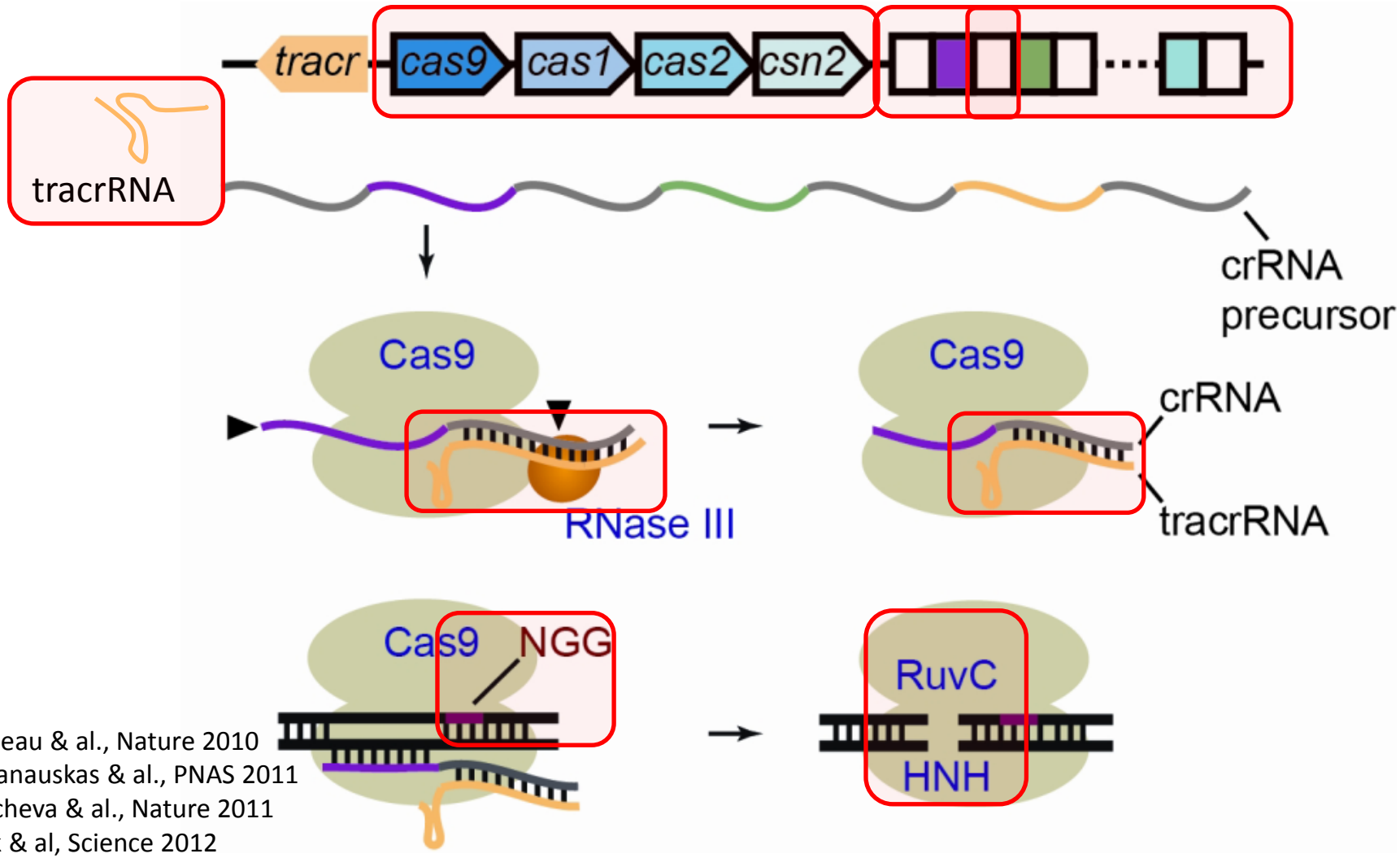
Genome Editing and Engineering

Course No: BT-637

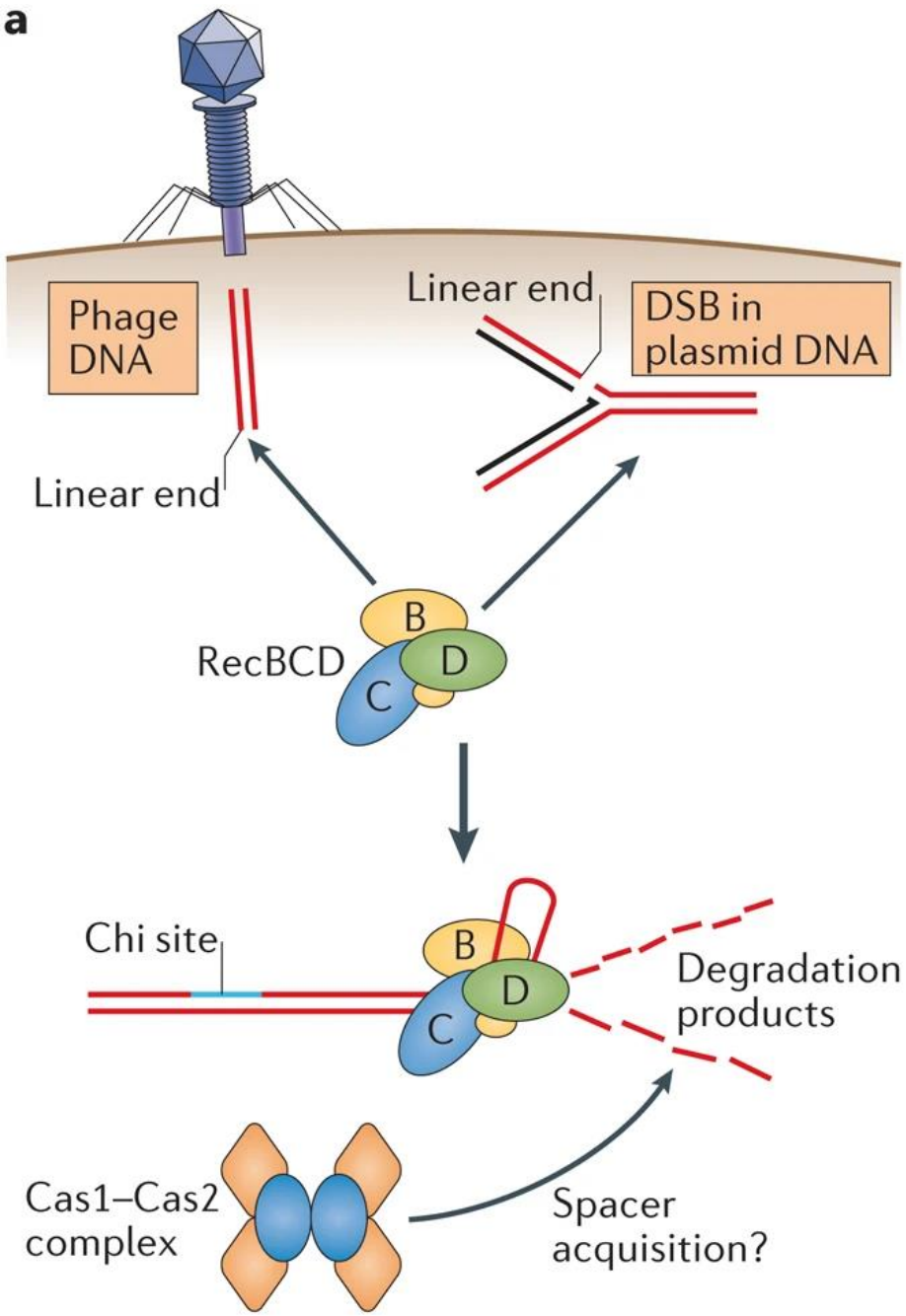
LECTURE-17

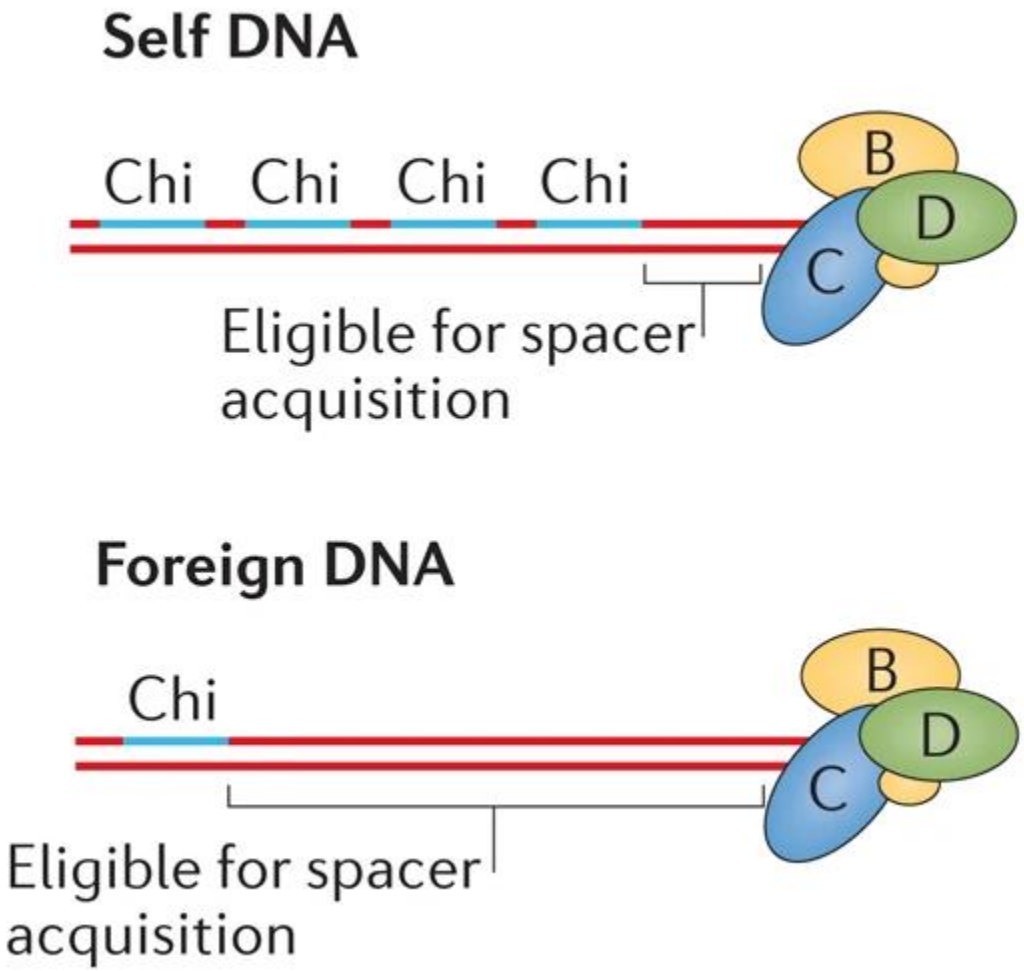
Dr. Kusum K. Singh
Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

