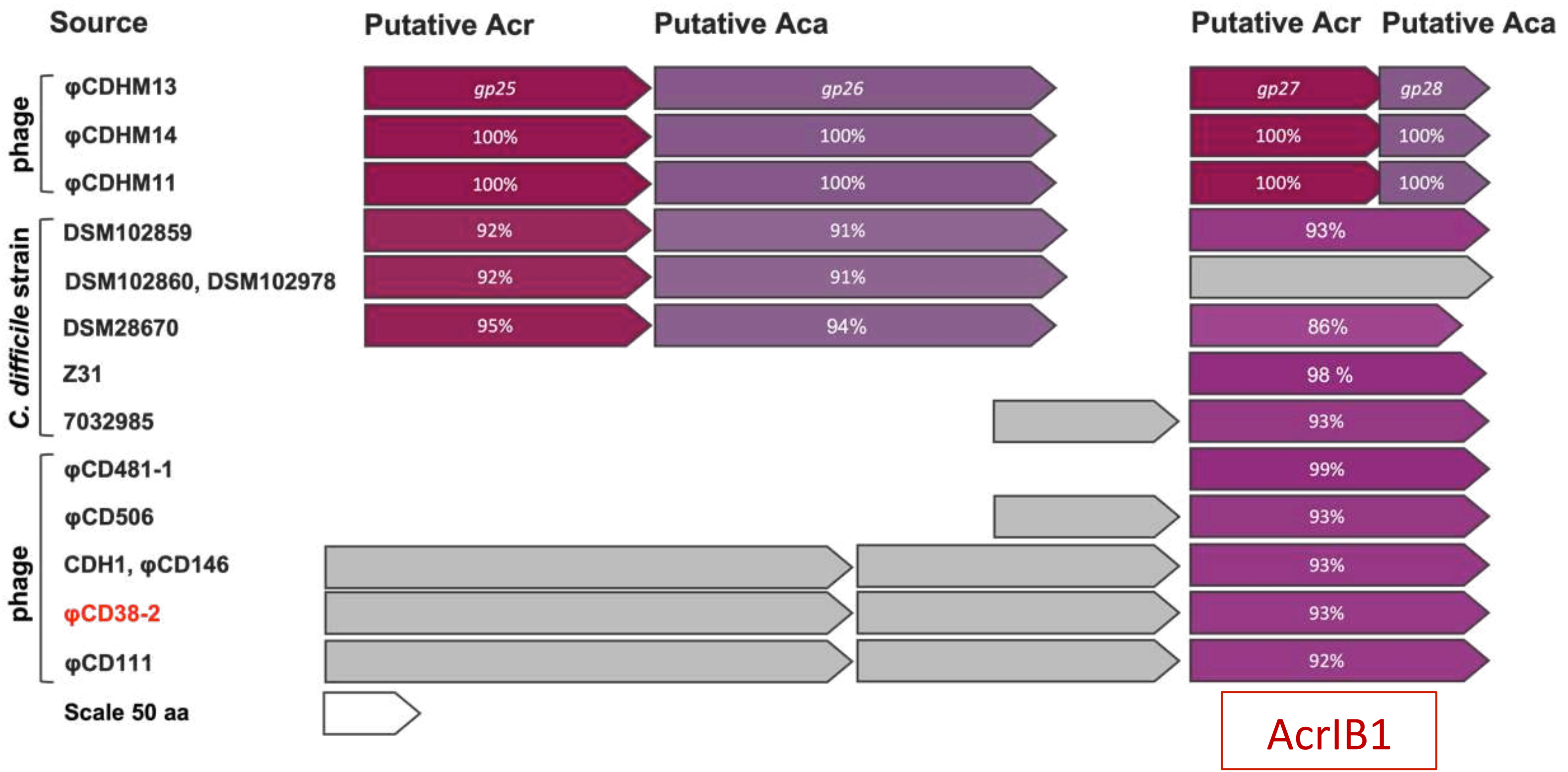
The use of Acr proteins in genome editing can provide:

- precise temporal control of CRISPR-Cas activity through "ON-OFF" regulation
- decreasing off-target effects
- reducing the toxicity of self-targeting CRISPR-Cas system

