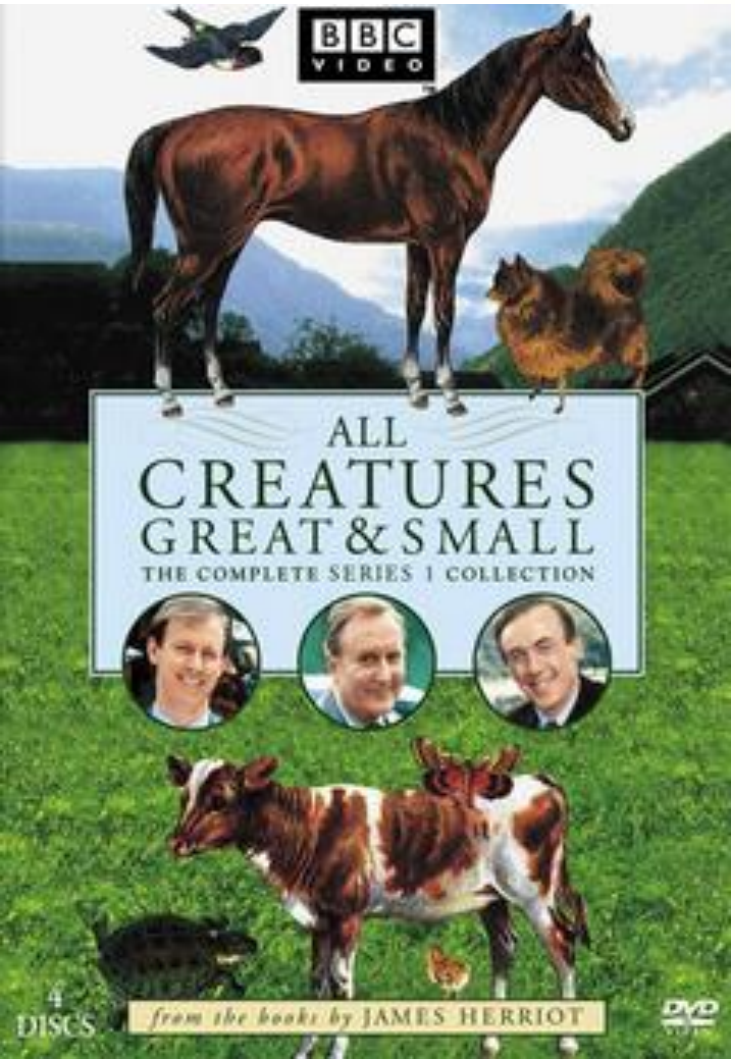
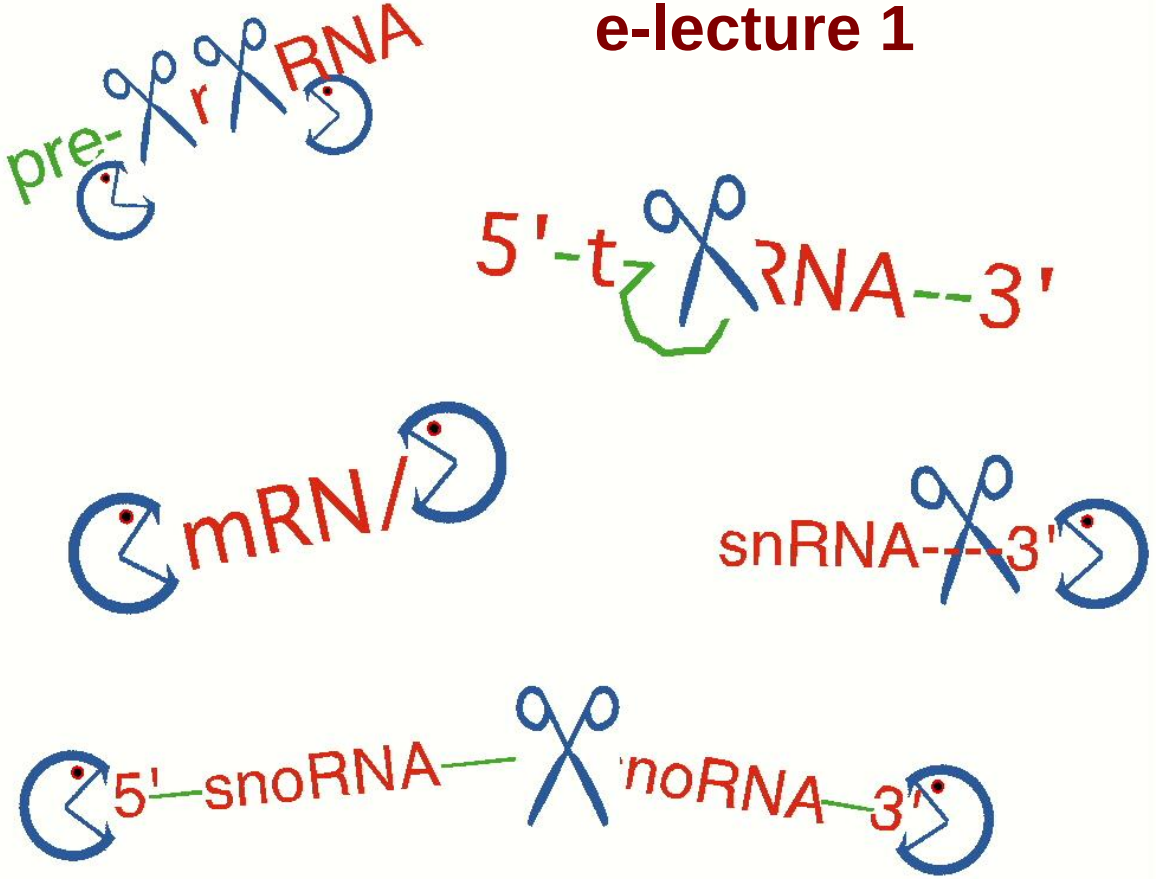
# All RNAs great and small

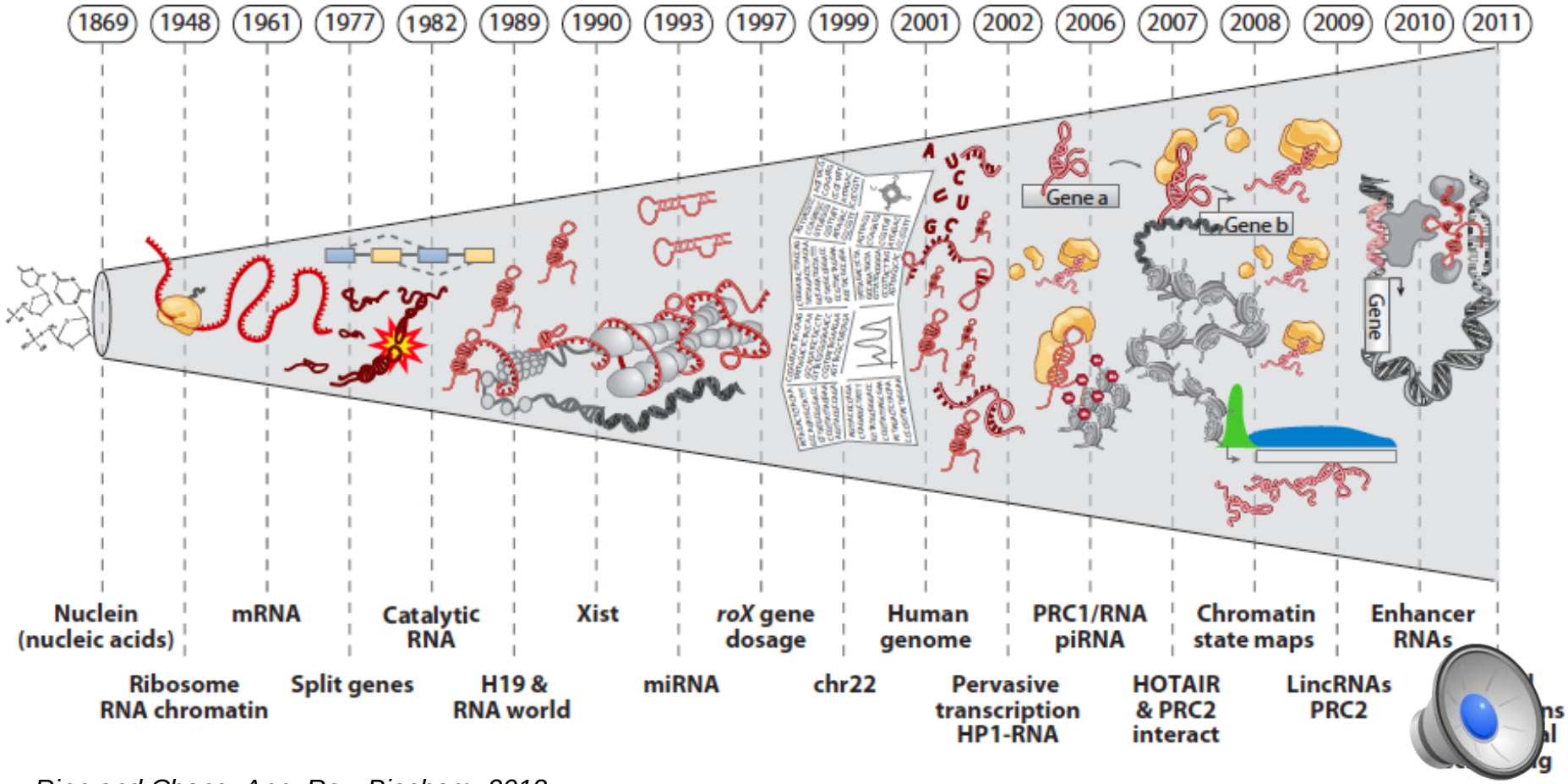



**e-lecture 1**



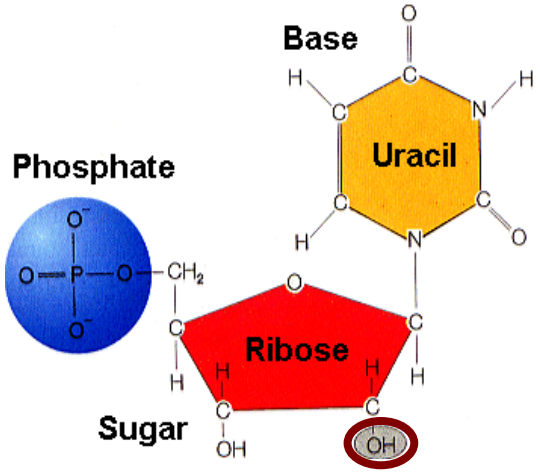

**Institute of Genetics and Biotechnology**
**University of Warsaw**

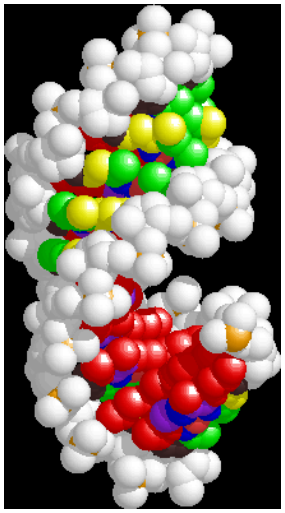
# HISTORY OF RNA

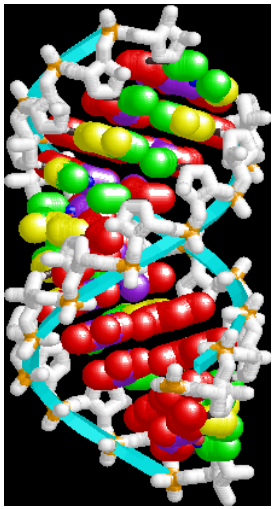



*Rinn and Chang, Ann. Rev. Biochem, 2012*

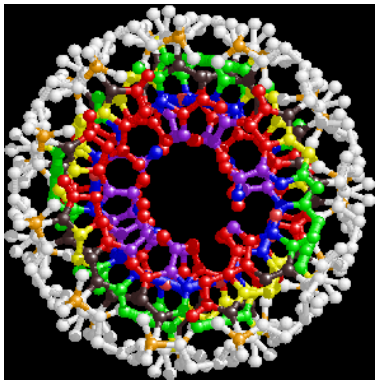
# RNA – *aka* My Favorite Molecule





**RNA form A helix**

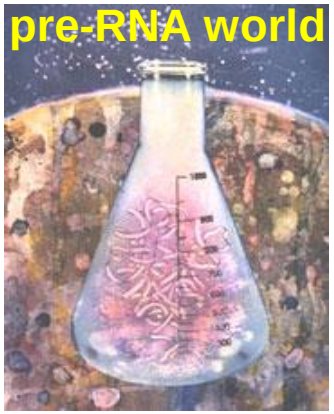


- **narrow inaccessible major groove** (red)
- **shallow minor groove** (green)

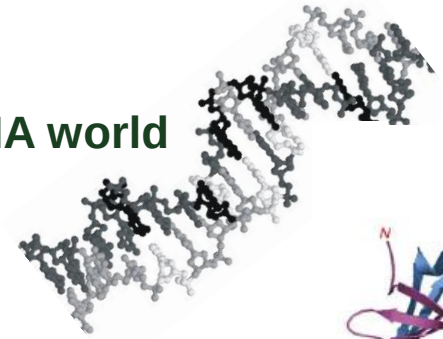
- **versatile and flexible**
- **catalytically active (splicing, translation, modification)**
- **self-sufficient?**
- **labile (regulation of expression)**
- **create complex 3D structures**
- **specific and unspecific interactions with proteins and other RNAs**

# „THE RNA WORLD" hypothesis

„primordial soup"
„prebiotic soup"



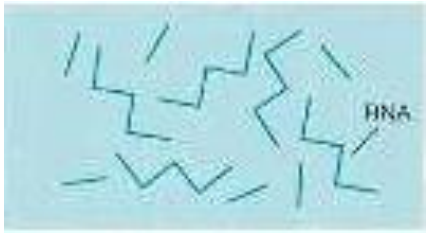

RNA world



RNA+proteins

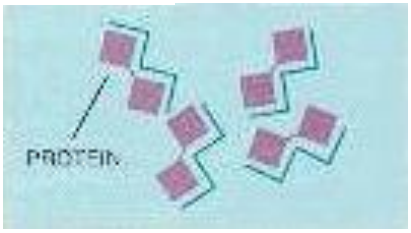


RNA+DNA+ proteins

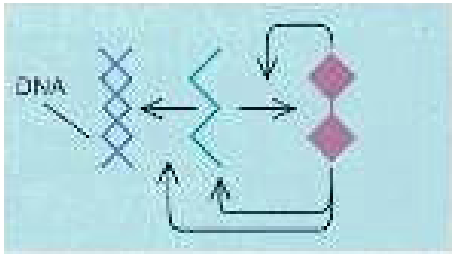



RNA made via condensation from ribose and other inorganic and organic sources

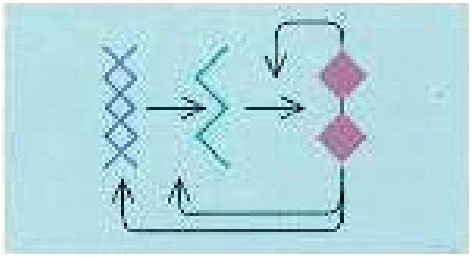
RNA evolution- molecules learns to replicate




RNA starts to join aminoacids and synthesises polypeptides and proteins




Proteins aid RNA to replicate and make proteins. dsRNA evolves into stable DNA.