S.pyogenes type II-A system

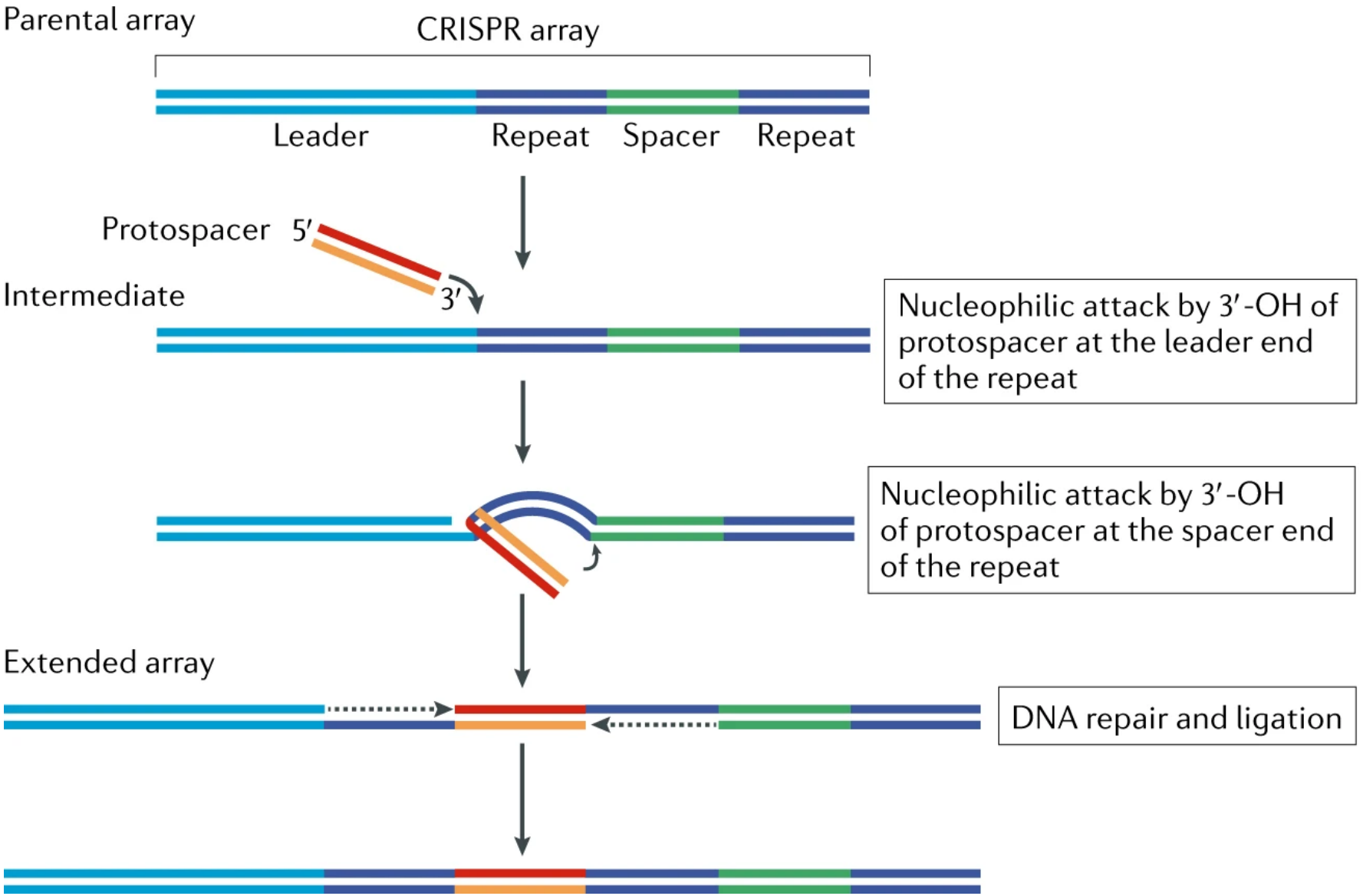


Garneau & al., Nature 2010
Sapranauskas & al., PNAS 2011
Deltcheva & al., Nature 2011
Jinek & al, Science 2012
Gasiunas & al., PNAS 2012
Sternberg & al., Nature 2014

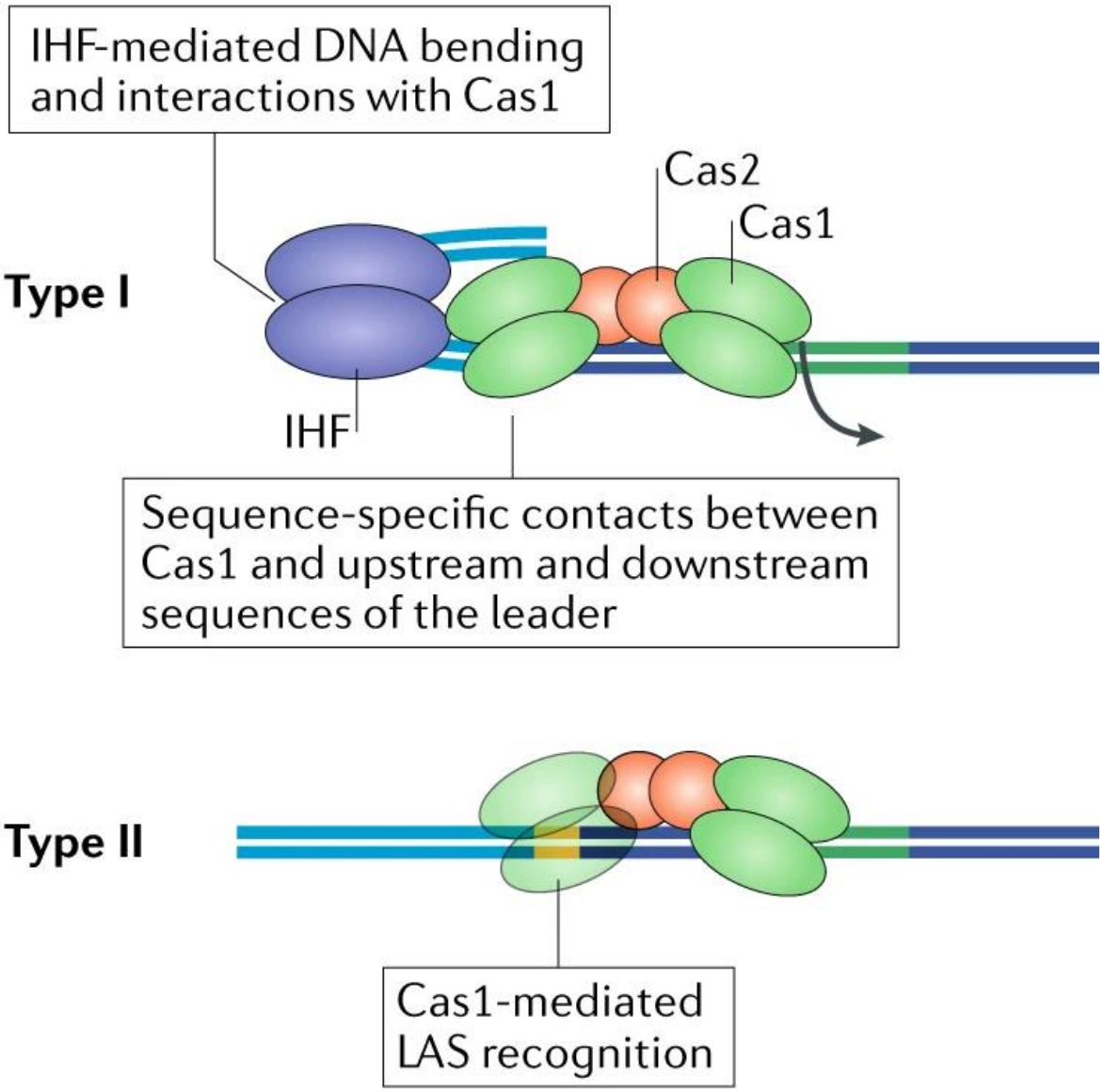

a
Phage DNA
Linear end
DSB in plasmid DNA
Linear end
B
RecBCD
D
C
Chi site
B
D
C
Degradation products
Cas1–Cas2 complex
Spacer acquisition?