Putative anti-CRISPR loci of *C. difficile* phages

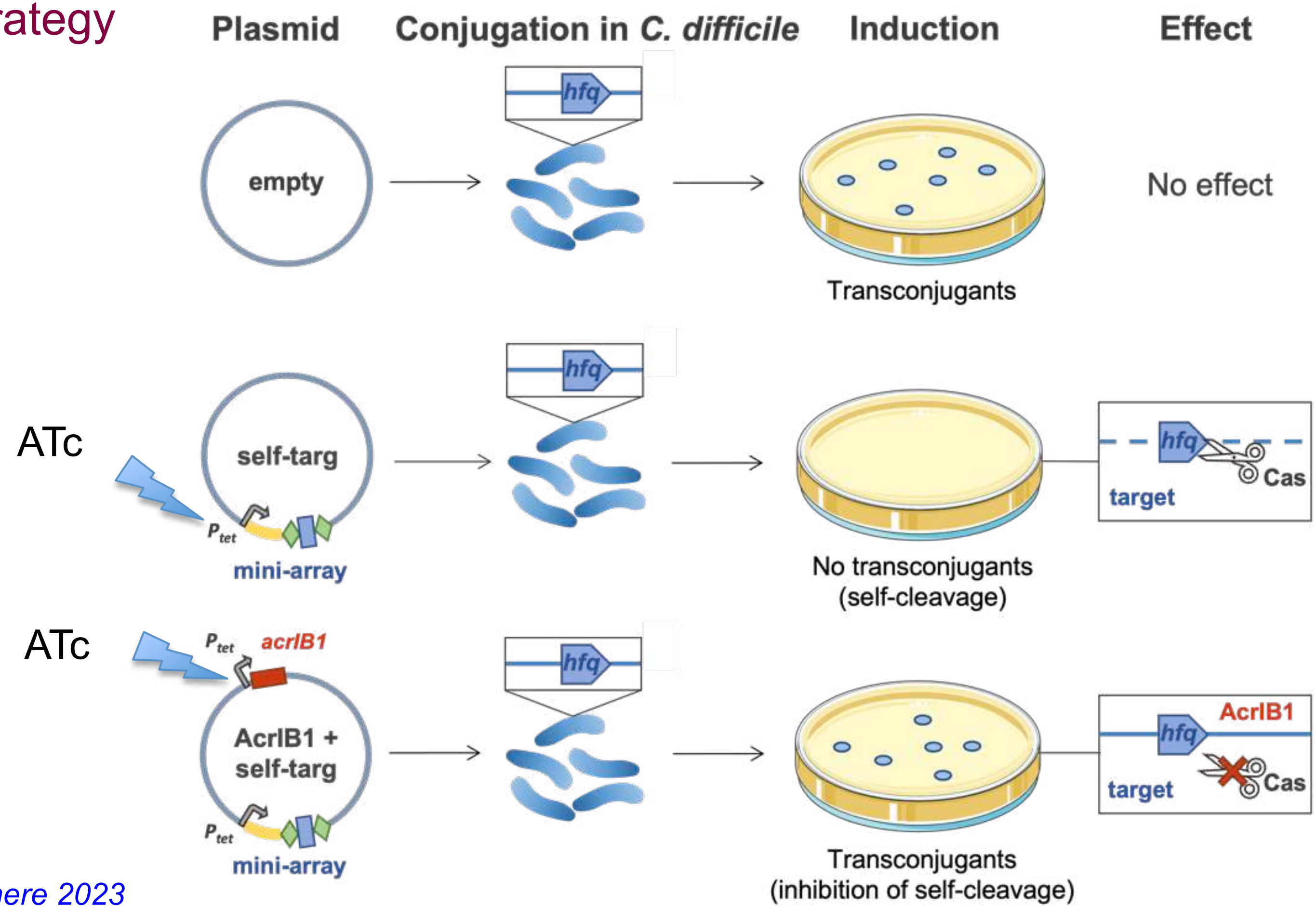
A. Borges, J. Bondy-Denomy

- putative anti-CRISPR (Acr)
- putative anti-CRISPR associated (Aca)



C. difficile conjugation experiment for AcrIB1 efficiency testing

Self-targeting strategy



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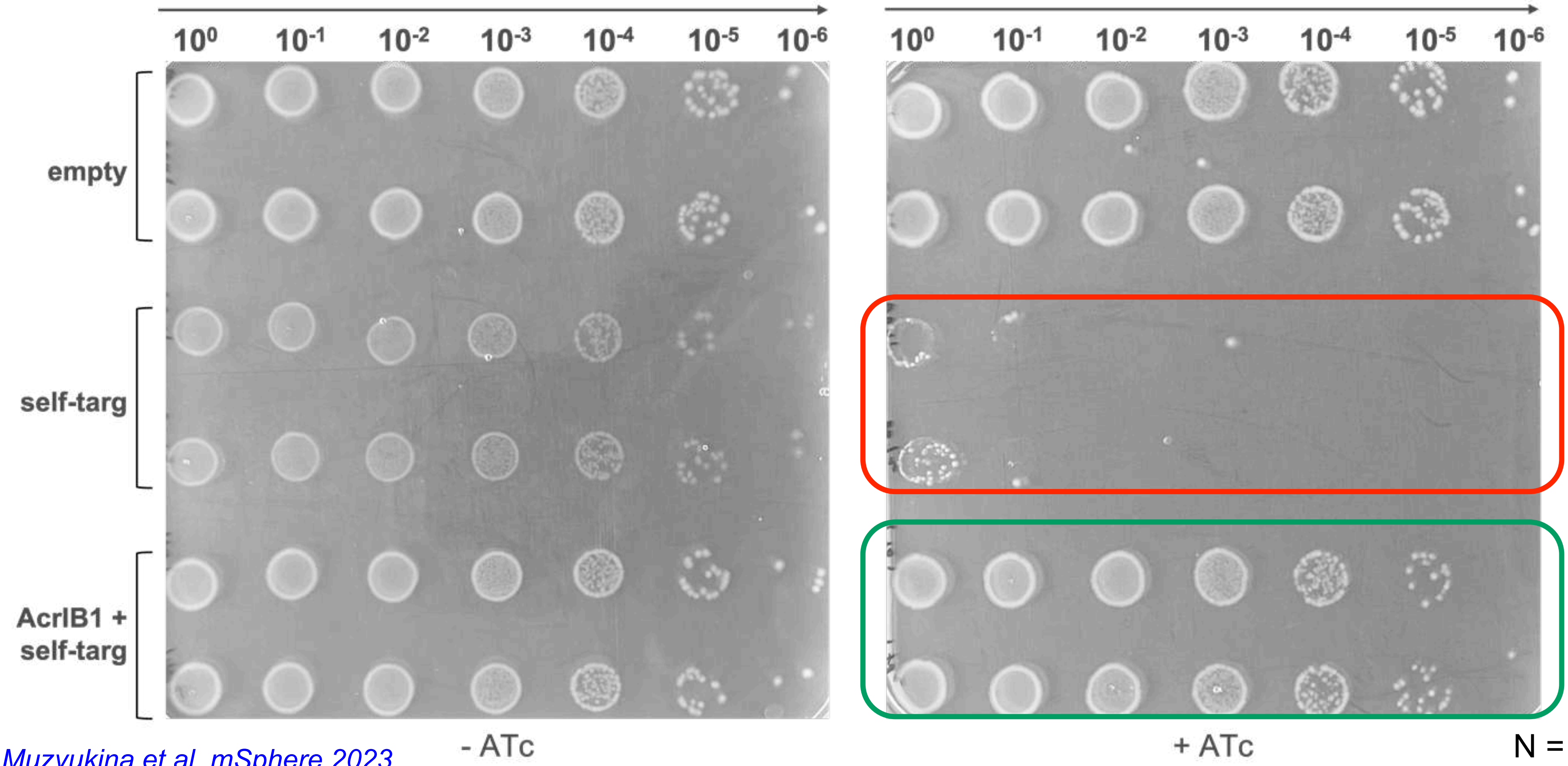
acrIB1 gene expression inhibits CRISPR interference in *C. difficile*