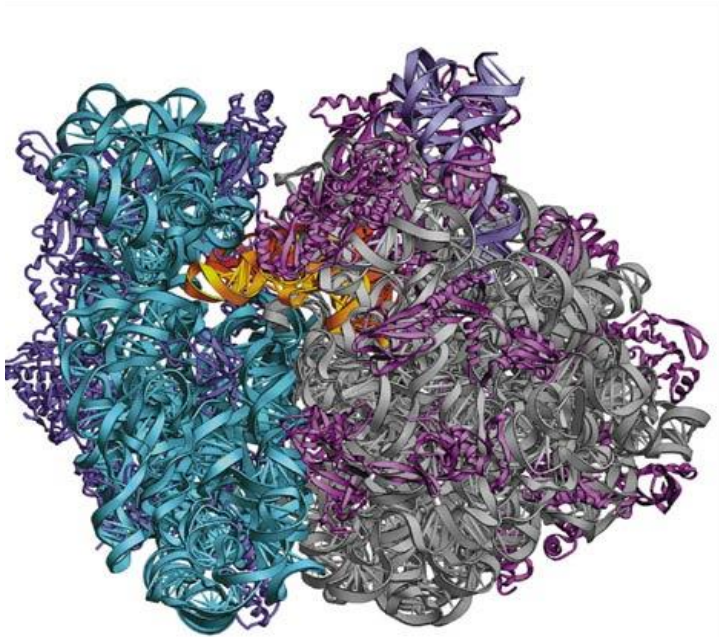


DNA and proteins take over major roles as genetic information and enzymes

# MODERN RNA WORLD

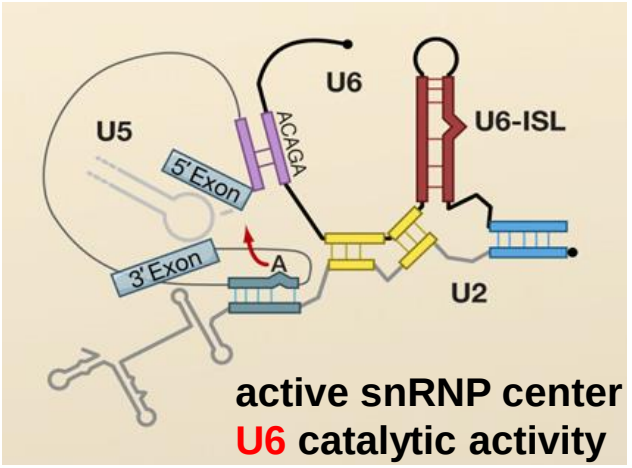
## RNA vestiges- catalytic RNAs with active centres made of RNA

**RIBOSOME** - protein synthesis

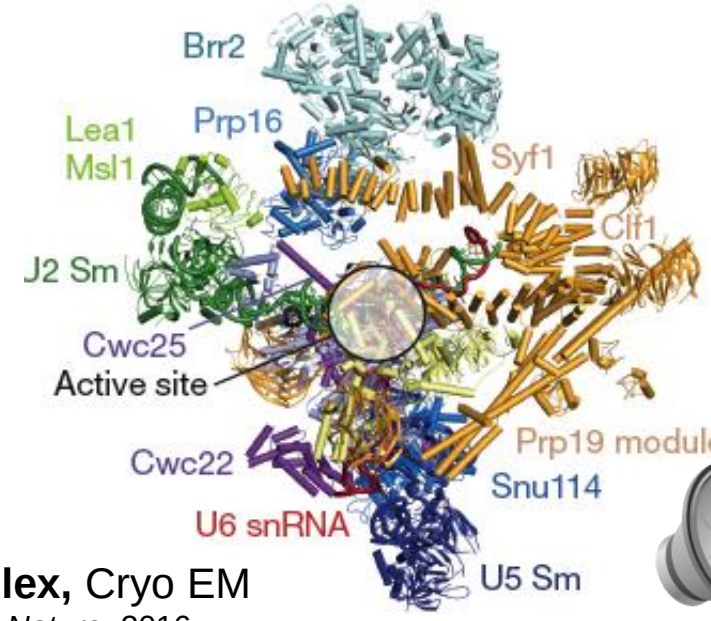


**Ribosome,** crystal structure
Cryo EM
*Ditlev Brodersen, Venki Ramakrishnan*

**SPLICEOSOME** - pre-mRNA splicing




**5 snRNAs**
**U1, U2, U4, U5, U6**




**C complex,** Cryo EM
Galej *et al, Nature,* 2016

# RNA

- coding: mRNAs
- non-coding: ncRNAs
  - structural (rRNA, tRNA)
  - regulatory (si/miRNA)

- stable
- unstable

- polyadenylated
- non-polyadenylated

**There are no „free” RNAs in the cell**

**All cellular RNAs exist as ribonucleoprotein particles (RNPs)**

**All RNA types are synthesised as precursors and undergo processing**

**RNA transcription, processing and decay are tightly coordinated**

**Several RNA processing steps occur co-transcriptionally**

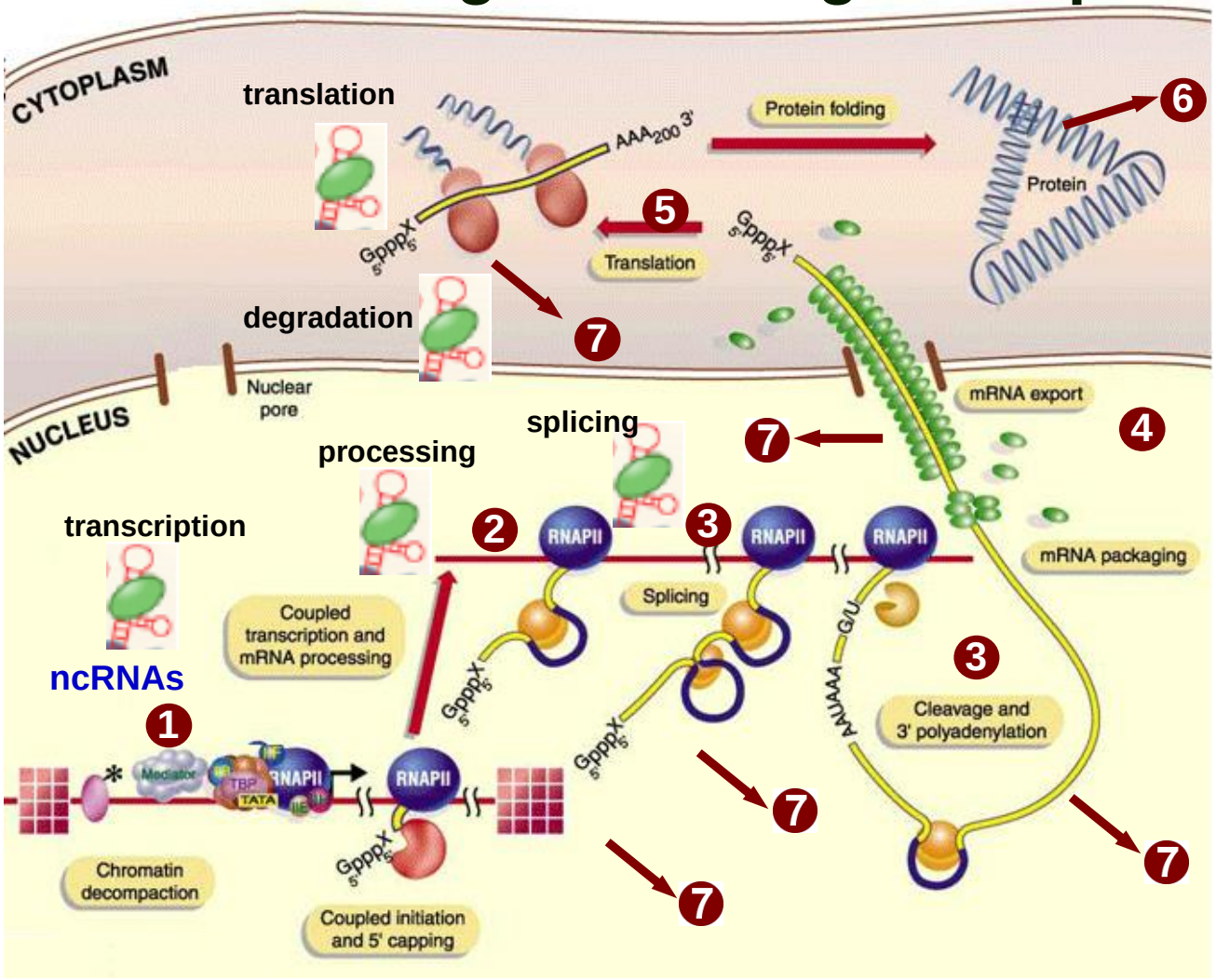
**Regulation of RNA biogenesis involves alternative processes:**

**aTSS, aTIS, AS, APA**

**Lecture on ncRNAs by Monika Zakrzewska-Placzek**

# RNA FLUX

## Regulation of gene expression




1) chromatin

2) transcription

3) RNA processing

4) RNA export

5) translation (mRNA)

6) protein stability

7) RNA degradation

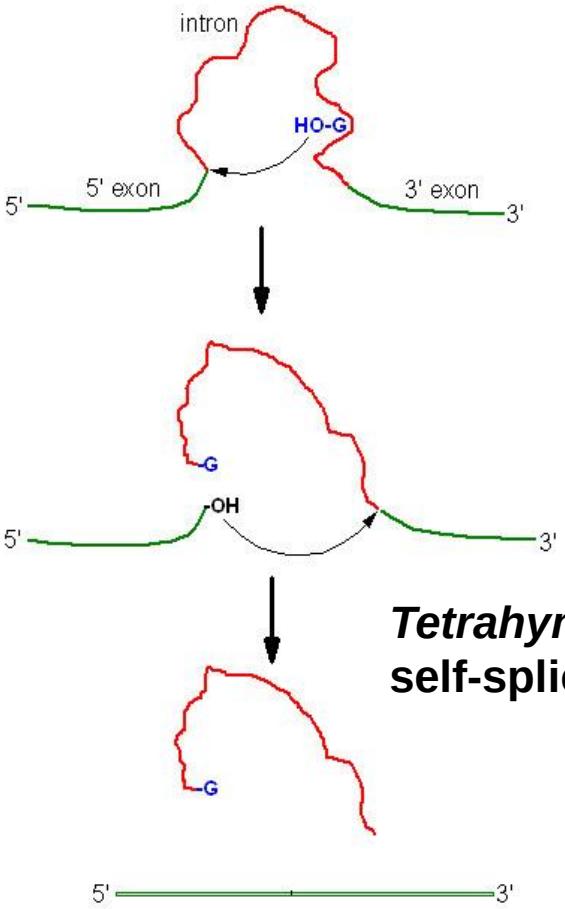
# RNA capacity - CATALYTIC RNAs

## Nobel 1989

RNA enzymes **– RIBOZYMES**

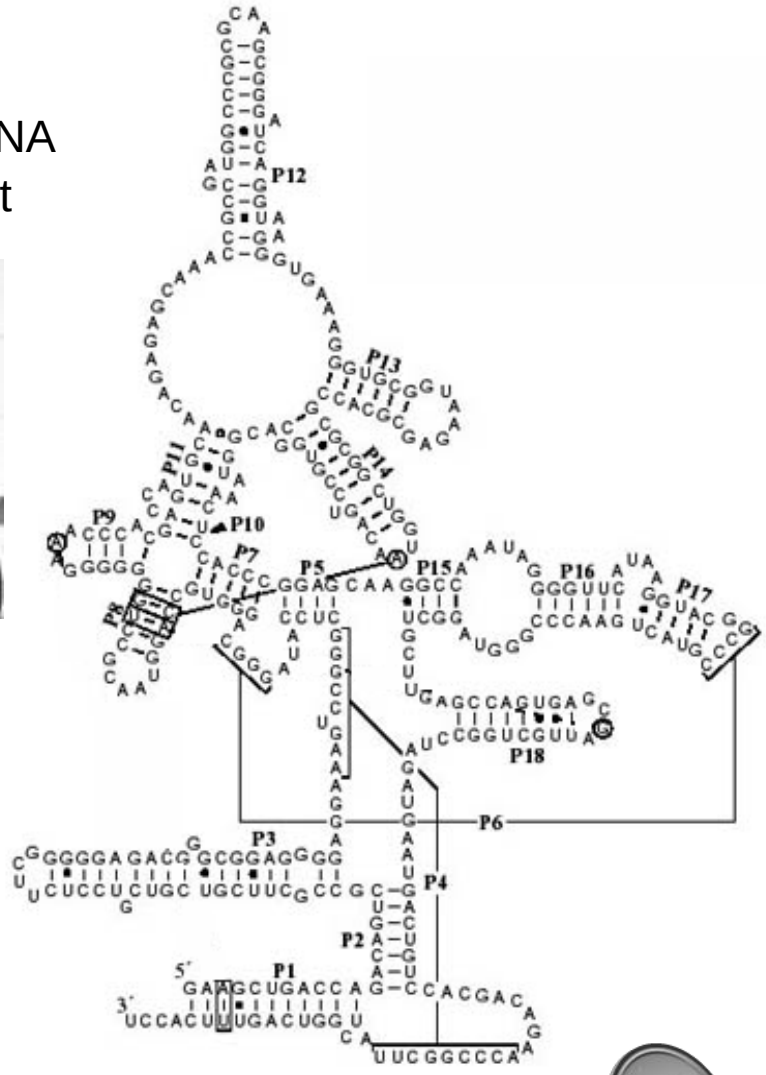
-1981/82 Tom Cech - self-splicing in *Tetrahymena* rRNA