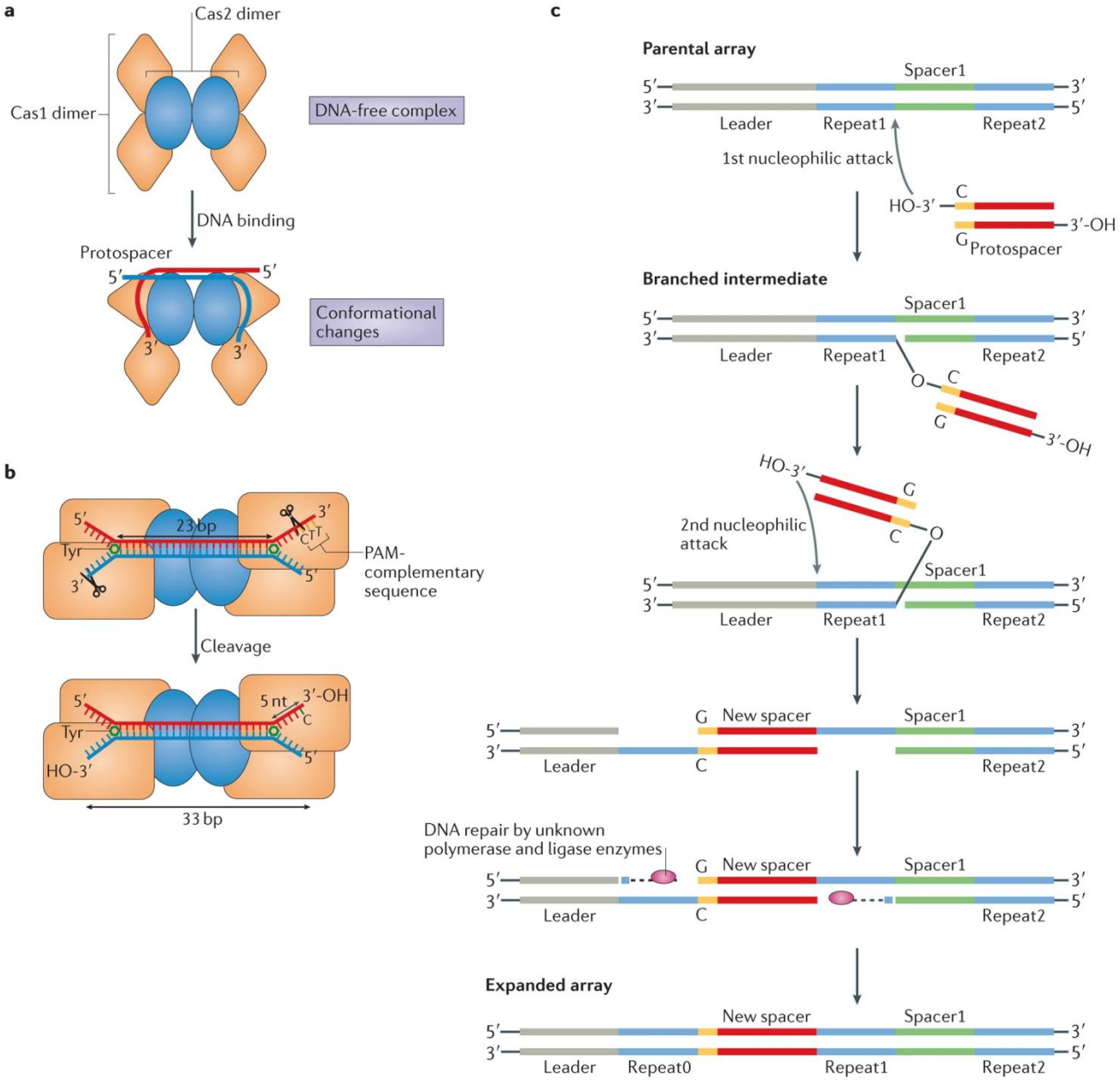


Amitai & Sorek 2016 Nature Reviews Microbiology

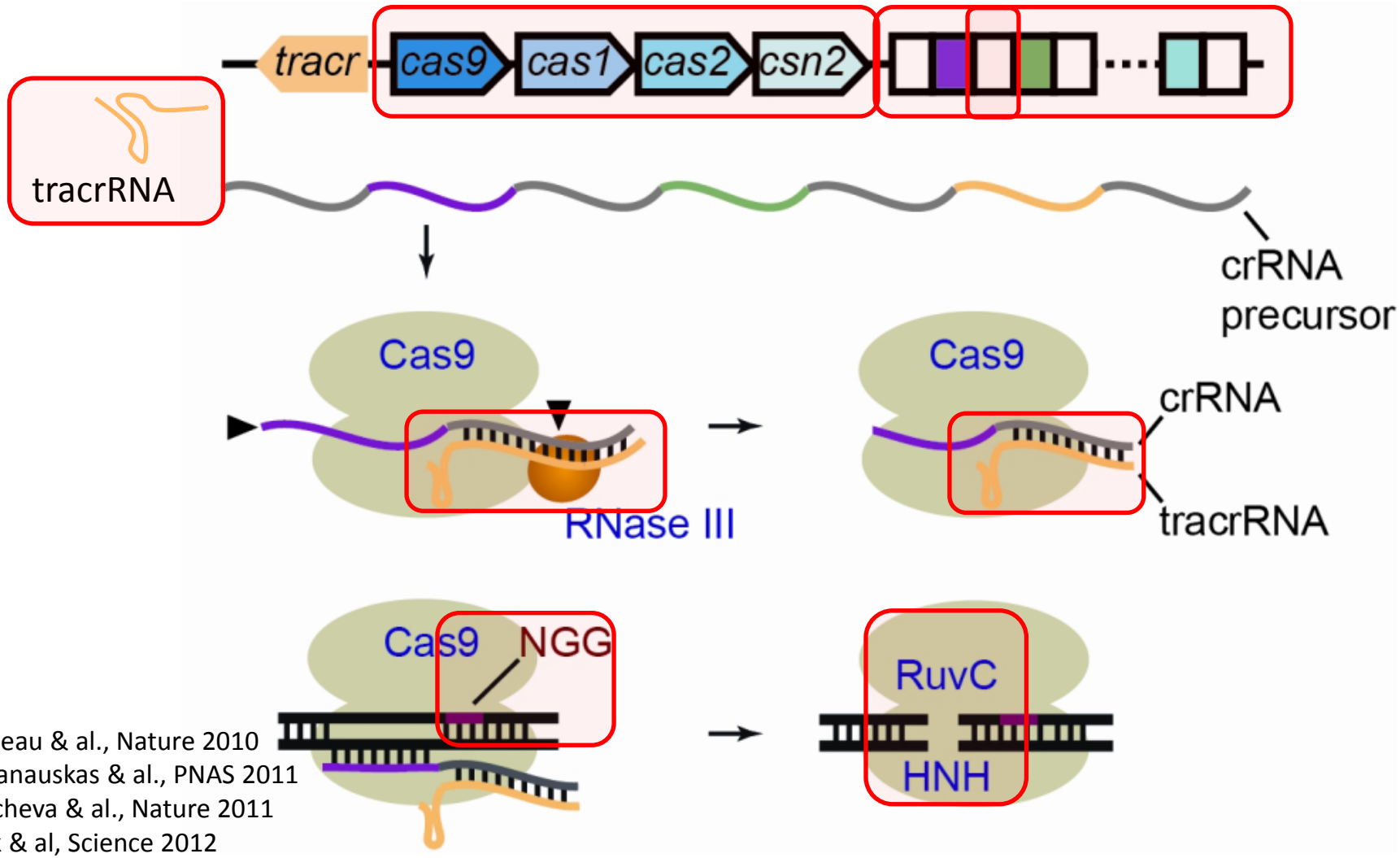


McGinn & Marraffini 2019 Nature Reviews Microbiology


IHF-mediated DNA bending and interactions with Cas1
Cas2
Cas1
Type I
IHF
Sequence-specific contacts between Cas1 and upstream and downstream sequences of the leader
Type II
Cas1-mediated LAS recognition


a
Cas2 dimer
Cas1 dimer
DNA-free complex
DNA binding
Protospacer
5′
5′
3′
3′
Conformational changes
b
5′
3′
23 bp
Tyr
3′
5′
CTT
PAM-complementary sequence
Cleavage
5′
5 nt
3′-OH
Tyr
C
HO-3′
5′
33 bp
c
Parental array
Spacer1
5′
3′
3′
5′
Leader
Repeat1
Repeat2
1st nucleophilic attack
HO-3′
C
3′-OH
G
Protospacer
Branched intermediate
Spacer1
Leader
Repeat1
Repeat2
O
C
G
3′-OH
HO-3′
G
C
O
2nd nucleophilic attack
Spacer1
Leader
Repeat1
Repeat2
G
New spacer
Spacer1
Leader
C
Repeat2
DNA repair by unknown polymerase and ligase enzymes
G
New spacer
Spacer1
Leader
C
Repeat2
Expanded array
New spacer
Spacer1
Leader
Repeat0
Repeat1
Repeat2