Self-targeting strategy



N = 3

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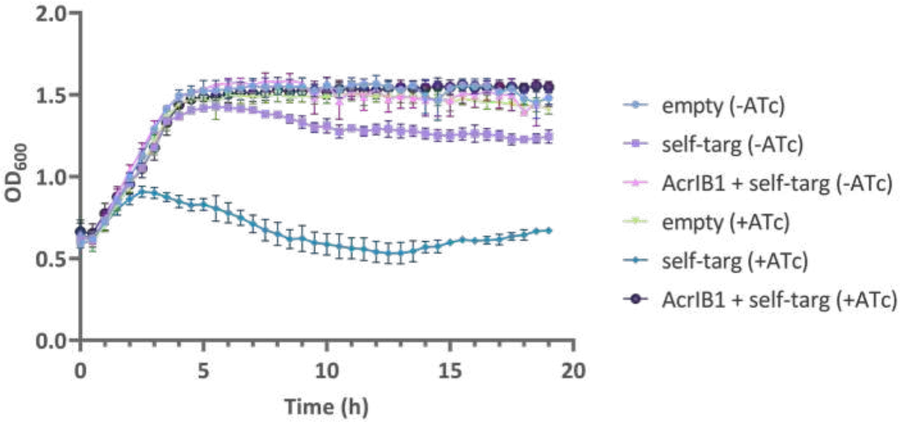
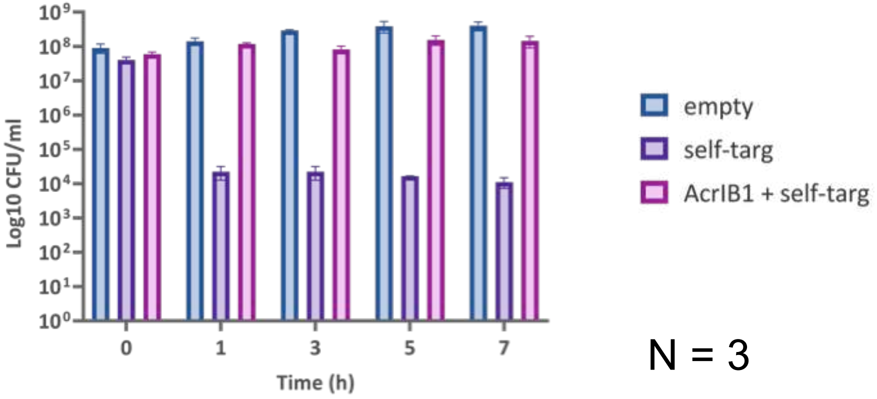
acrIB1 gene expression inhibits CRISPR interference in *C. difficile*

Effect of anti-CRISPR self-targeting inhibition on bacterial growth in BHI liquid medium





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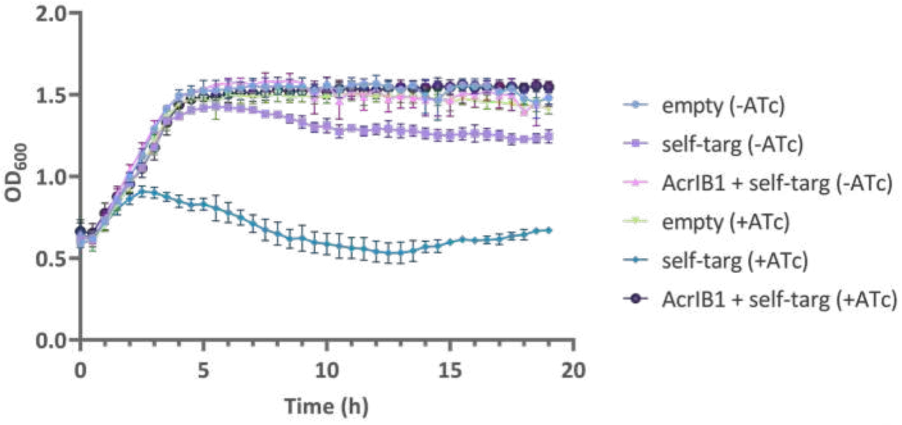
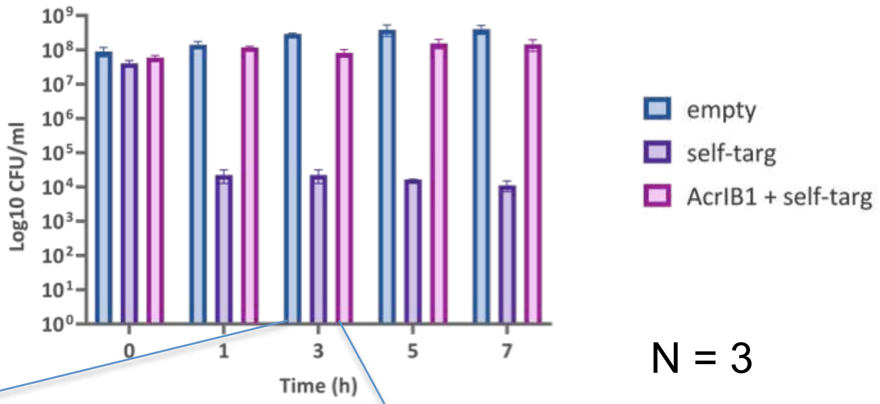
acrIB1 gene expression inhibits CRISPR interference in *C. difficile*

Effect of anti-CRISPR self-targeting inhibition on bacterial growth in BHI liquid medium