-1982 Sidney Altman - bacterial RNaseP RNA subunit




**Thomas Cech**
**Sidney Altman**

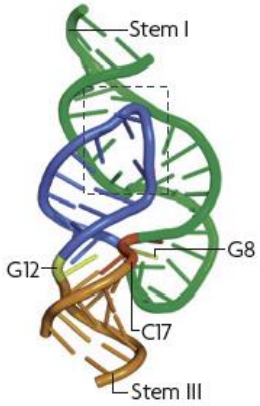
***Tetrahymena* group I self-splicing intron**

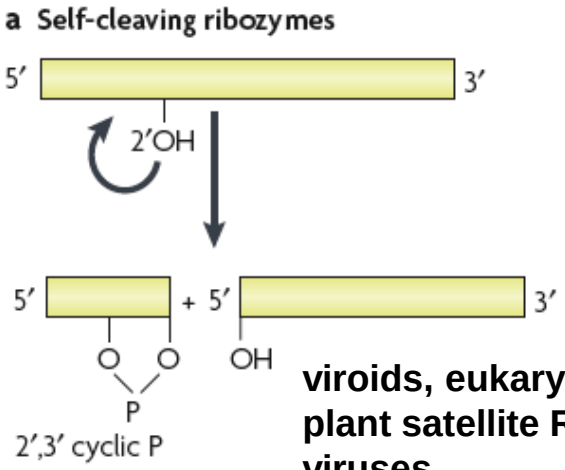



***Escherichia coli* RNaseP R**

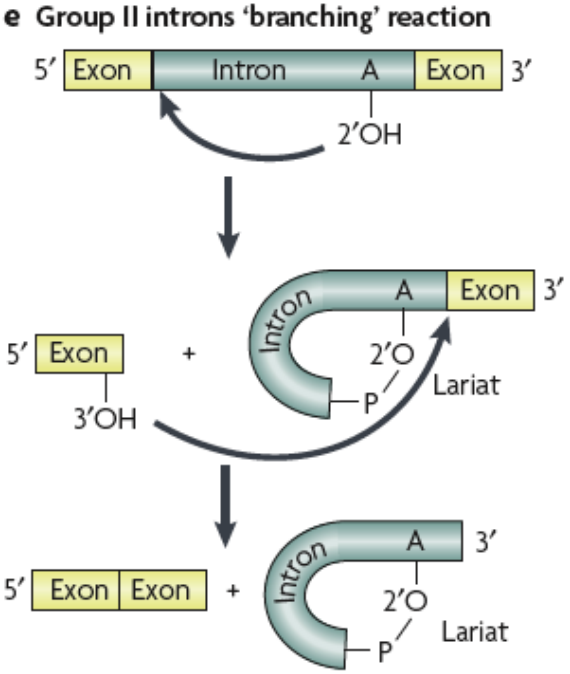
# RIBOZYMES

**Hammerhead, Hairpin, HDV**

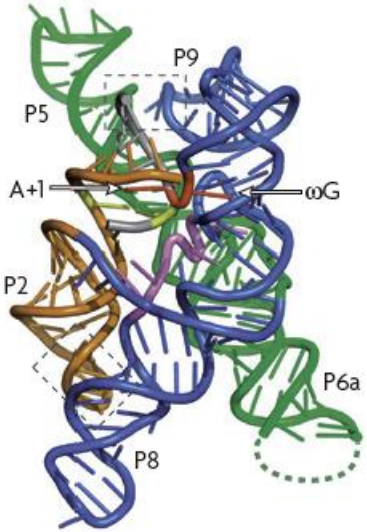


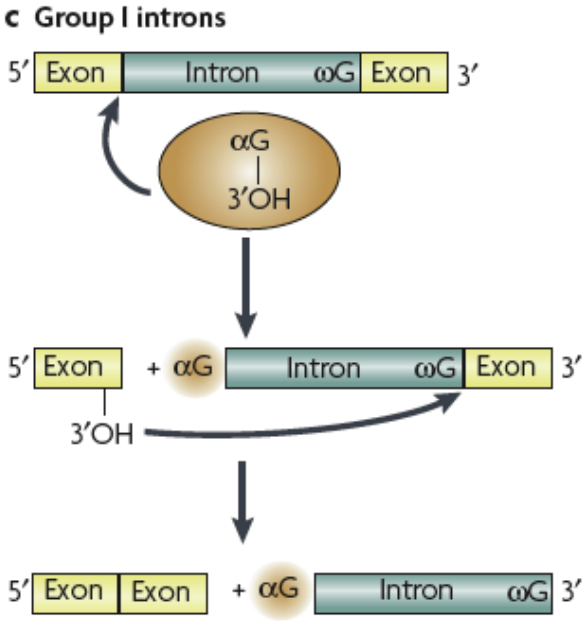

**viroids, eukaryotes plant satellite RNA, viruses**

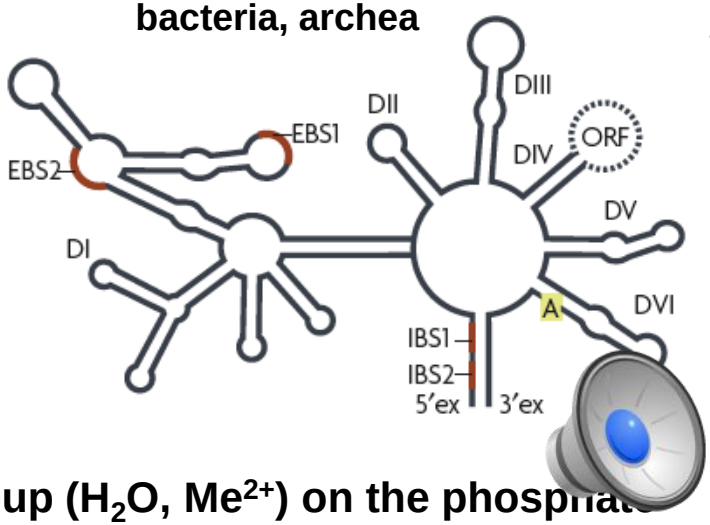



**mRNA splicing-like organelles (fungi, plants), bacteria, archea**




**organelles (fungi, plants), bacteria, mitochondria (animals)**



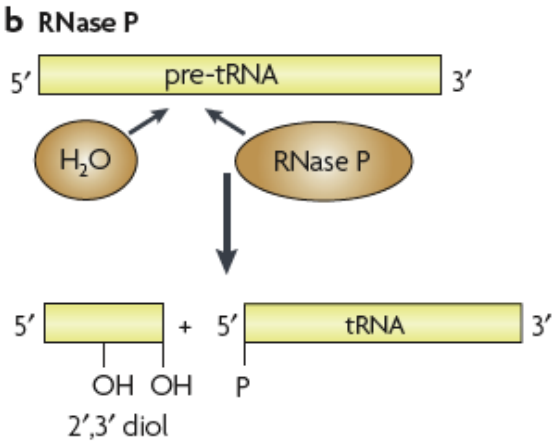

Serganov and Patel, *Nat. Rev. Genet.*, 2007

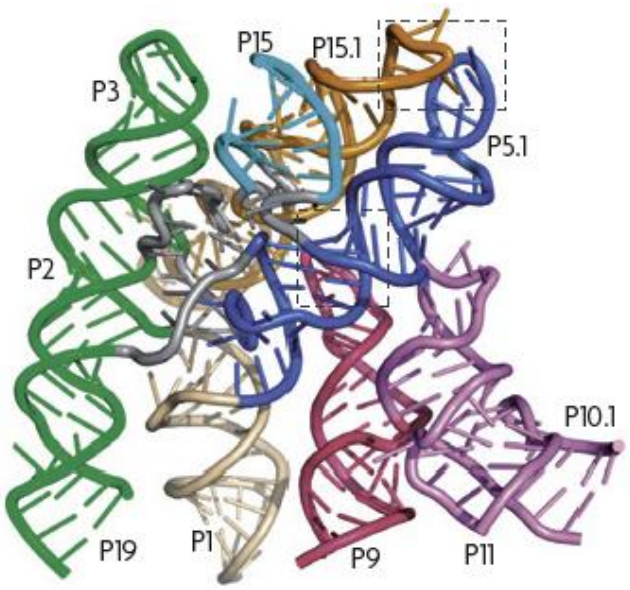
**Mechanism: nucleophilic attack of the ribose -OH group ($H_2O$, $Me^{2+}$) on the phosphat**

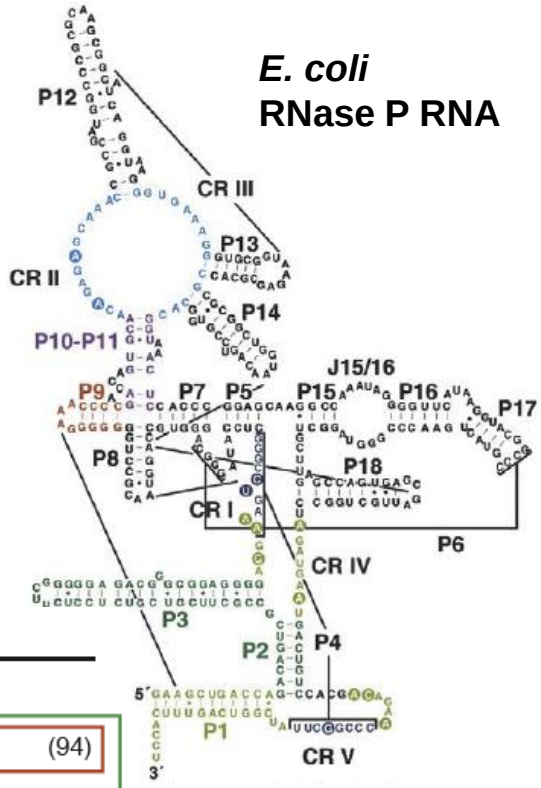
# RNase P RNA – a true enzyme

## *tRNA processing, multiple turnover*

Serganov and Patel, *Nat Rev Genet*, 2007; Evans et al, *TiBS*, 2006

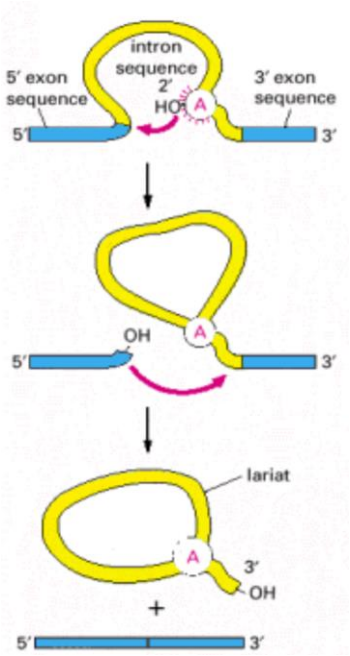


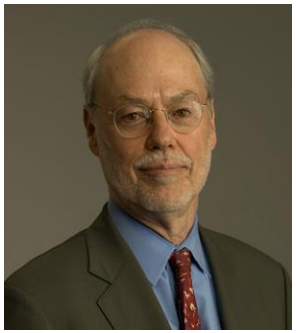




***E. coli***
**RNase P RNA**

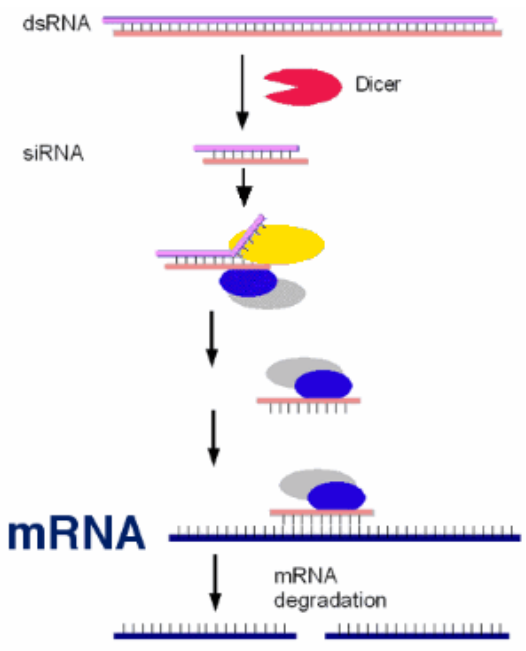
| Bacteria | | Eukarya | | | | Archaea | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Eco* | | *Sce* | | *Hsa* | | *Pfu* | | *Pho* | | *Mth* | |
| RNA | (121) | RNA | (118) | RNA | (109) | RNA | (106) | RNA | (106) | RNA | (94) |
| RnpA | (13.8) | | | | | | | | | | |
| | | Pop5 | (19.6) | hPop5 | (18.8) | PF1378 | (13.8) | PH1481* | (14.0) | MTH687 | (14.6) |
| | | Rpp1 | (32.2) | Rpp30 | (29.3) | PF1914 | (24.5) | PH1877 | (24.7) | MTH688 | (27.7) |
| | | Rpr2 | (16.3) | Rpp21* | (17.6) | PF1613 | (14.3) | PH1601* | (14.6) | MTH1618 | (17.0) |
| | | Pop4 | (32.9) | Rpp29* | (25.4) | PF1816 | (15.0) | PH1771* | (15.1) | MTH11 | (10.7) |
| | | Pop1 | (100.5) | hPop1 | (114.7) | | | | | | |
| | | Pop3 | (22.6) | Rpp38 | (31.8) | | | | | | |
| | | Pop7 | (15.8) | Rpp20 | (15.7) | | | | | | |
| | | Pop6 | (18.2) | | | | | | | | |
| | | Pop8 | (15.5) | | | | | | | | |
| | | | | Rpp40 | (34.6) | | | | | | |
| | | | | Rpp25 | (20.6) | | | | | | |
| | | | | Rpp14 | (13.7) | | | | | | |