S.pyogenes type II-A system



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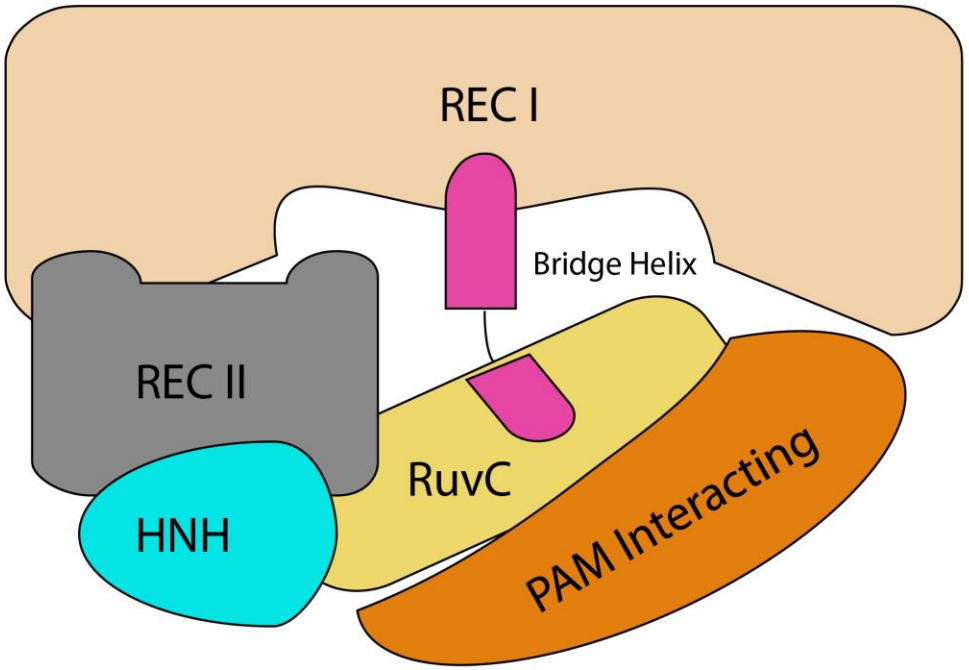
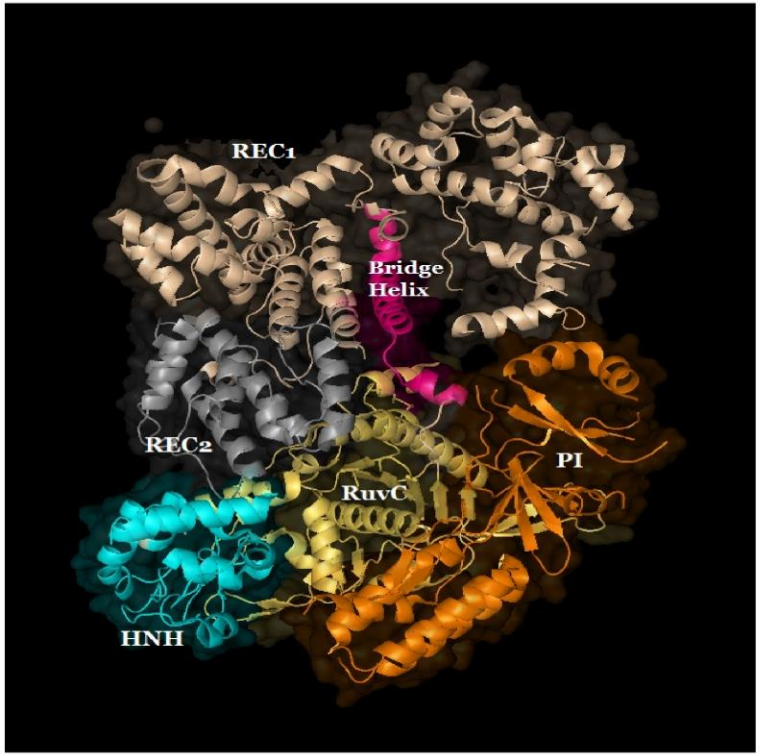
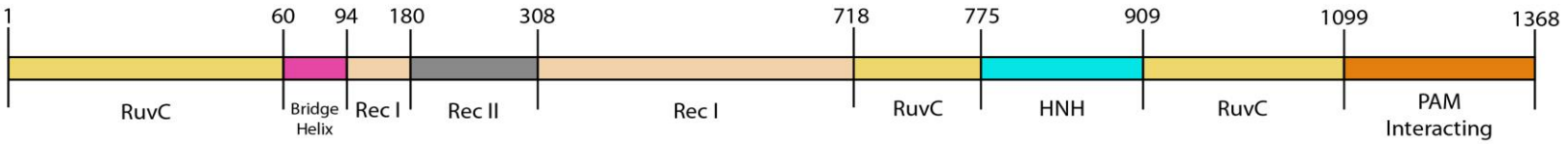
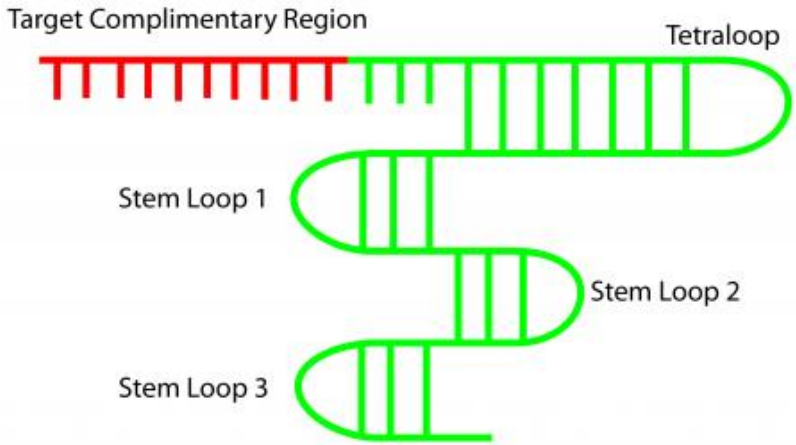


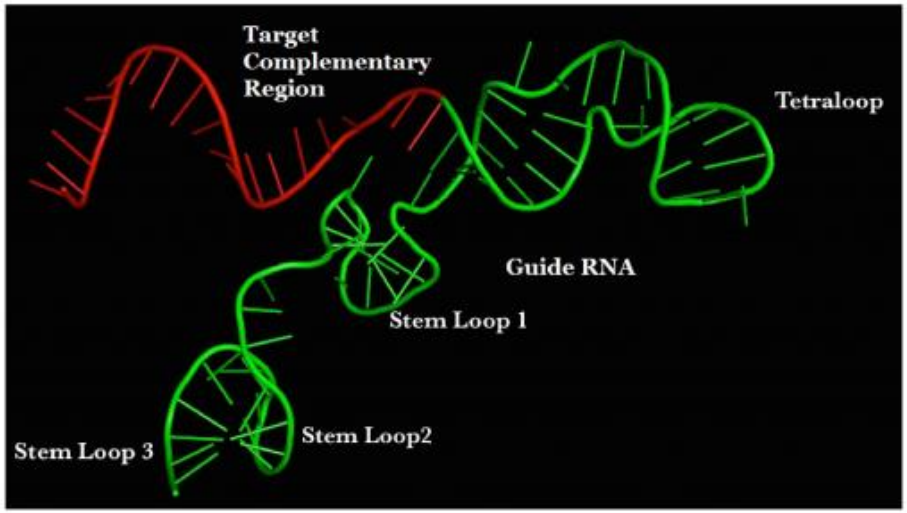




https://sites.tufts.edu/crispr/crispr-mechanism/

S.pyogenes type II-A system





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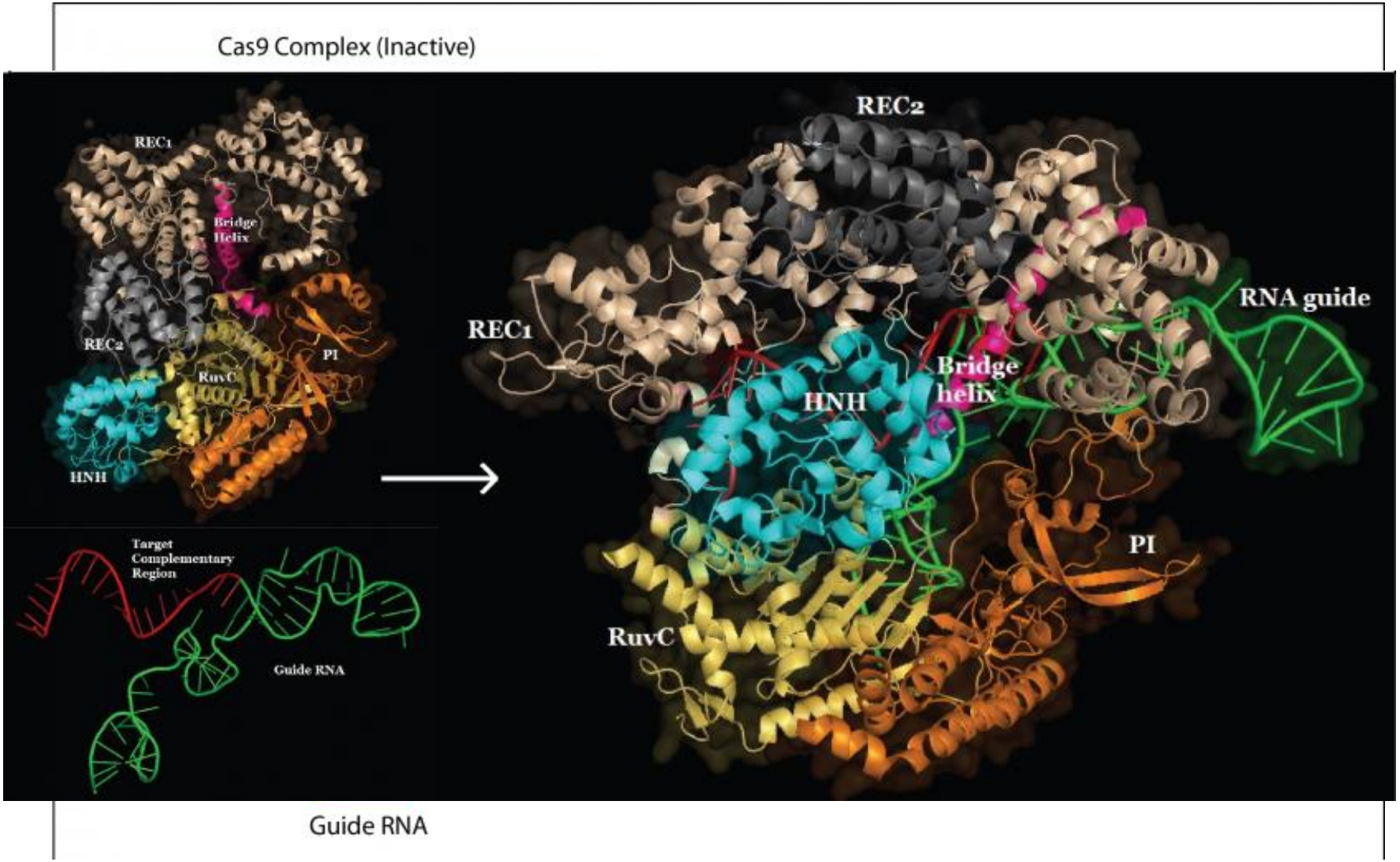
S.pyogenes type II-A system