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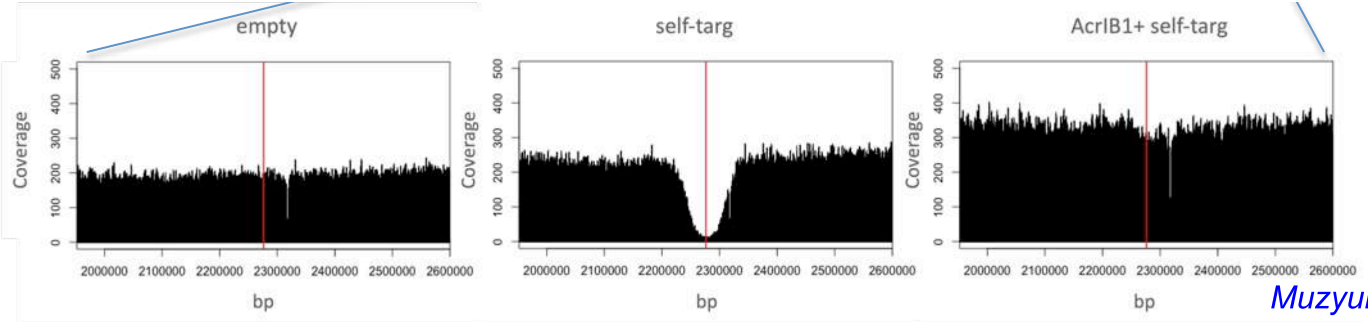
Effect of self-targeting and anti-CRISPR inhibition of self-targeting on genomic DNA content



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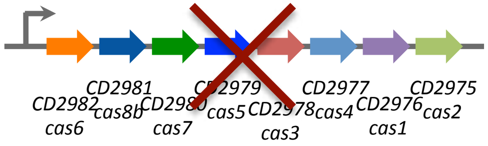
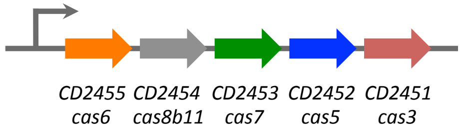
Deletion of partial *cas* operon disables CRISPR inhibitory activity

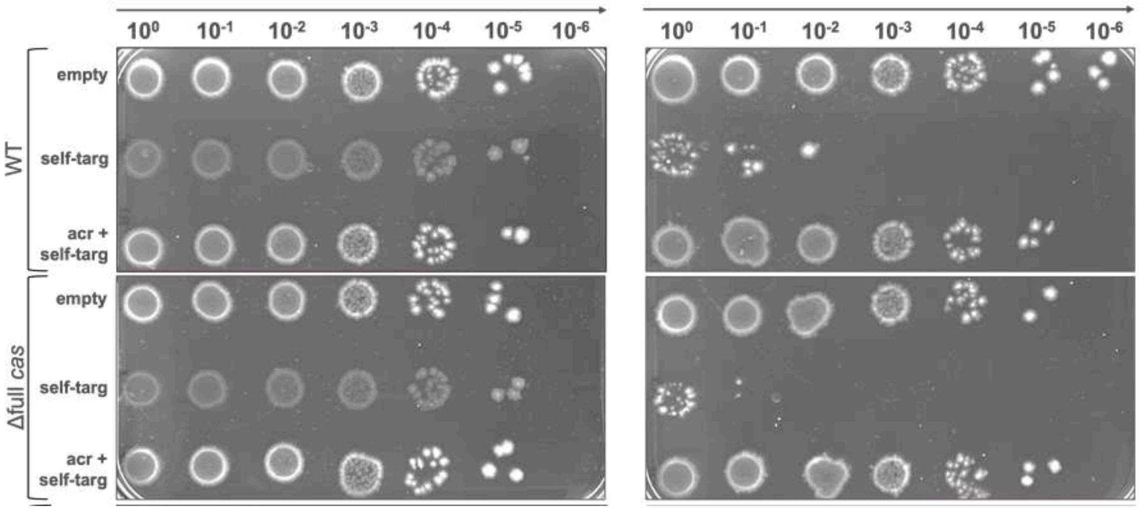


effector module

adaptation module



effector module



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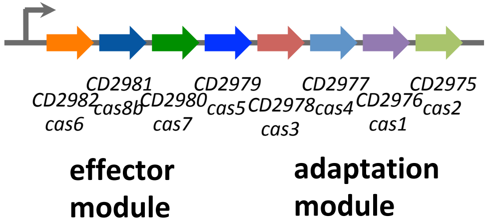
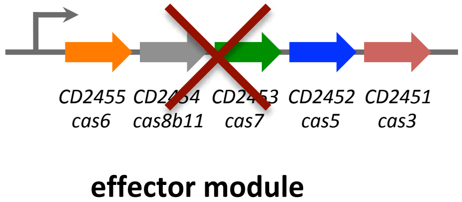
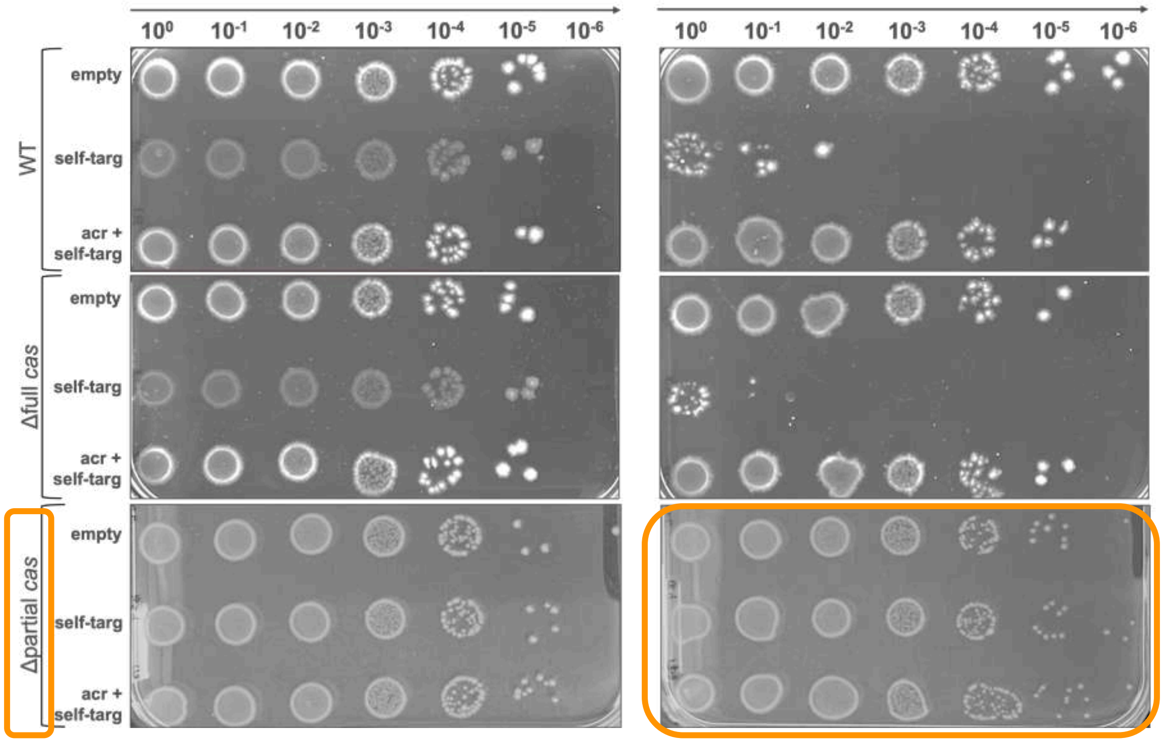
N = 3