# mRNA SPLICING Nobel 1993



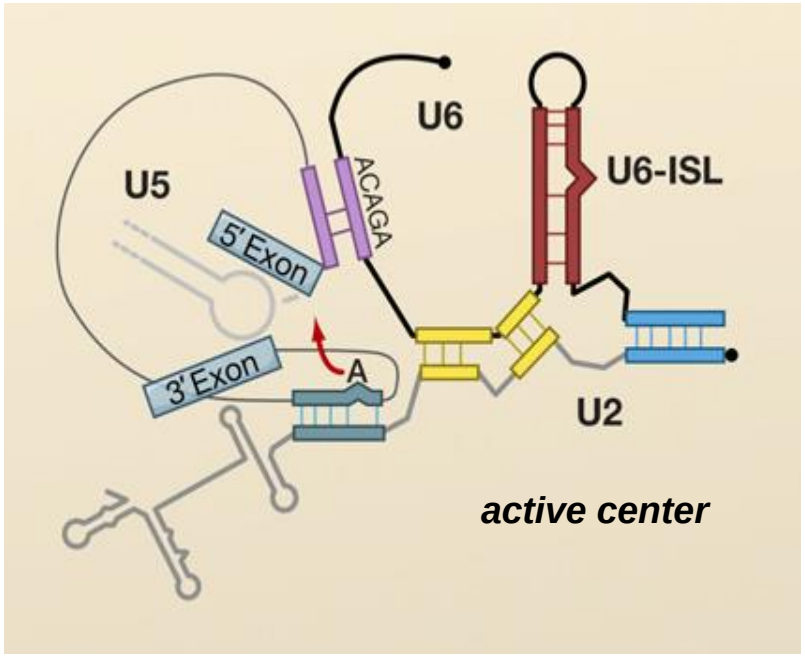
**Phil Sharp**
**Richard Roberts**

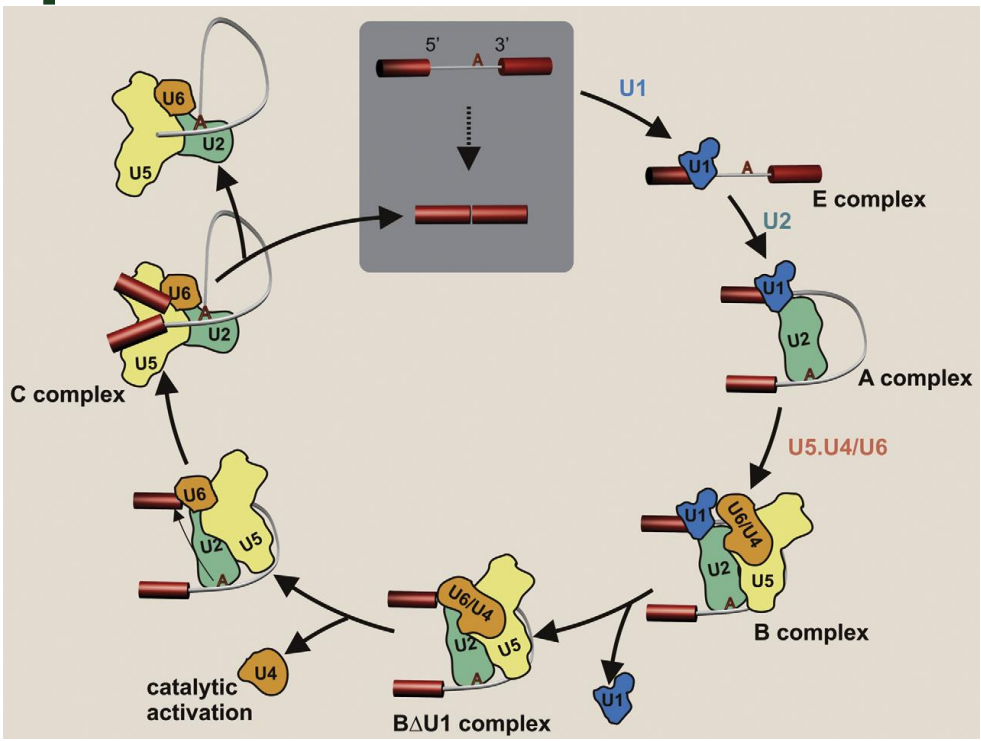



# RNAi Nobel 2006

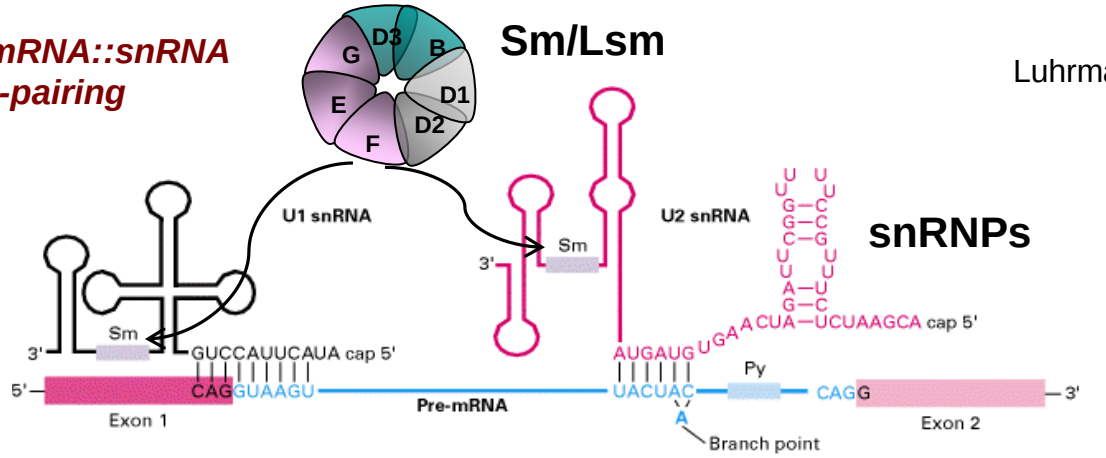
**Andrew Fire**
**Craig Mello**

# SPLICEOSOME: pre-mRNA SPLICING




*pre-mRNA::snRNA base-pairing*

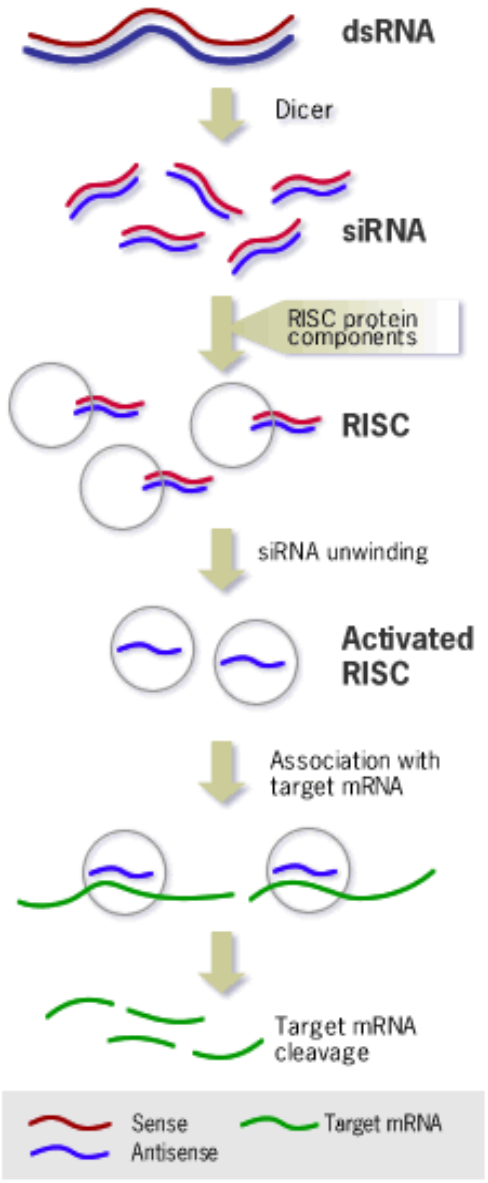



Luhrmann and Stark, *Curr. Op. Str. Biol.*, 2009

**SPLICEOSOME -**ribonucleoprotein complex (RNP) organised around snRNAs

# GENE SILENCING - RNAi

***DISCOVERY OF 2002: ncRNAs in RNAi***


**siRNAs/miRNAs:**

**- double stranded small noncoding RNAs**

**- complementary to mRNA targets**

**- participate in gene silencing**

**- mediate:**

***TRANSCRIPTIONAL GENE SILENCING (TGS)***

- **transcription inhibition**

***POST-TRANSCRIPTIONAL GENE SILENCING (PTGS)***

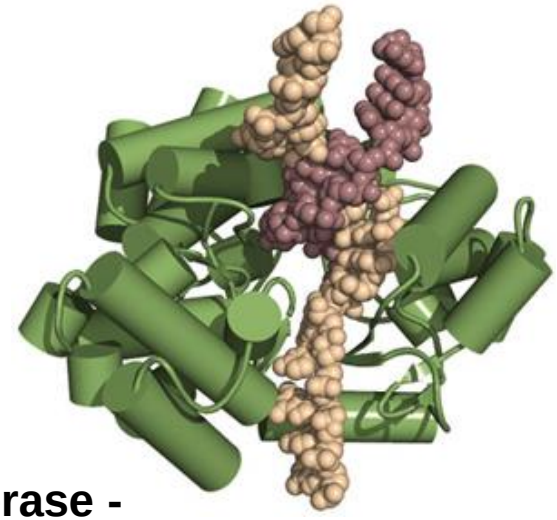
- **mRNA cleavage** *or*
- **translation inhibitio**
- **translation activat**