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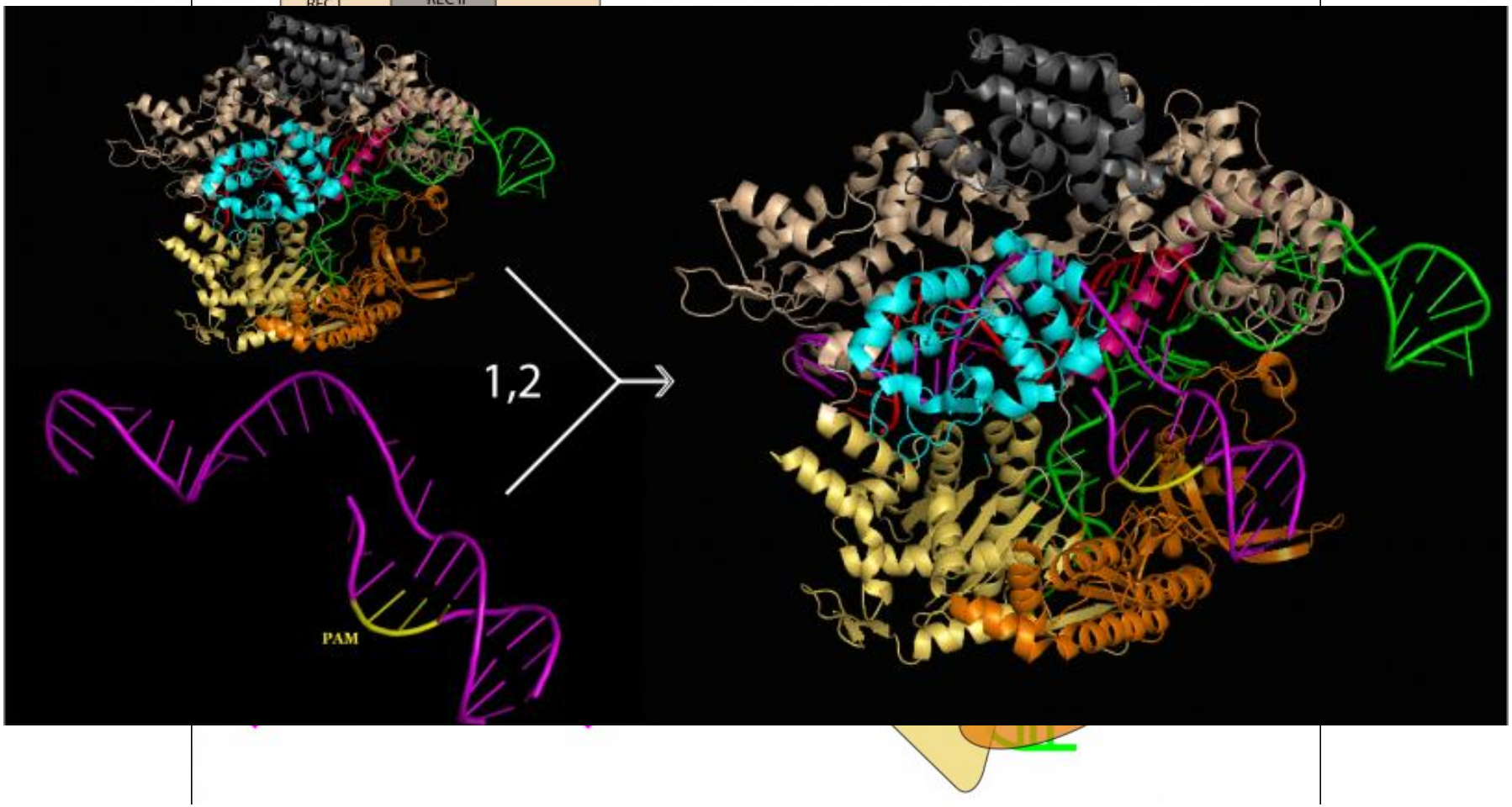
S.pyogenes type II-A system



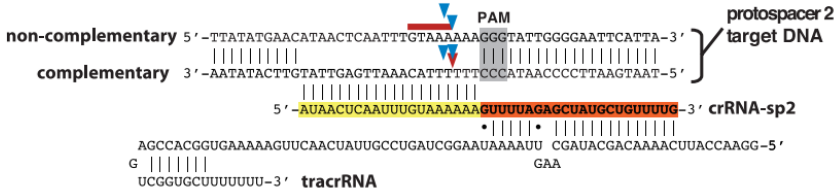
https://sites.tufts.edu/crispr/crispr-mechanism/

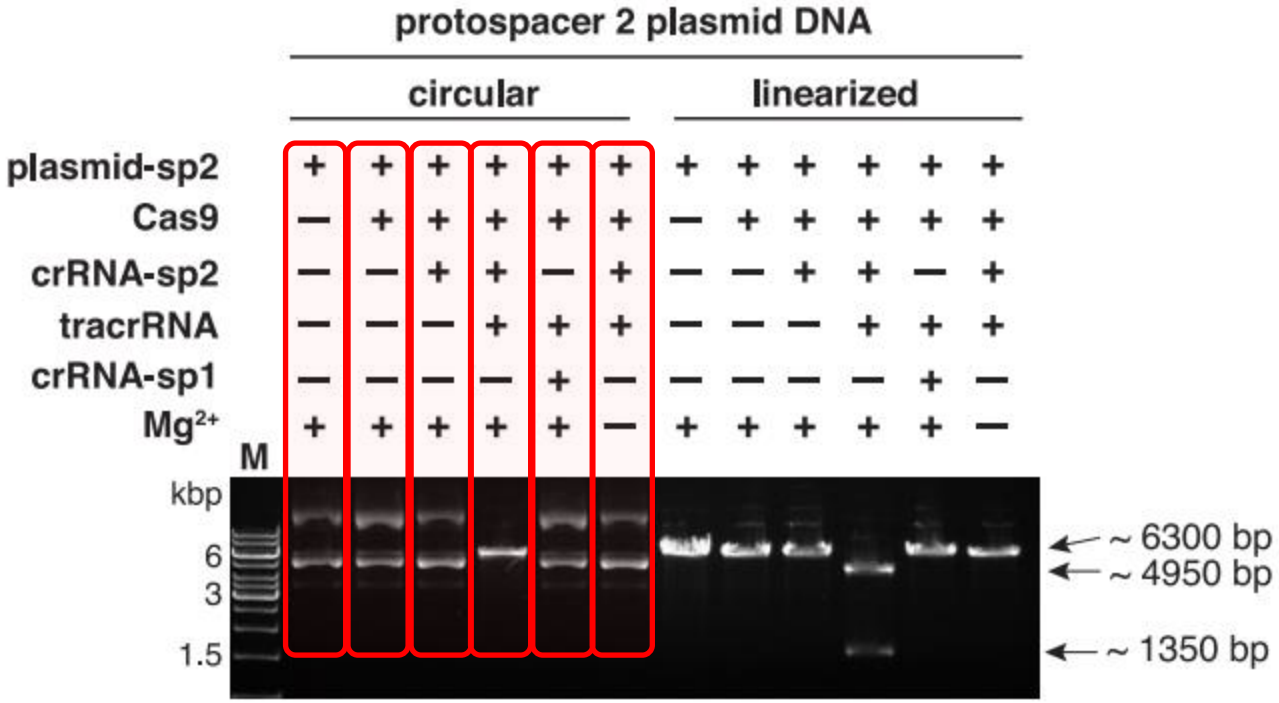
S.pyogenes type II-A system

A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity

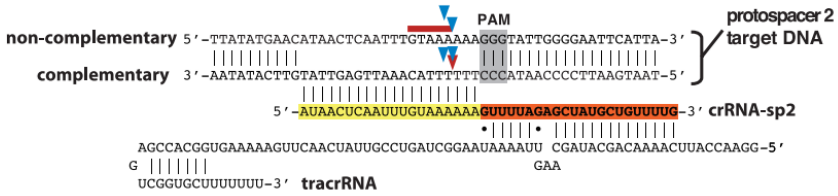
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Hauer,[2]†
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