Deletion of partial *cas* operon disables CRISPR inhibitory activity







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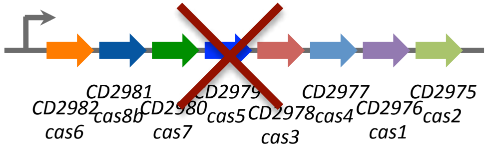
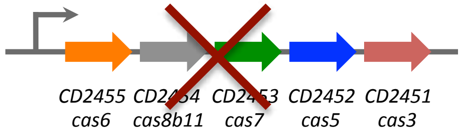
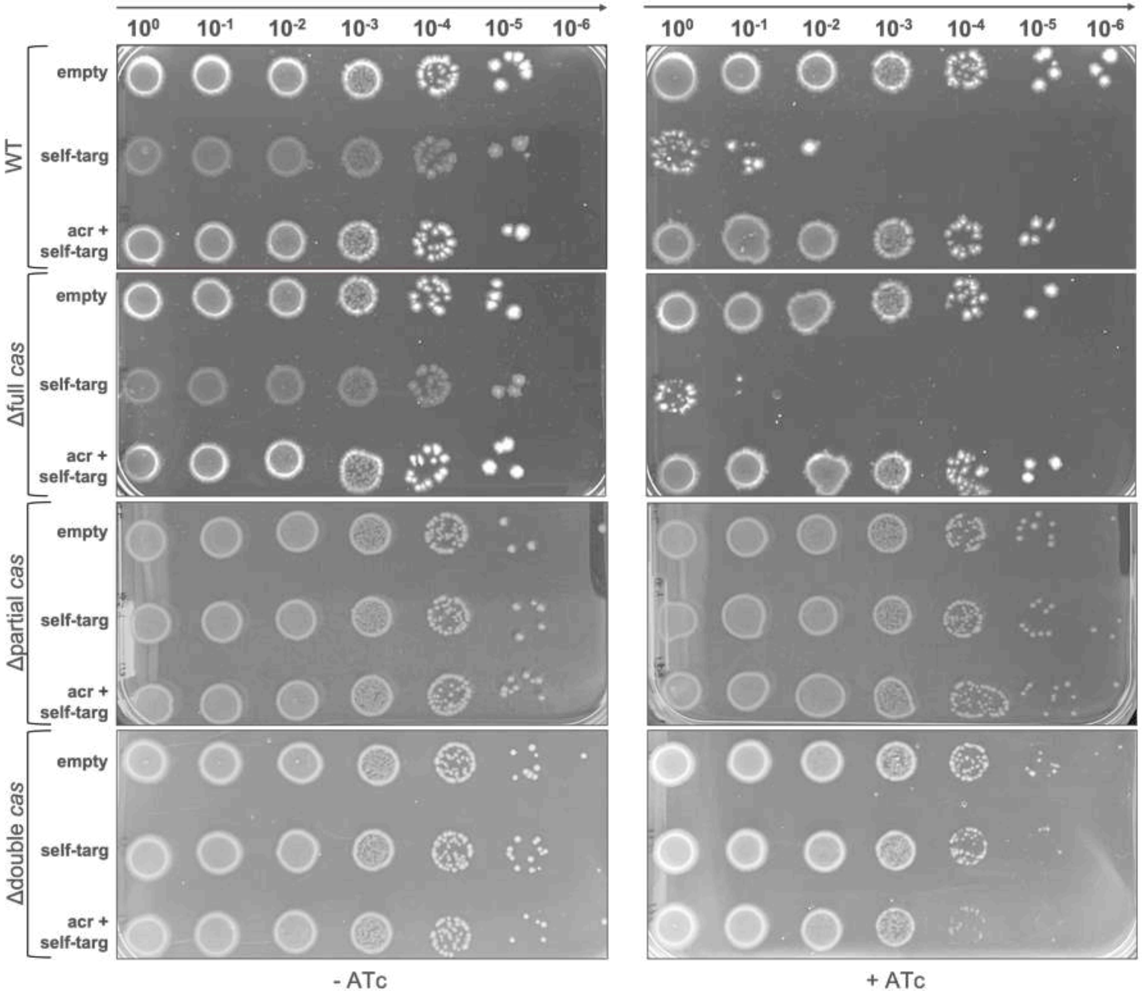
N = 3