# RNAs – STRUCTURE AND FUNCTION

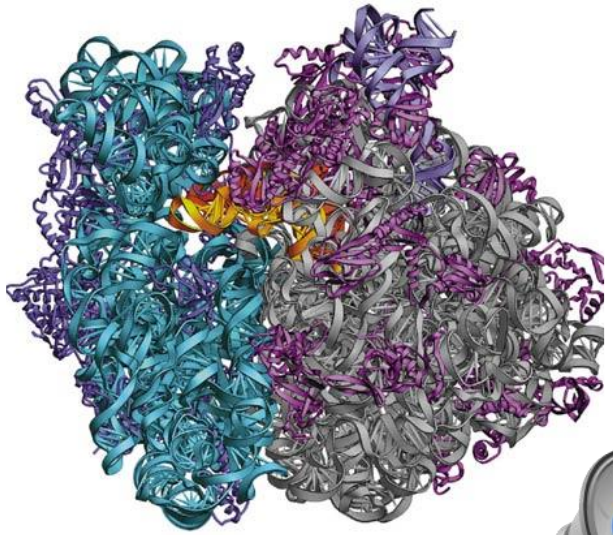
## Nobel 2009

**Elizabeth Blackburn**
**Jack Szostak**
**Carol Greider**

**Venkatraman Ramakrishnan**
**Ada Yonath**
**Thomas Steitz**

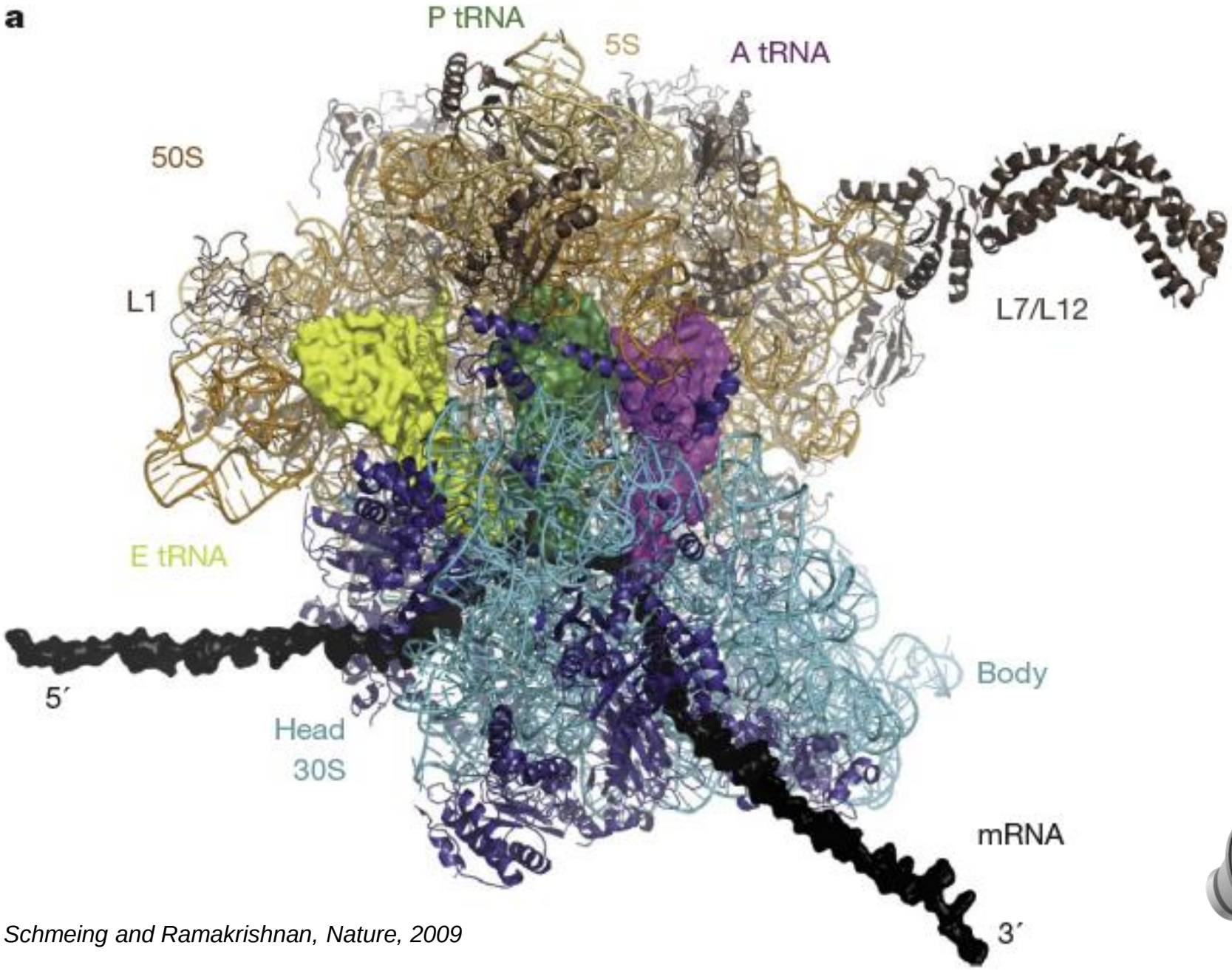


**Telomerase -**
**maintaing chromosome ends**



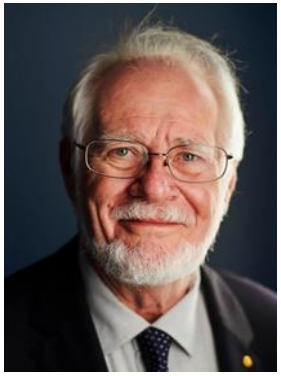
**Crystal structure of the ribos**

# THE RIBOSOME




*Schmeing and Ramakrishnan, Nature, 2009*

# RNPs - STRUCTURE/METHODOLOGY



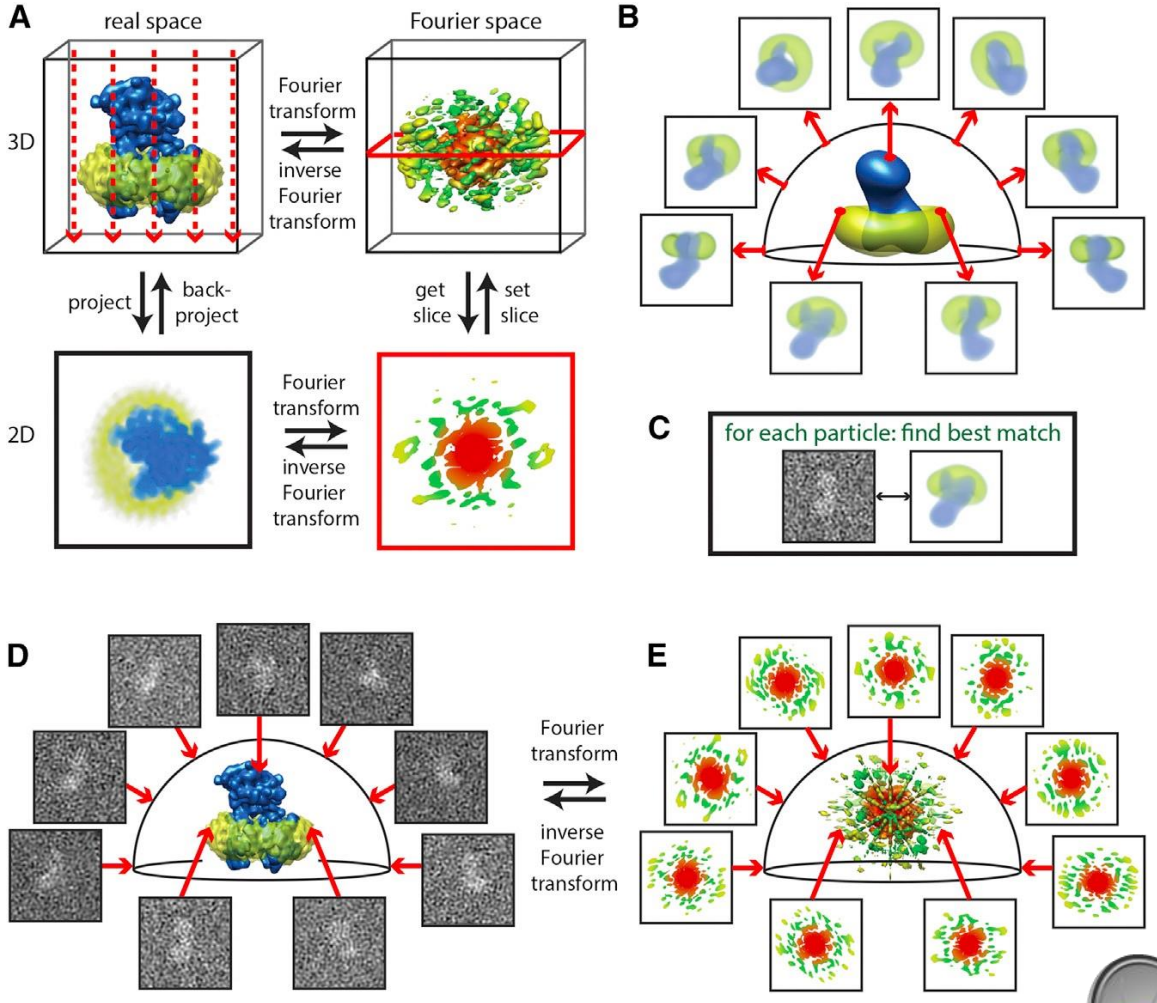
Jacques Dubochet

Joachim Frank

Richard Henderson

## Nobel 2017

## *CRYO-EM*




*Nogales and Scheres, Mol Cell 2015*

Lecture on crystallography and CryoEM by Marcin N

# CRISPR-Cas: CRISPR-based genome editing

## Nobel 2020

**Emmanuelle Charpentier**
**Max Planck Institute**

**Jenifer Doudna**
**University of California**

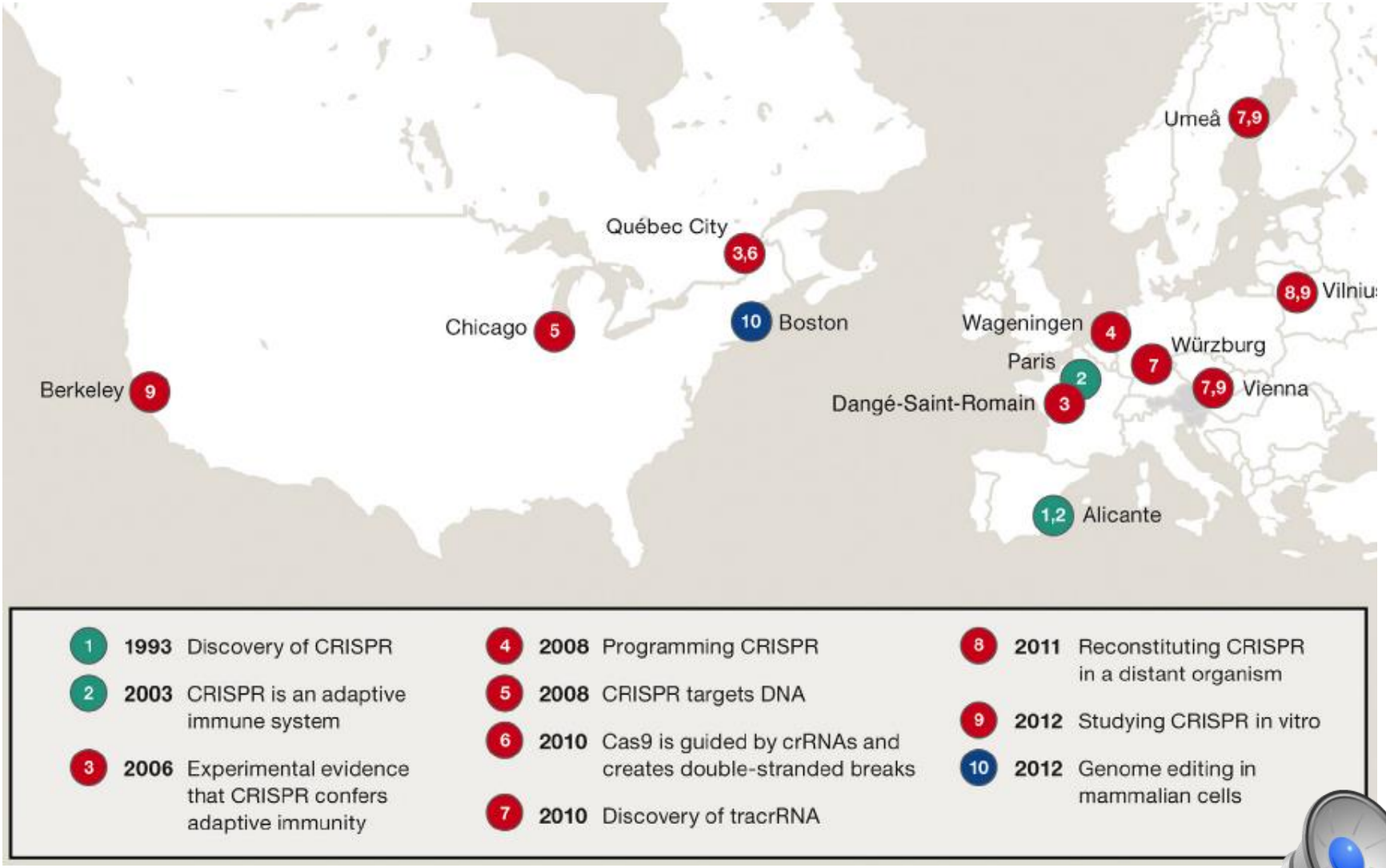
**CRISPR RNA maturation by *trans*-encoded small RNA and host factor RNase III**

Elitza Deltcheva[1,2], Krzysztof Chylinski[1,2]*, Cynthia M. Sharma[3]*, Karine Gonzales[2], Yanjie Chao[3,4], Zaid A. Pirzada[2], Maria R. Eckert[2], Jörg Vogel[3,4] & Emmanuelle Charpentier[1,2]

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

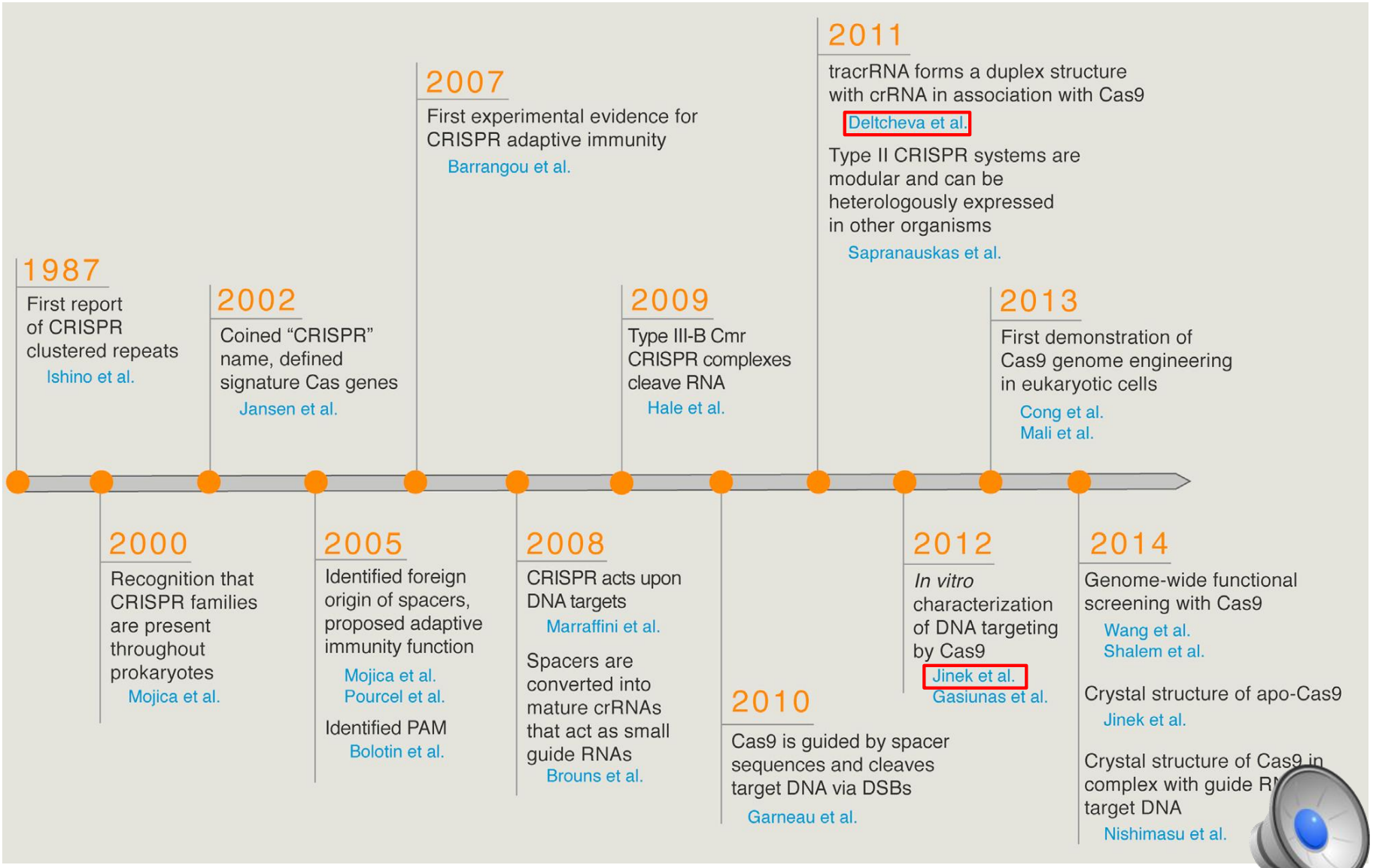
Martin Jinek,[1,2]* Krzysztof Chylinski,[3,4]* Ines Fonfara,[4] Michael Haue
Jennifer A. Doudna,[1,2,5,6]‡ Emmanuelle Charpentier[4]‡