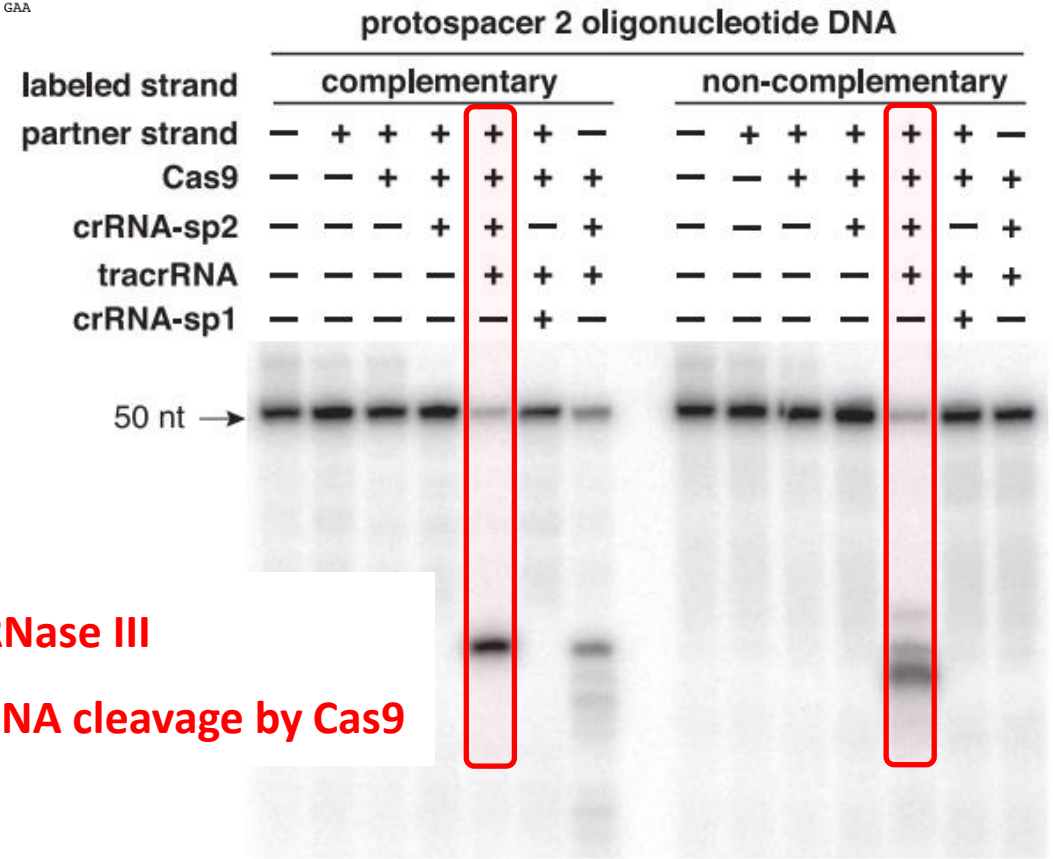
Cas9 DNA endonuclease guided by two RNA





Cas9 DNA endonuclease guided by two RNA

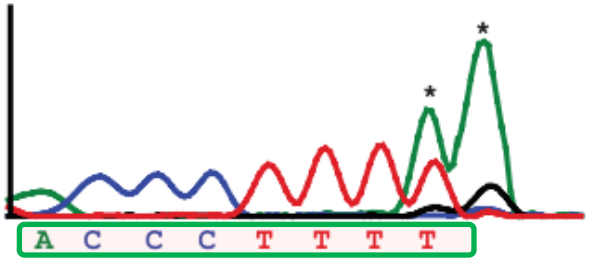




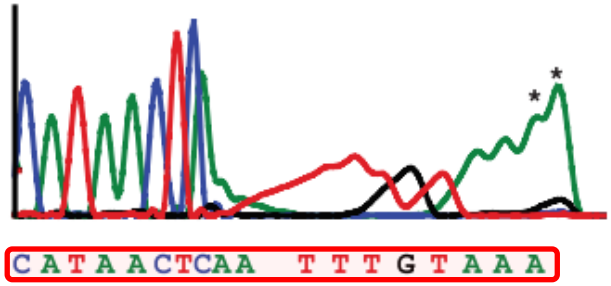
- pre-crRNA processing by RNase III
- activating crRNA-guided DNA cleavage by Cas9

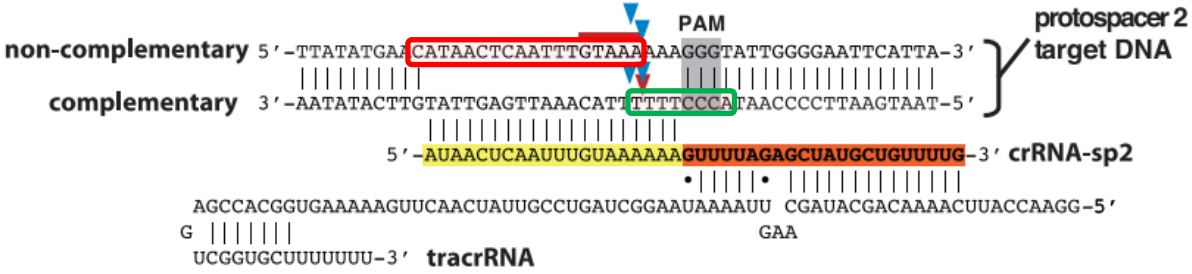
Cas9 DNA endonuclease guided by two RNA

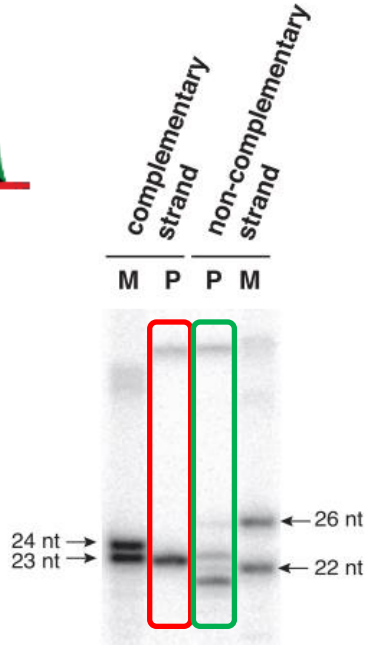
non-complementary strand binding primer



complementary strand binding primer

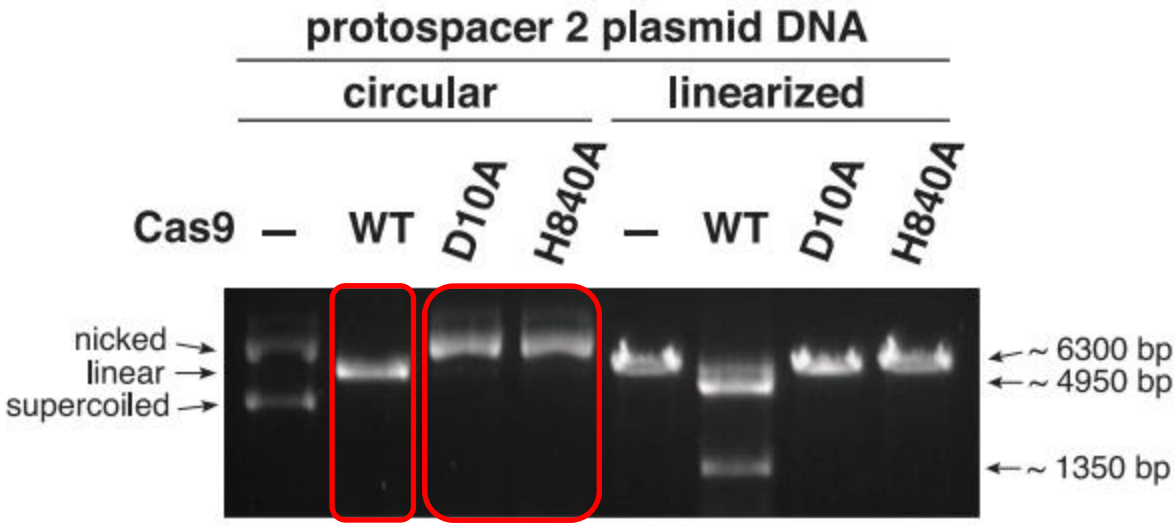






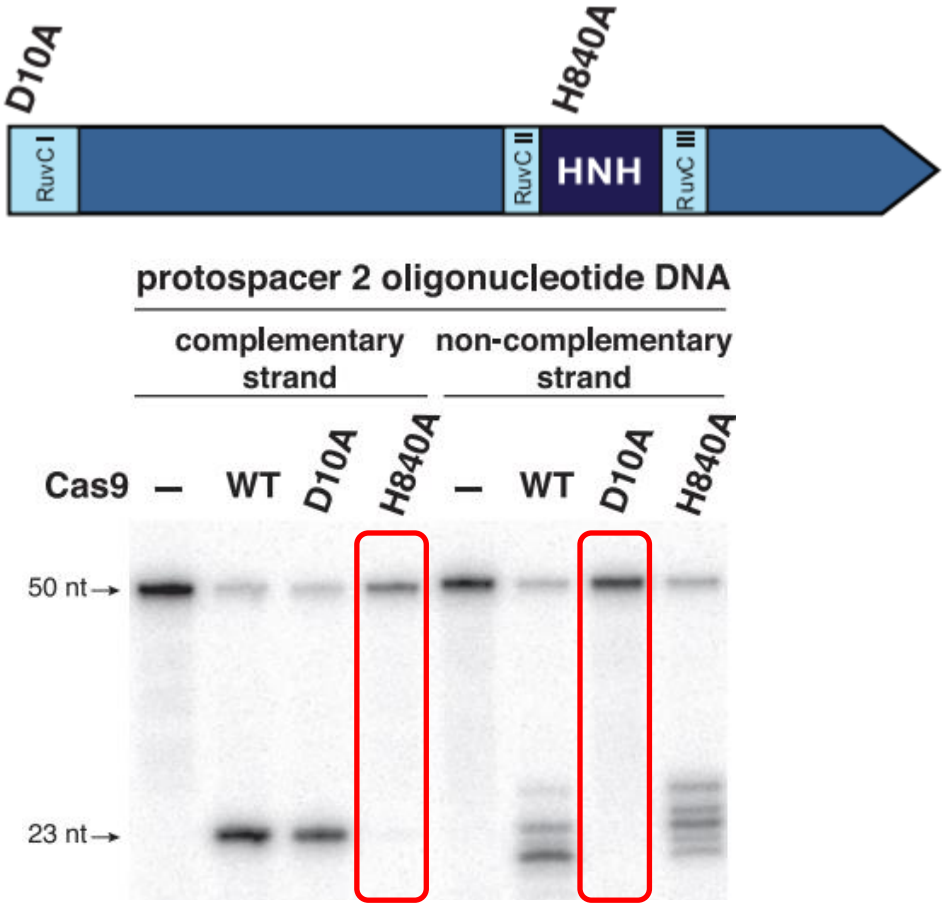
- Non complementary = 1st cleaved endonuclease
- 3’-5’ exonuclease