Deletion of partial *cas* operon disables CRISPR inhibitory activity







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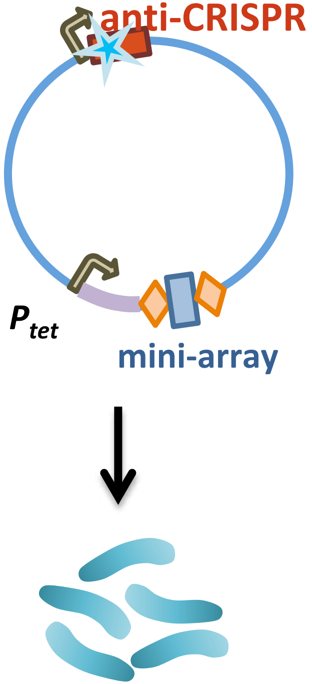
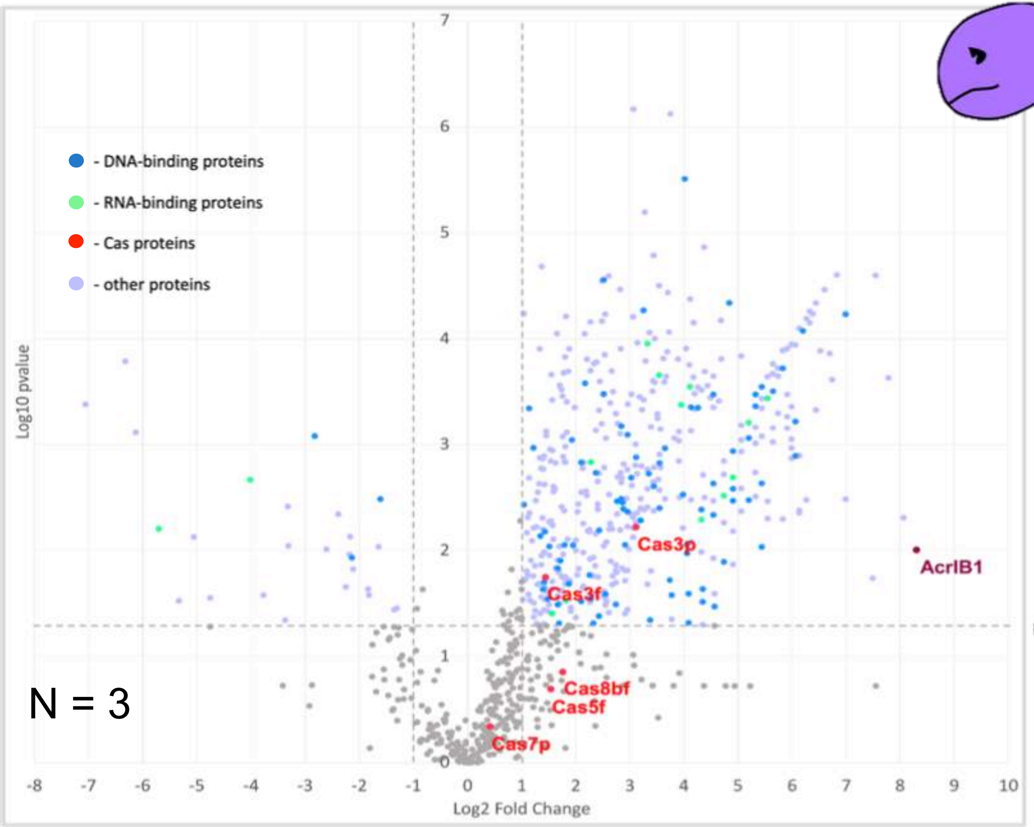
Identification of AcrIB1 interacting proteins

Pull-down results for AcrIB1-N-Strep sample by LC-MS/MS

STREP-tag





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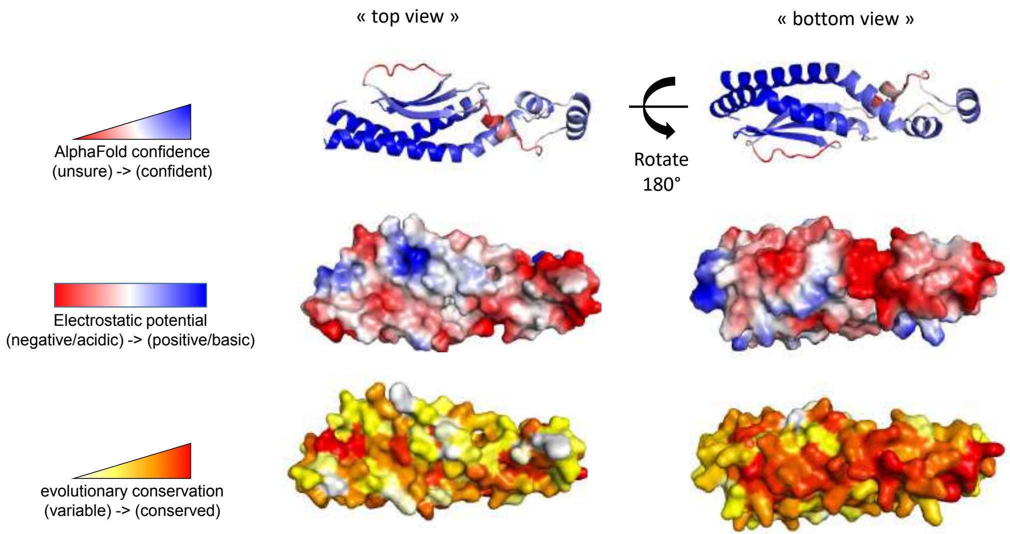
Potential DNA mimicry mechanism of action of AcrIB1

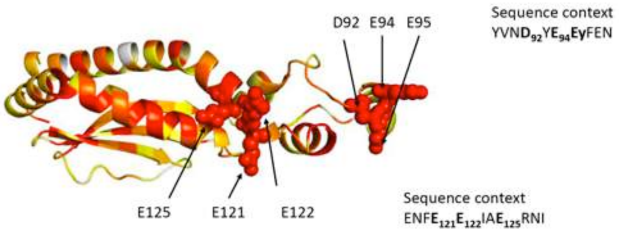
Negatively charged amino acids (**D** – aspartic; **E** – glutamic acid)
Aromatic amino acids (**F** – phenylalanine; **Y** – tyrosine; **W** - tryptophan)

J. Andreani

```
>AcrIB1
MWSHPQFEKNKQKARRFLRVIDMNIDKIEEEAIKAFKESCLIKETNNIKIYIDIQGKVEAIAVQTWAKLLG
DDKEINIFTLNQAPTHLNDMLGEICYVNDYEEFENWCENEWENLDWDSYKKFNKENFEEIAERNIDDSTSV
FLEELQKGIESCKQELQNVIEN
```