# CRISPR/Cas history



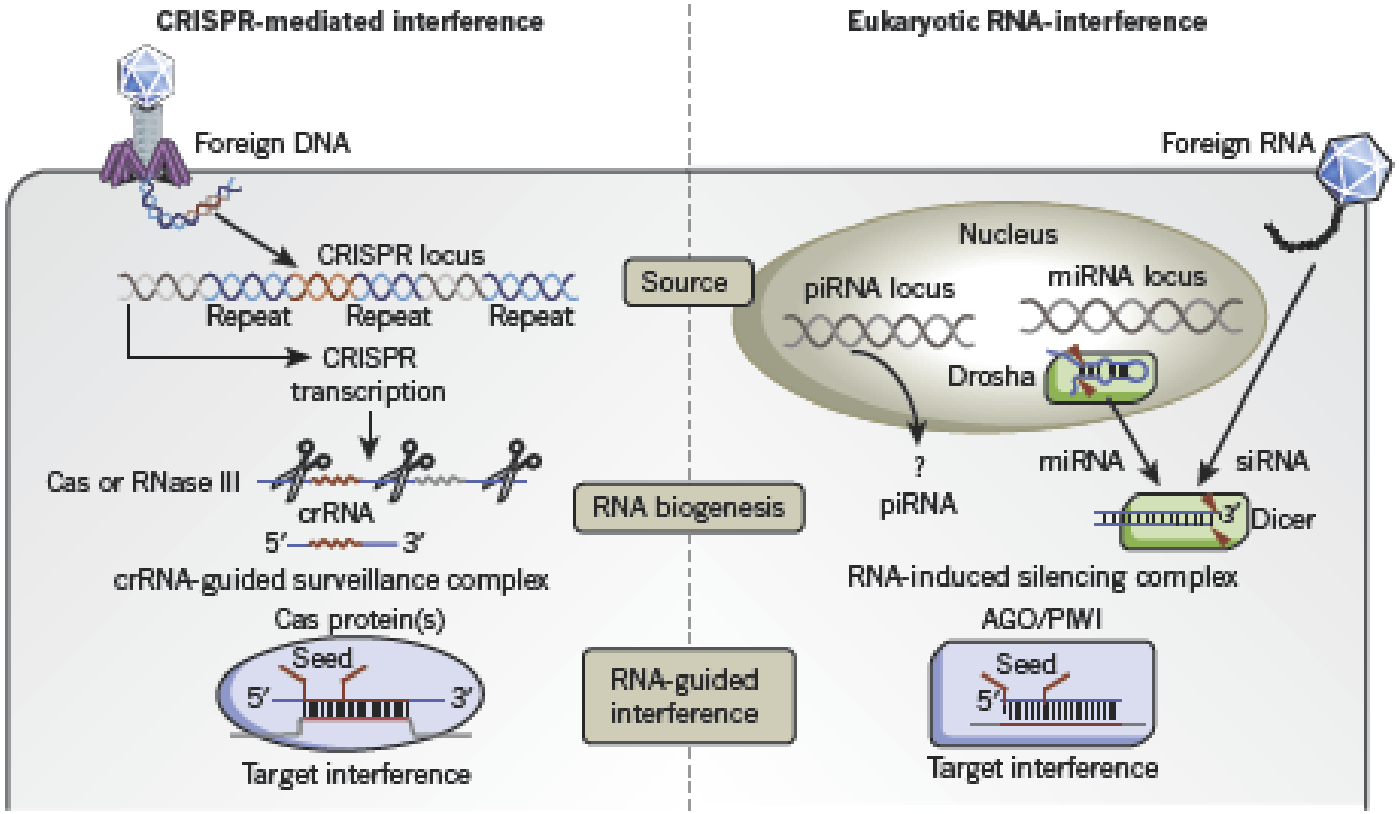

*Lander, Cell, 2016*

# CRISPR/Cas history



# CRISPR/Cas adaptive bacterial immunity
# RNA-guided RNAi in Bacteria and Archaea

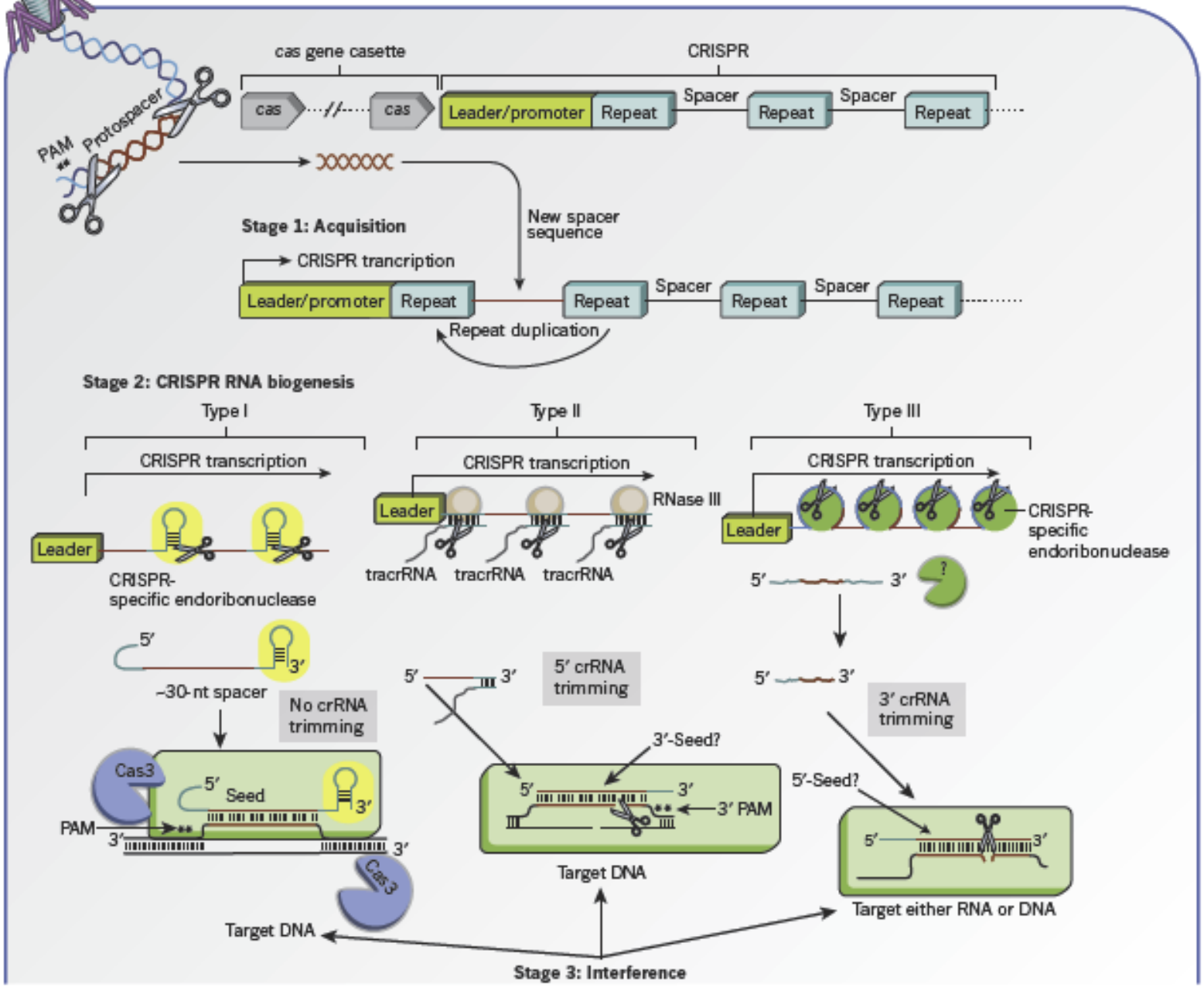
**CRISPR Clustered Regularly Interspaced Short Palindromic Repeat**
**Cas- CRISPR associated**




- **CRISPR: foreign DNA is integrated into the CRISPR locus**
- **long CRISPR transcripts are processed by Cas or RNase III nuclease**
- **short crRNAs assemble into surveillance complexes**
- **target invading DNAs or RNAs recognized by crRNA „seed" are destroyed**