Each Cas9 nuclease domain cleaves one DNA strand



- **HNH = one plasmid strand**
- **RuvC = one plasmid strand**

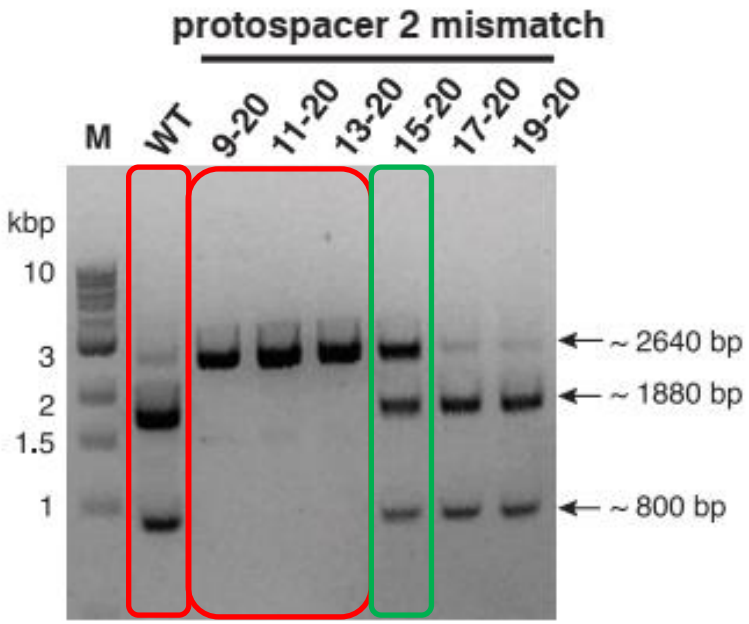
Each Cas9 nuclease domain cleaves one DNA strand