Alpha Fold predictions



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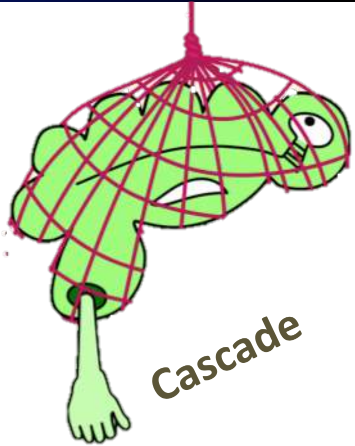
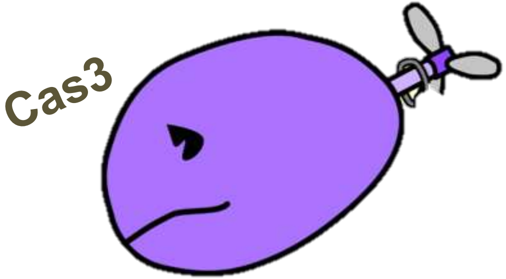
Conclusions



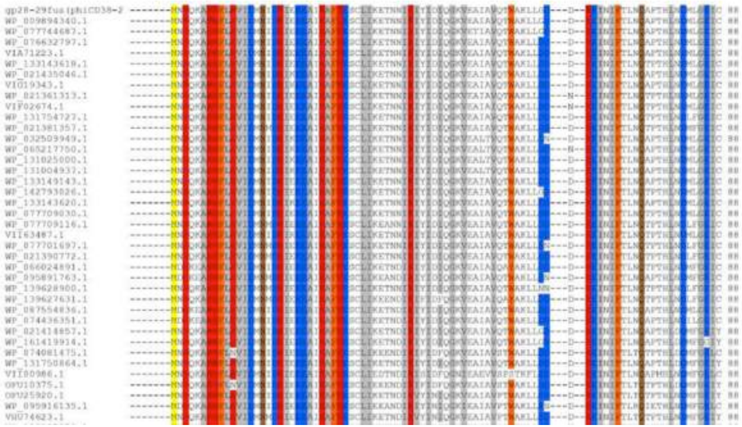
- Identification of first anti-CRISPR protein that inhibits subtype I-B CRISPR-Cas system in the human pathogen *C. difficile*
- DNA mimicry mechanism of AcrIB1 from structure predictions

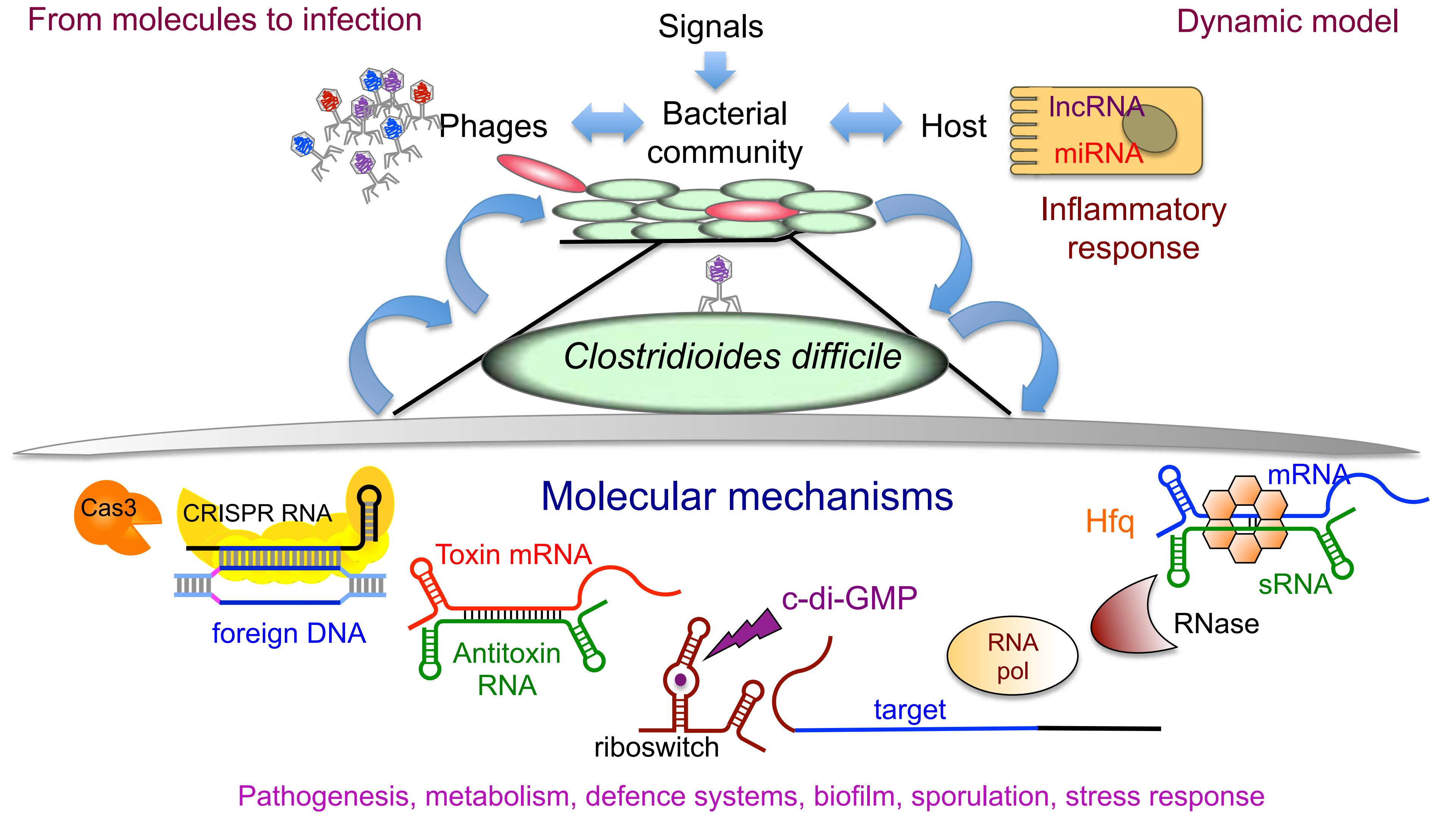
Perspectives

- Analysing AcrIB1 partner protein(s) and mechanism of action
- Testing new Acr candidates