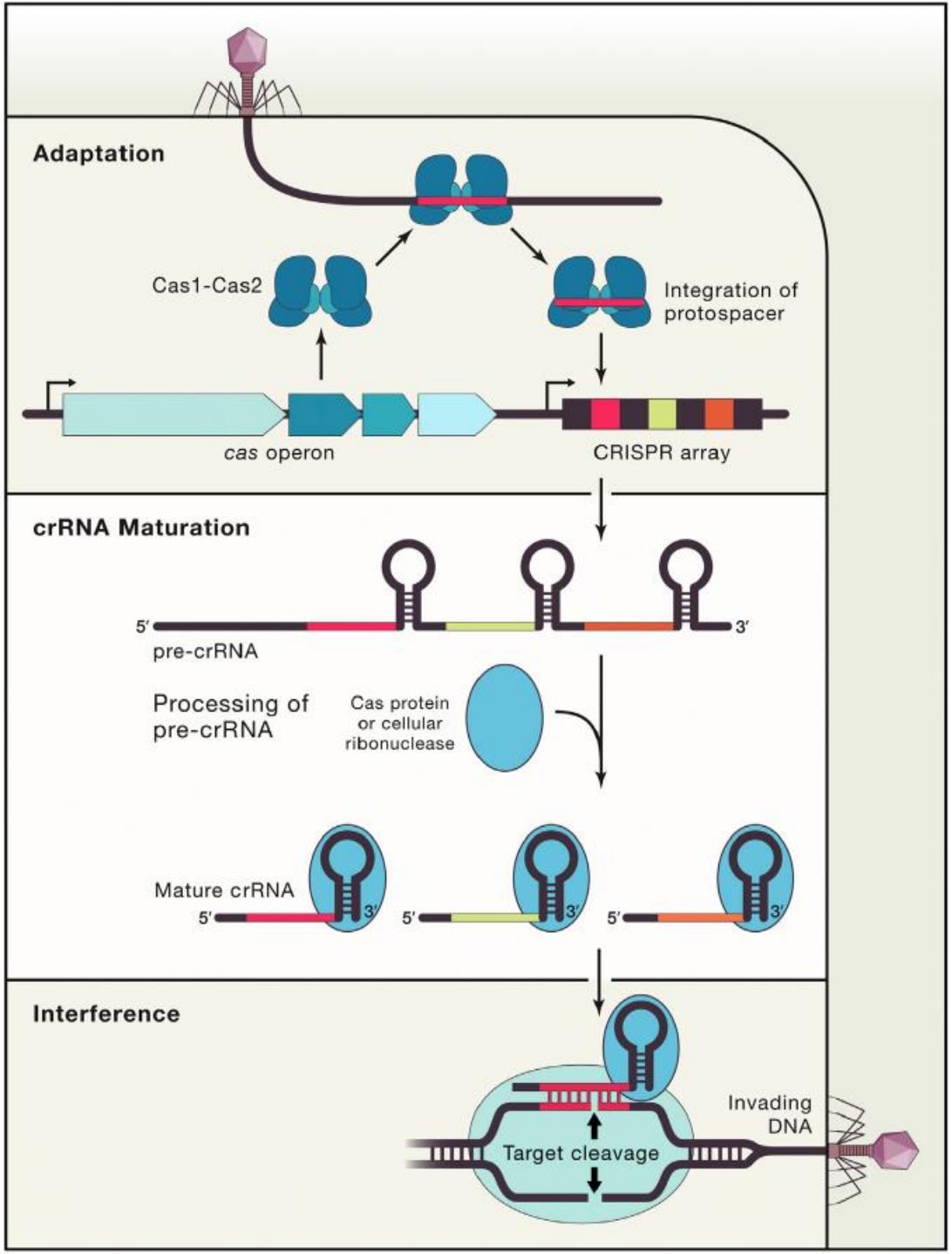
*Wiedenheft et al, Nature, 2012*

# CRISPR/Cas adaptive bacterial immunity



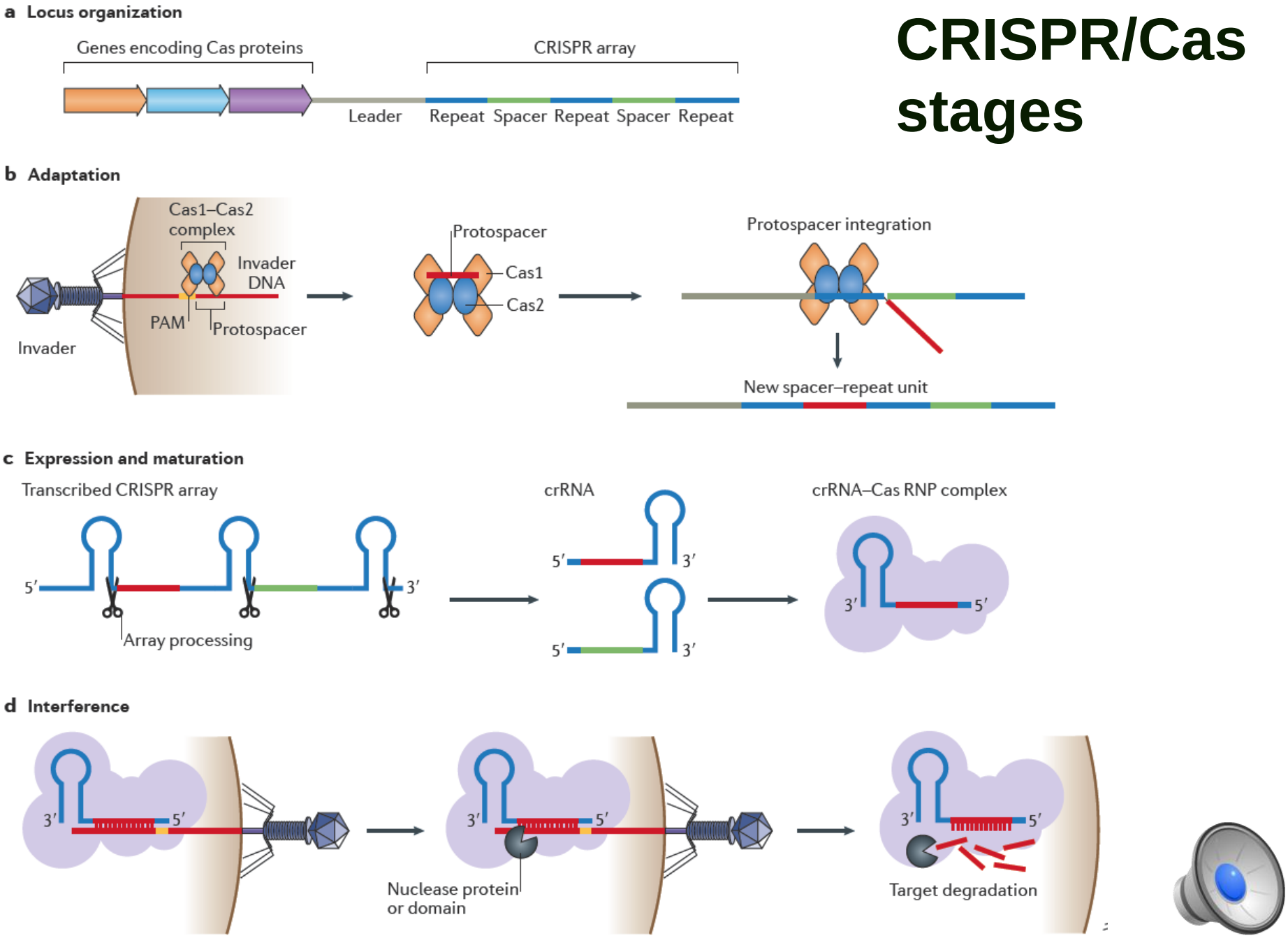

Wiedenheft et al, *Nature*, 2012

# CRISPR/Cas stages



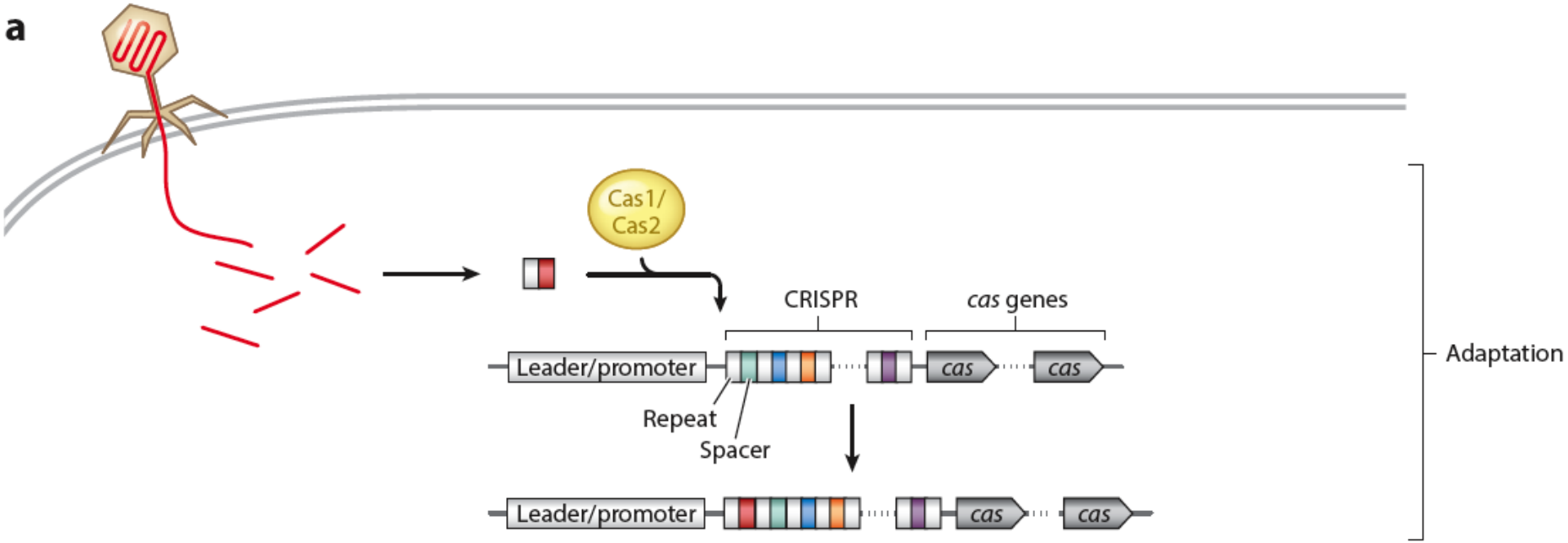

*Hille et al, Cell, 2018*

# CRISPR/Cas stages




*Amitai and Sorek, NatRevMicro, 2016*

# CRISPR/Cas: adaptation and spacer acquisition



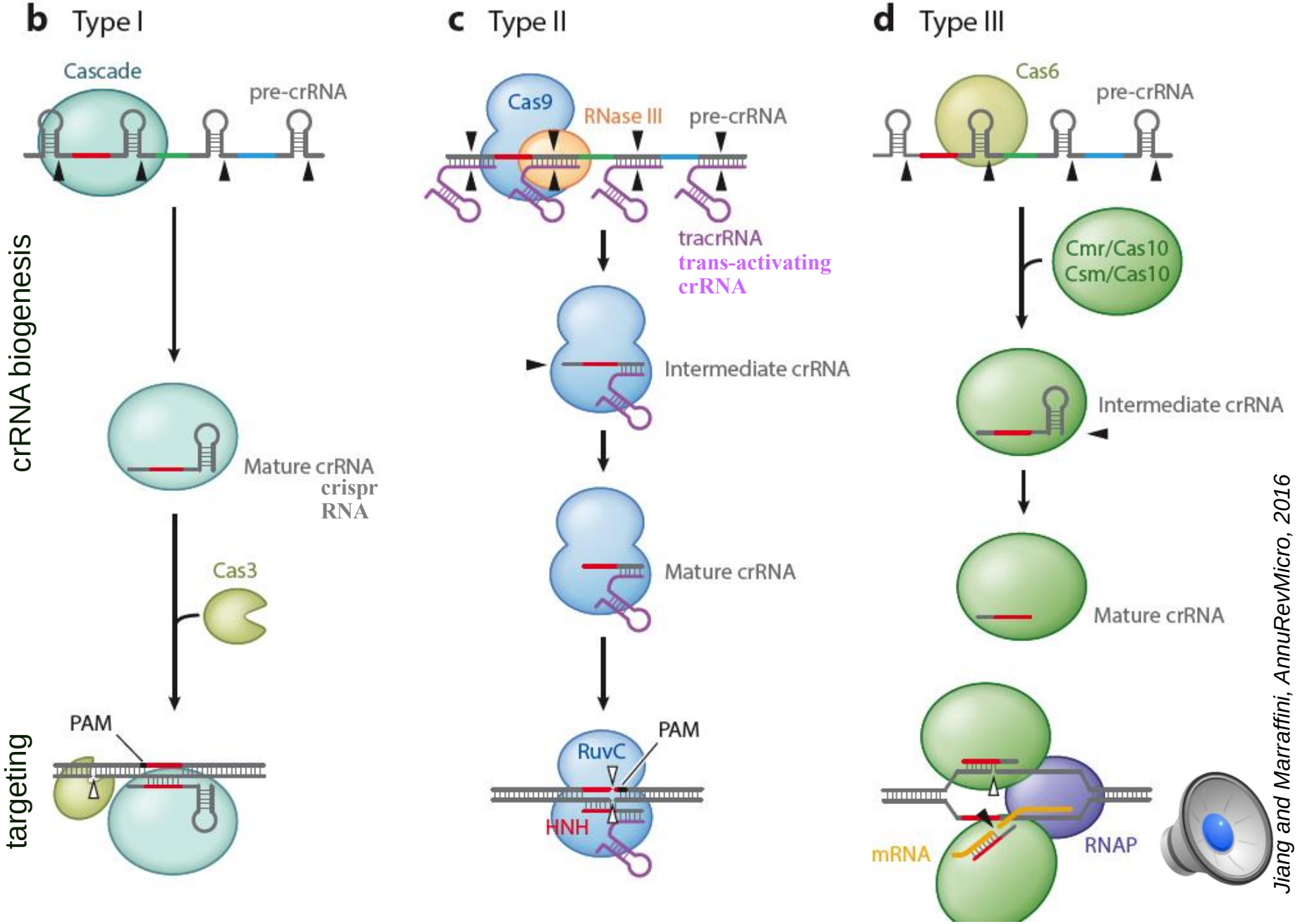

**PAM protospacer-adjacent motif in most CRISPR-Cas systems**

**- e.g. in type I immunity usually tri-nucleotide (AWG in *E. coli*) recognized by the Cascade complex (CasA in *E. coli*)**

**- probably allows tolerance to self (prevents autoimmunity against spacer DNA sequ complementary to crRNAs they encode)**

*Jiang and Marraffini, AnnuRevMicro, 2016*

# CRISPR/Cas: crRNA biogenesis, targeting




*Jiang and Marraffini, AnnuRevMicro, 2016*

# CRISPR/Cas types

**Table 1. Classification and Examples of CRISPR Systems**

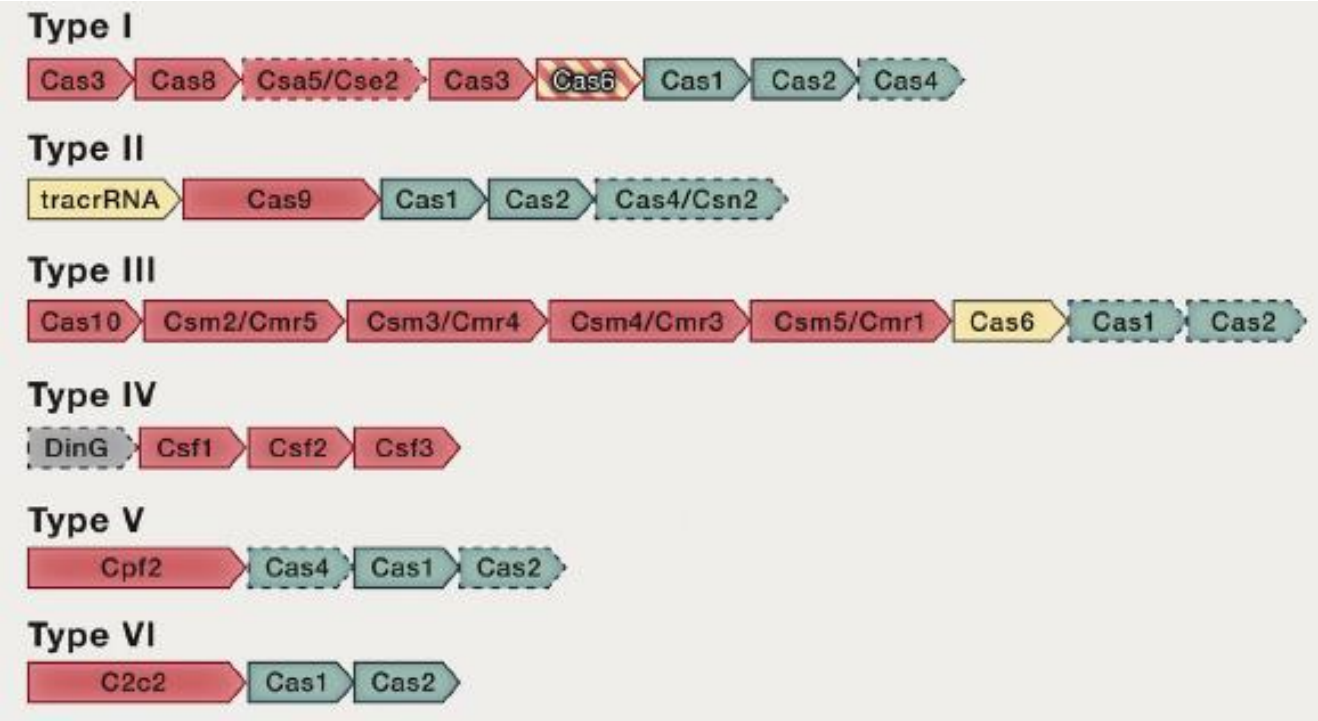
| Class | Type | Subtype | Hallmarks | Example effector | Example organism | Studies Cited |
|---|---|---|---|---|---|---|
| Class 1 | Type I | | multisubunit effector complex; Cas3 | Cascade | *E. coli* | Brouns et al., 2008 |
| | Type III | III-A | multisubunit effector complex; Csm effector module; DNA targeting | Cas10-Csm | *S. epidermidis* | Marraffini and Sontheimer, 2008 |
| | | III-B | multisubunit effector complex; Cmr effector module; RNA targeting | Cmr | *P. furiosus* | Hale et al., 2009 |
| Class 2 | Type II | | single protein effector; tracrRNA | Cas9 | *S. thermophilus* | Bolotin et al., 2005; Barrangou et al., 2007; Sapranauskas et al., 2011; Gasiunas et al., 2012 |
| | | | | | *S. pyogenes* | Deltcheva et al., 2011; Jinek et al., 2012; Cong et al., 2013; Mali et al., 2013 |
| | Type V | | single protein effector; single-RNA guided | Cpf1 | *F. novicida* | Zetsche et al., 2015 |

| Class | Class 1 Multi-subunit crRNA-effector complex | | | Class 2 Single-subunit crRNA-effector complex | | |
|---|---|---|---|---|---|---|
| Type | Type I | Type III | Type IV | Type II | Type V | Type VI |
| Effector complex | Cascade | Csm and Cmr | n.d. | Cas9 | Cpf1, C2c1, C2c3 | C2c2 |
| Target | dsDNA | ssRNA/ ssDNA | n.d. | dsDNA | dsDNA | ssRNA |