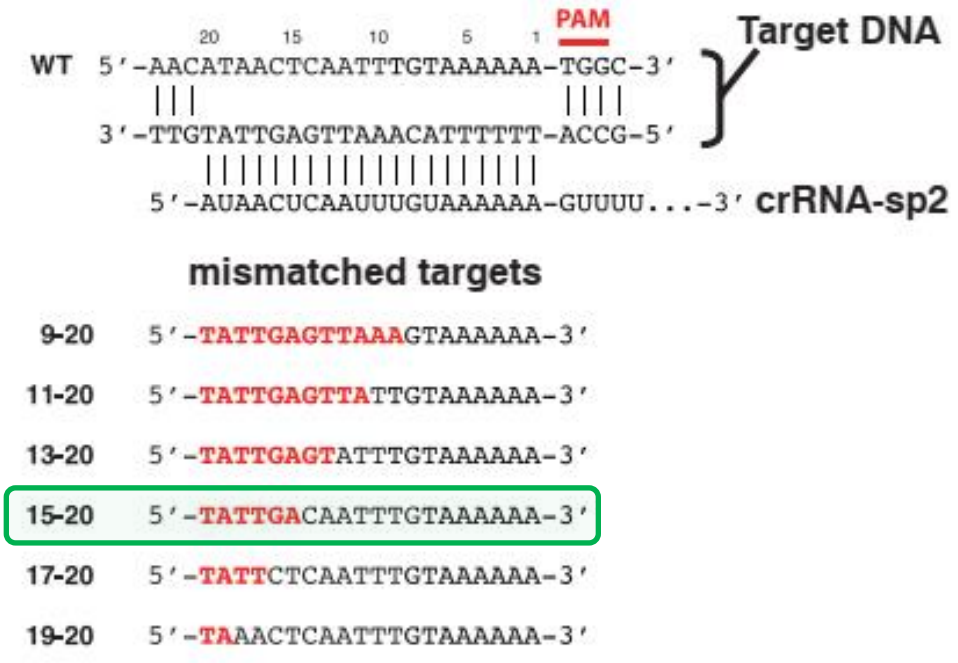


- **HNH = complementary strand**
- **RuvC = non complementary strand**

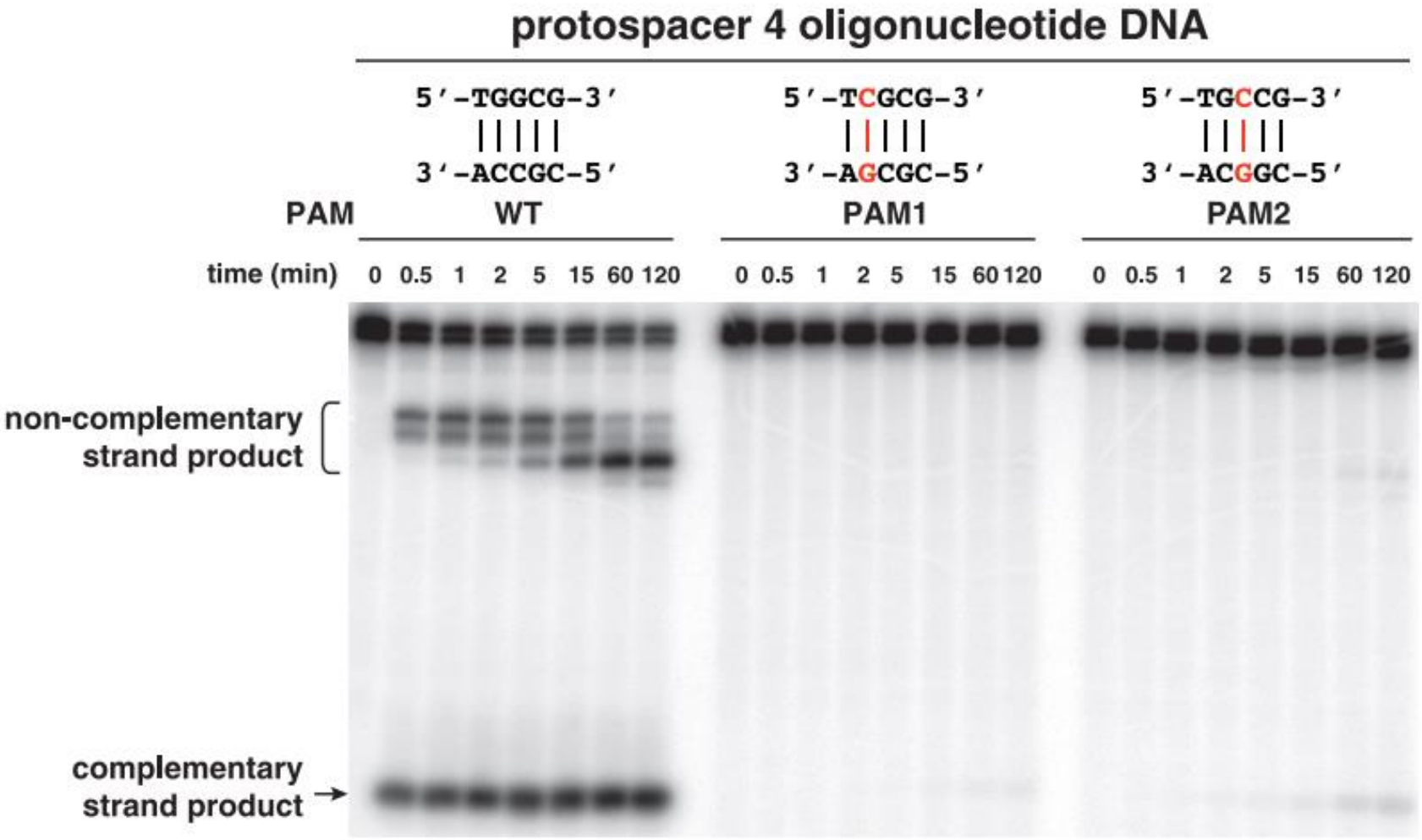
Dual RNA requirements

Protospacers sequence



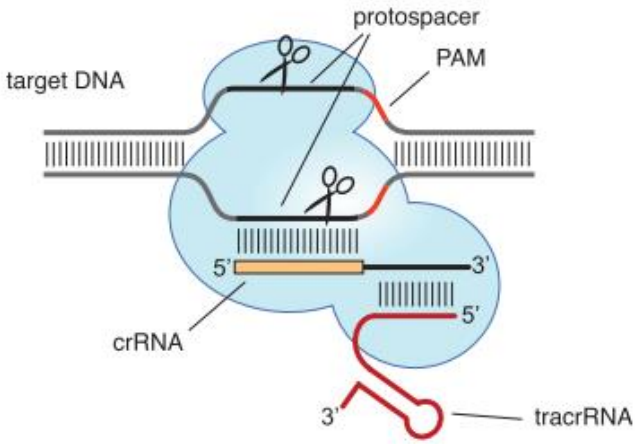


PAM sequence

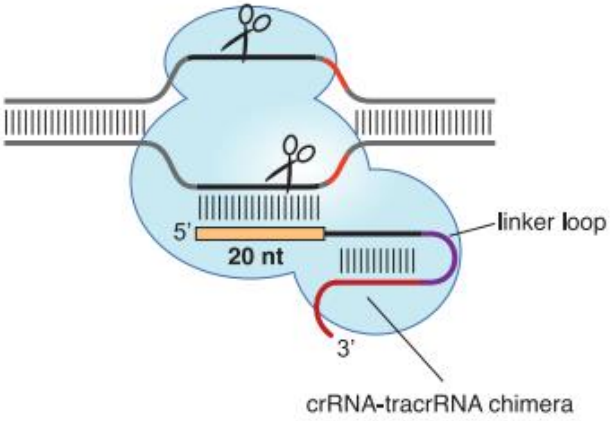


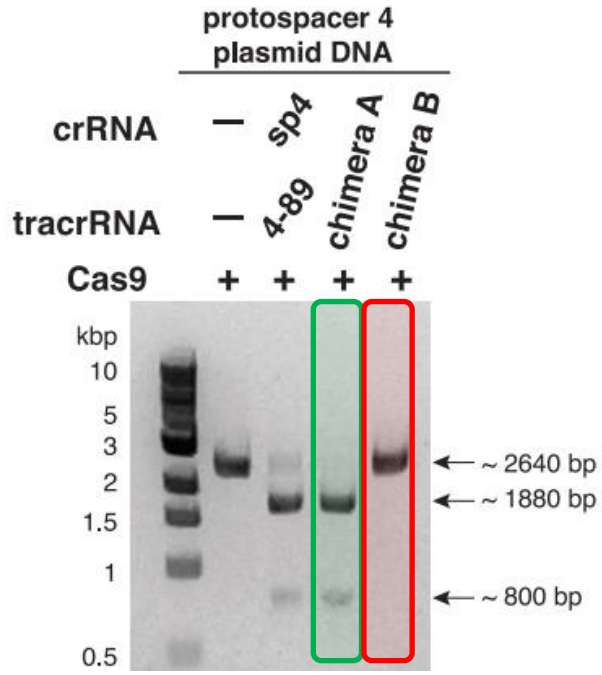
Single chimeric RNA

Cas9 programmed by crRNA:tracrRNA duplex

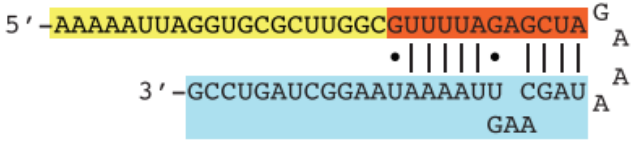


Cas9 programmed by single chimeric RNA

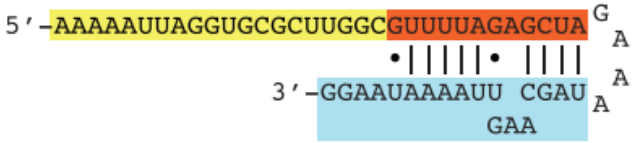




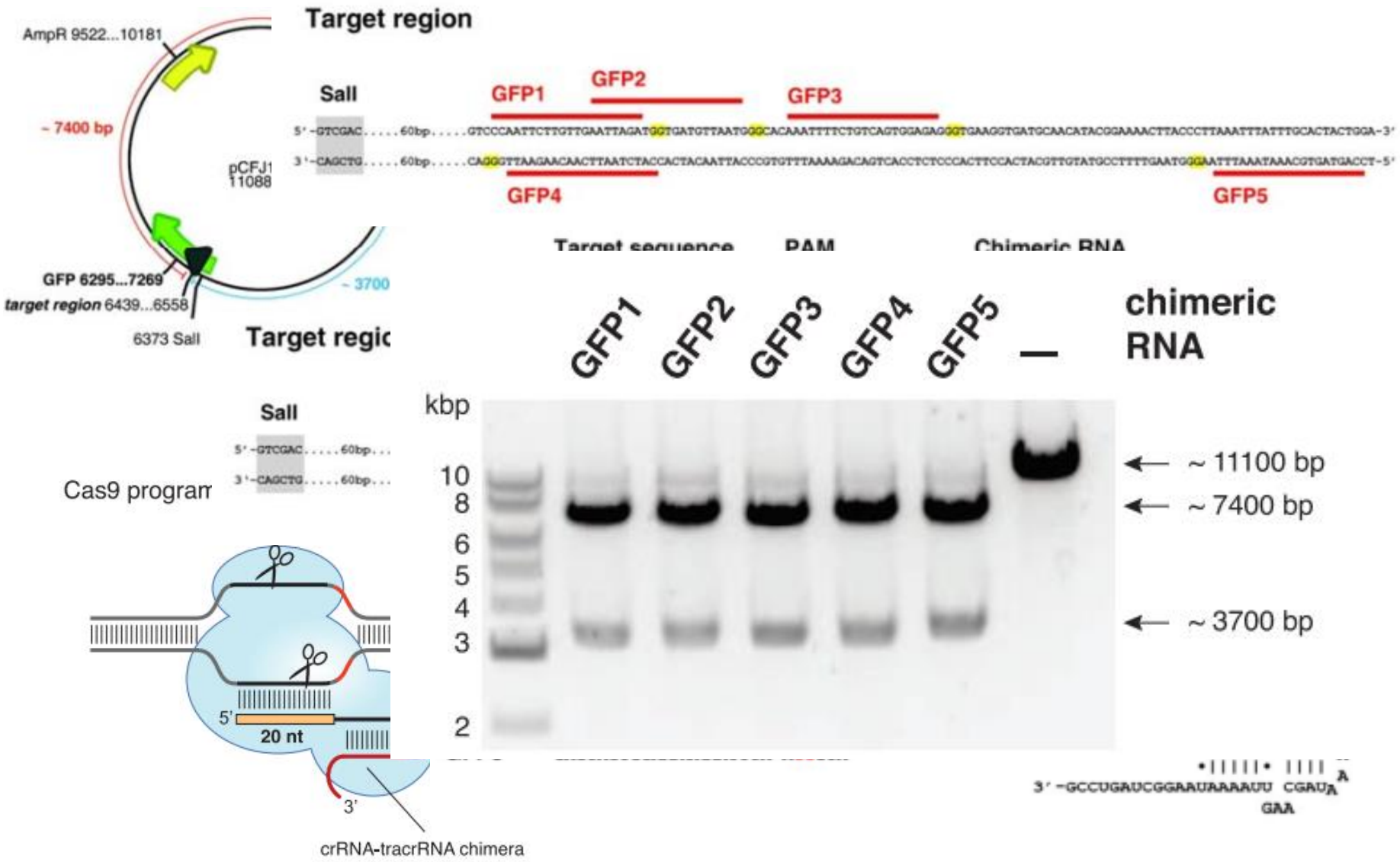
chimera A



chimera B



Engineered site specific guide RNA



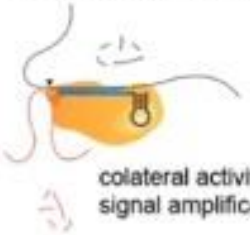
Conclusions of Lecture-17

- Type IIA CRISPR-Cas System
- Molecular requirements (Cas9, tracrRNA, crRNA, PAM in target DNA)
- Engineered Single guide RNA
- System = simple, efficient, versatile and programmable
- RNA-programmed Cas9 = gene-targeting and genome-editing

Abudayyeh *et al.*, *Science* (2016)
East-Seletsky *et al.*, *Nature* (2016)
Cox *et al.*, *Science* (2017)
Abudayyeh *et al.*, *Nature* (2017)
Smargon *et al.*, *Mol. Cell* (2017)
Konermann *et al.*, *Cell* (2018)
Yan *et al.*, *Mol. Cell* (2018)
...

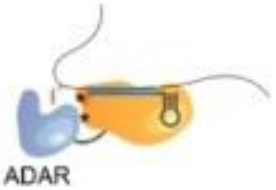
RNA knockdown

Virus detection/degradation



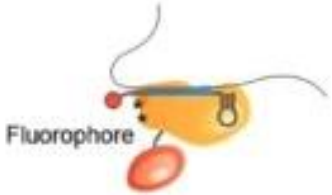
Gootenberg *et al.*, *Science* (2017)
Gootenberg *et al.*, *Science* (2018)
Joung *et al.*, (2020)

A-to-I editing



Cox *et al.*, *Science* (2017)

RNA tracing



Wang *et al.*, *Science* (2019)
Yang *et al.*, *Mol. Cell* (2019)

RNA demethylation

Li *et al.*, *NAR* (2020)

Alternative splicing

Konermann *et al.*, *Cell* (2018)
Jillette *et al.*, *bioRxiv* (2019)

Proximity labeling

Zhang *et al.*, *NAR* (2020)
Han *et al.*, *bioRxiv* (2020)
Lin *et al.*, *bioRxiv* (2020)
Li *et al.*, *bioRxiv* (2020)

Alternative polyadenylation

Anderson *et al.*, *bioRxiv* (2019)

Questions??