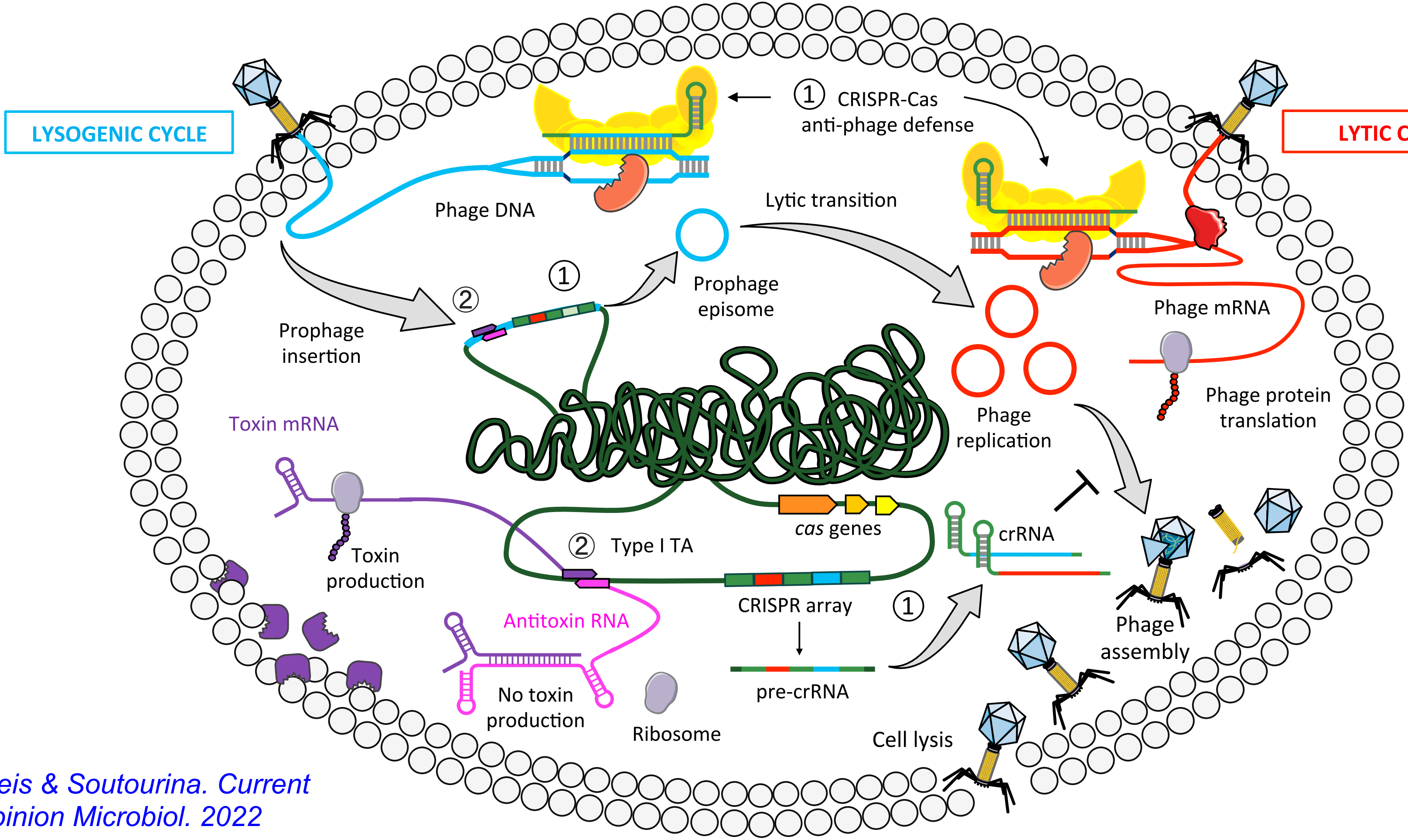


Protein alignment of 79 homologs of AcrIB1



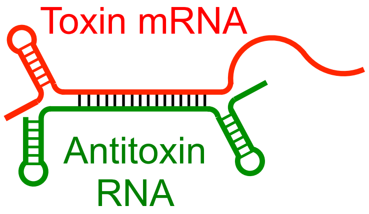

From molecules to infection
Signals
Dynamic model
Phages
Bacterial
community
Host
lncRNA
miRNA
Inflammatory
response
Clostridioides difficile
Molecular mechanisms
Cas3
CRISPR RNA
foreign DNA
Toxin mRNA
Antitoxin
RNA
c-di-GMP
riboswitch
target
RNA
pol
Hfq
mRNA
sRNA
RNase
Pathogenesis, metabolism, defence systems, biofilm, sporulation, stress response

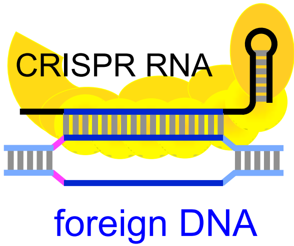
Summary of RNA-based mechanisms in *C. difficile*-phage interactions

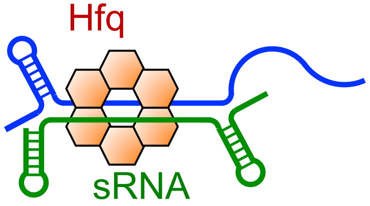


Kreis & Soutourina. Current Opinion Microbiol. 2022

Potential RNA applications in *C. difficile*







- **Applications**

- **Biotechnology**

(endogenous CRISPR editing & TA-based tools, Anti-CRISPR)

- **Epidemiology**

(CRISPR typing, ncRNA markers for epidemic strains)

- **New antimicrobials**

(Antisense RNA, sRNAs as drug targets

CRISPR autoimmunity, TA induction for self-targeting
Anti-CRISPR for phage therapy

Riboswitches targeting, Secreted RNAs …)