*Lander, Cell, 2016; Tamulaitis, TiMicro 2016*

# CRISPR/Cas types

## Gene organization

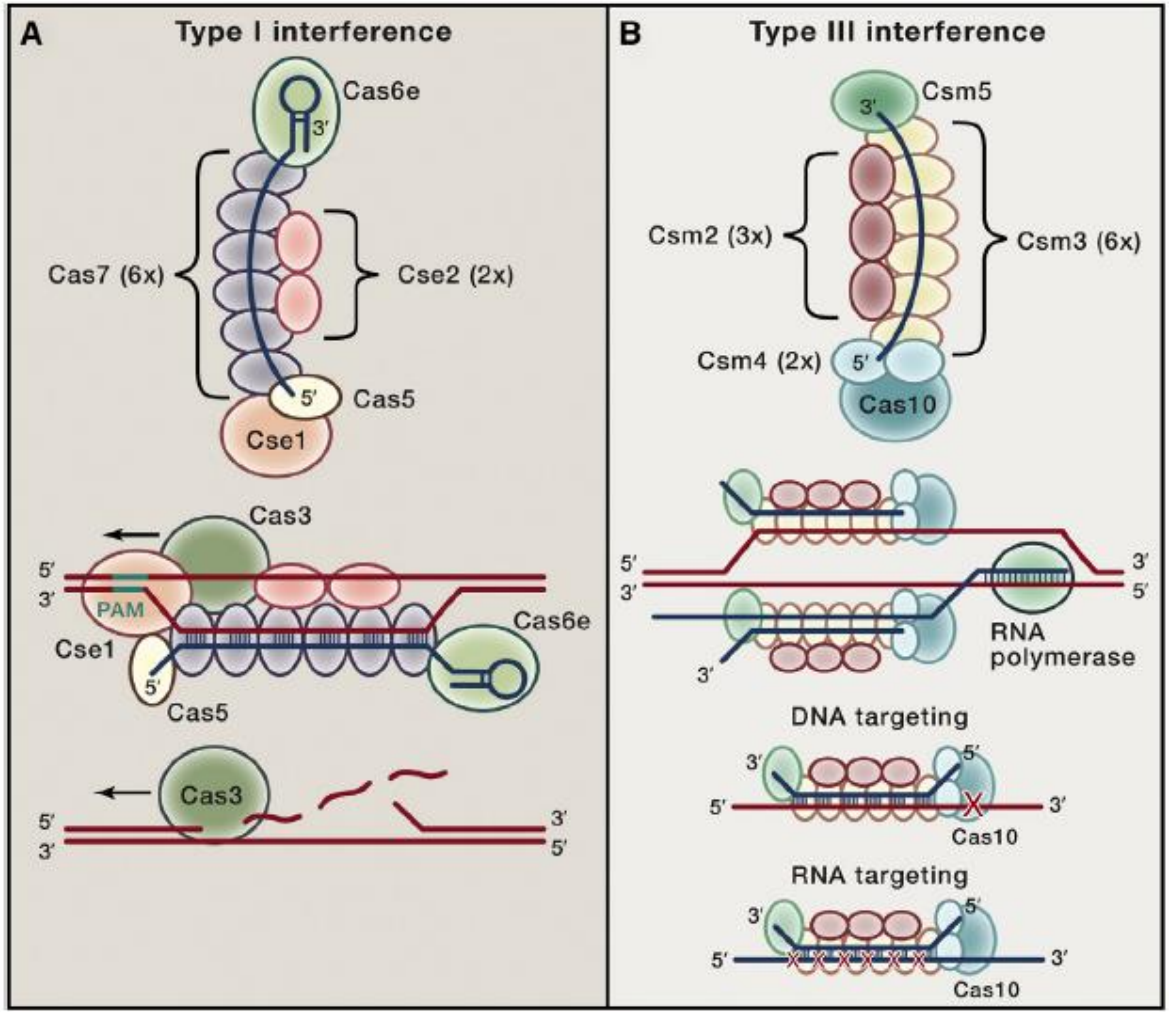



*Wright et al, Cell, 2016*

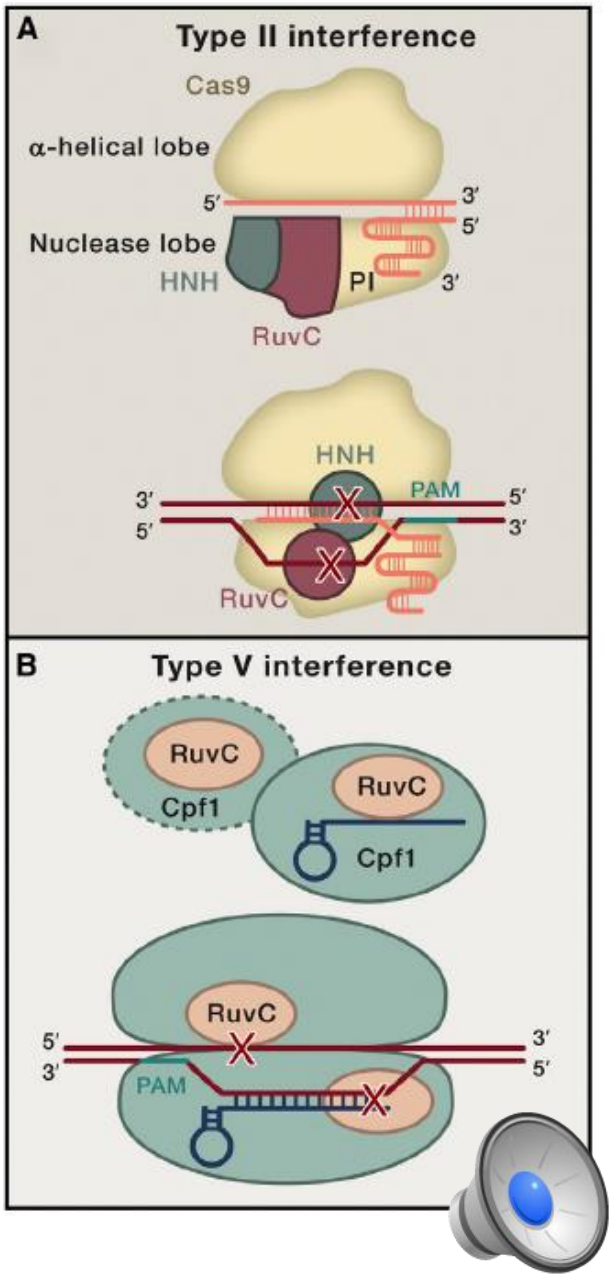
# CRISPR/Cas types

**targets DNA**

**targets RNA and actively transcribed DNA**



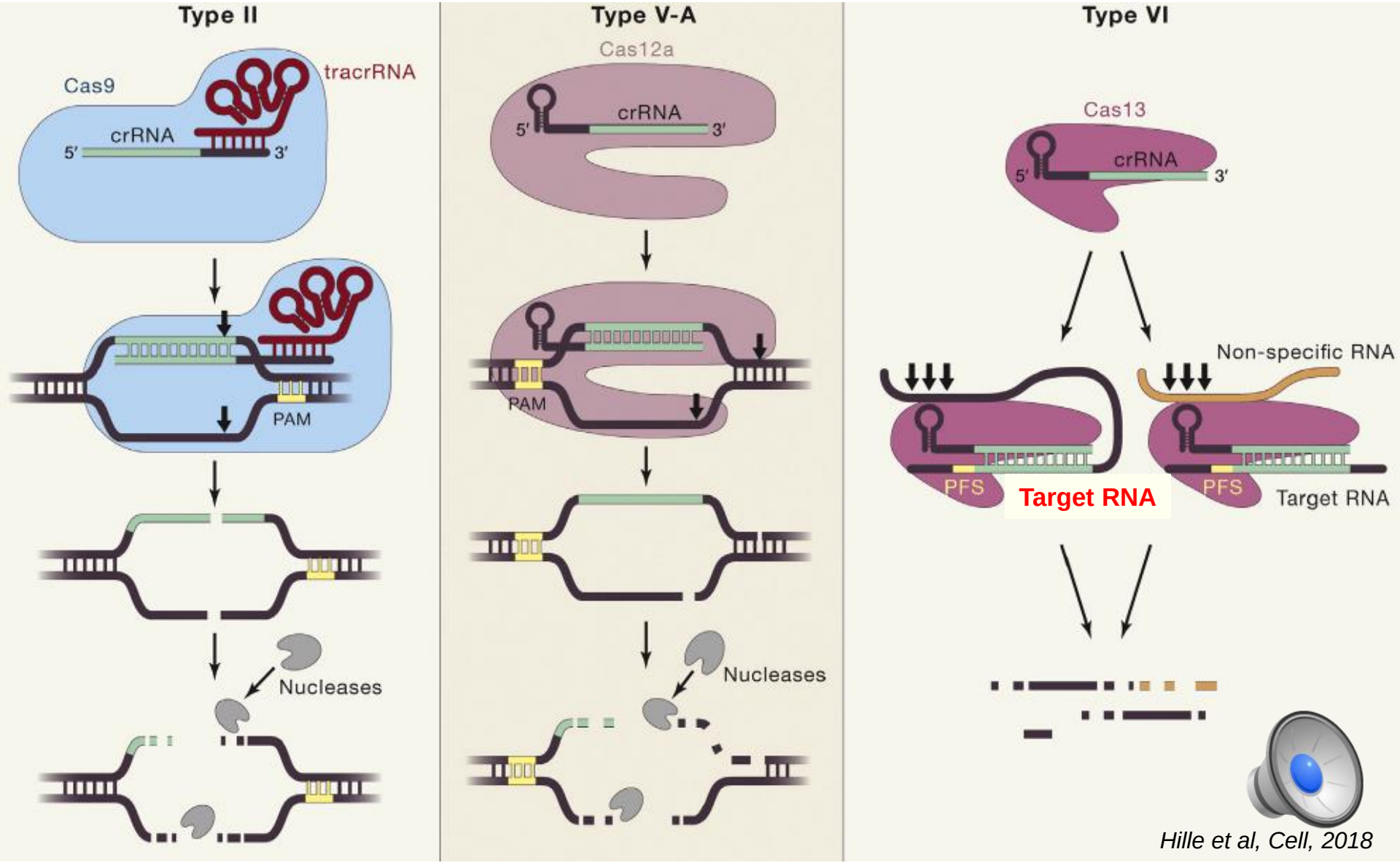

**target DNA**




*Wright et al, Cell, 2016*

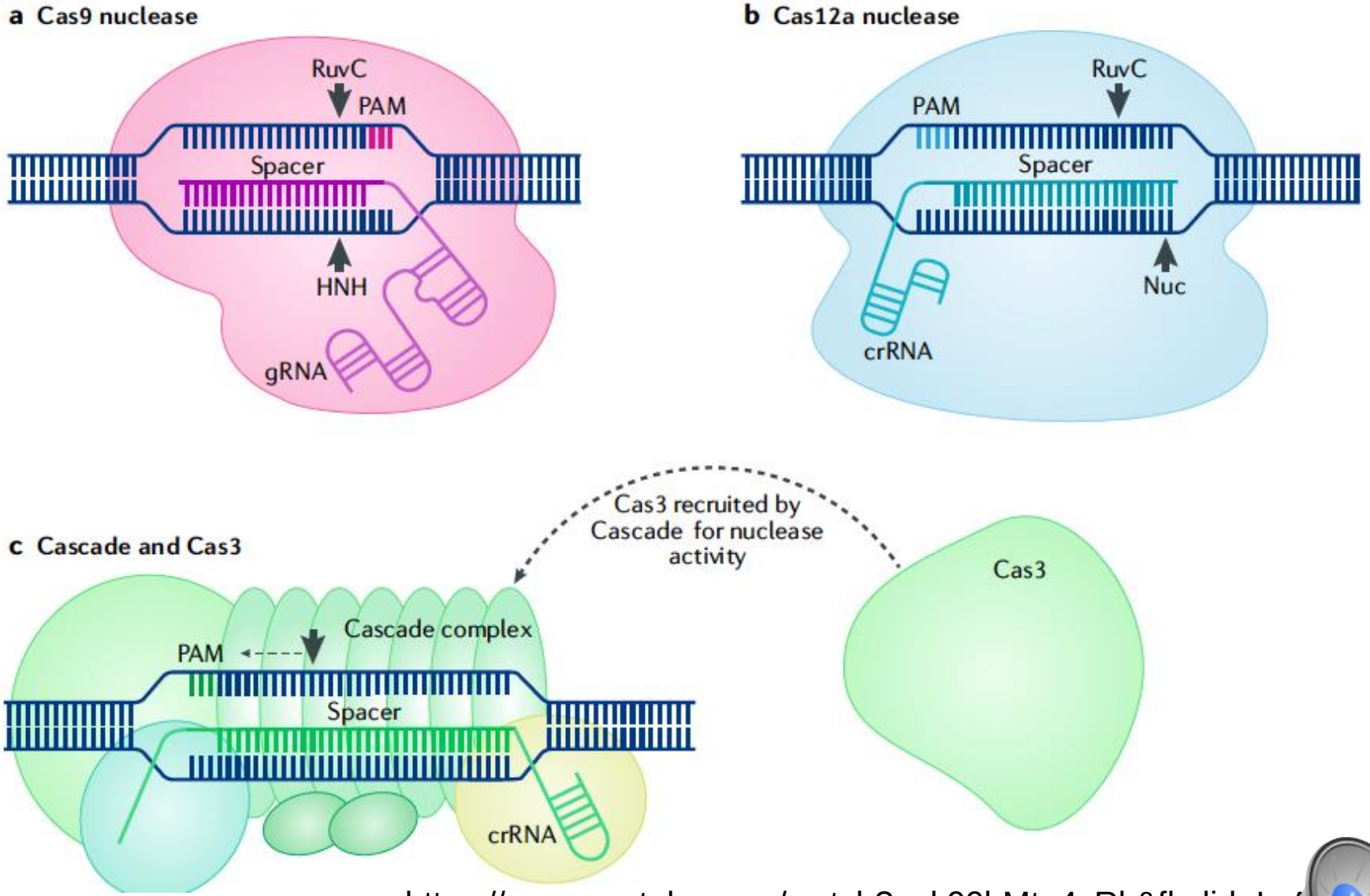
# Interference of Class 2 CRISPR/Cas

**One protein effector: Cas9, Cas12a or Cas13**




*Hille et al, Cell, 2018*

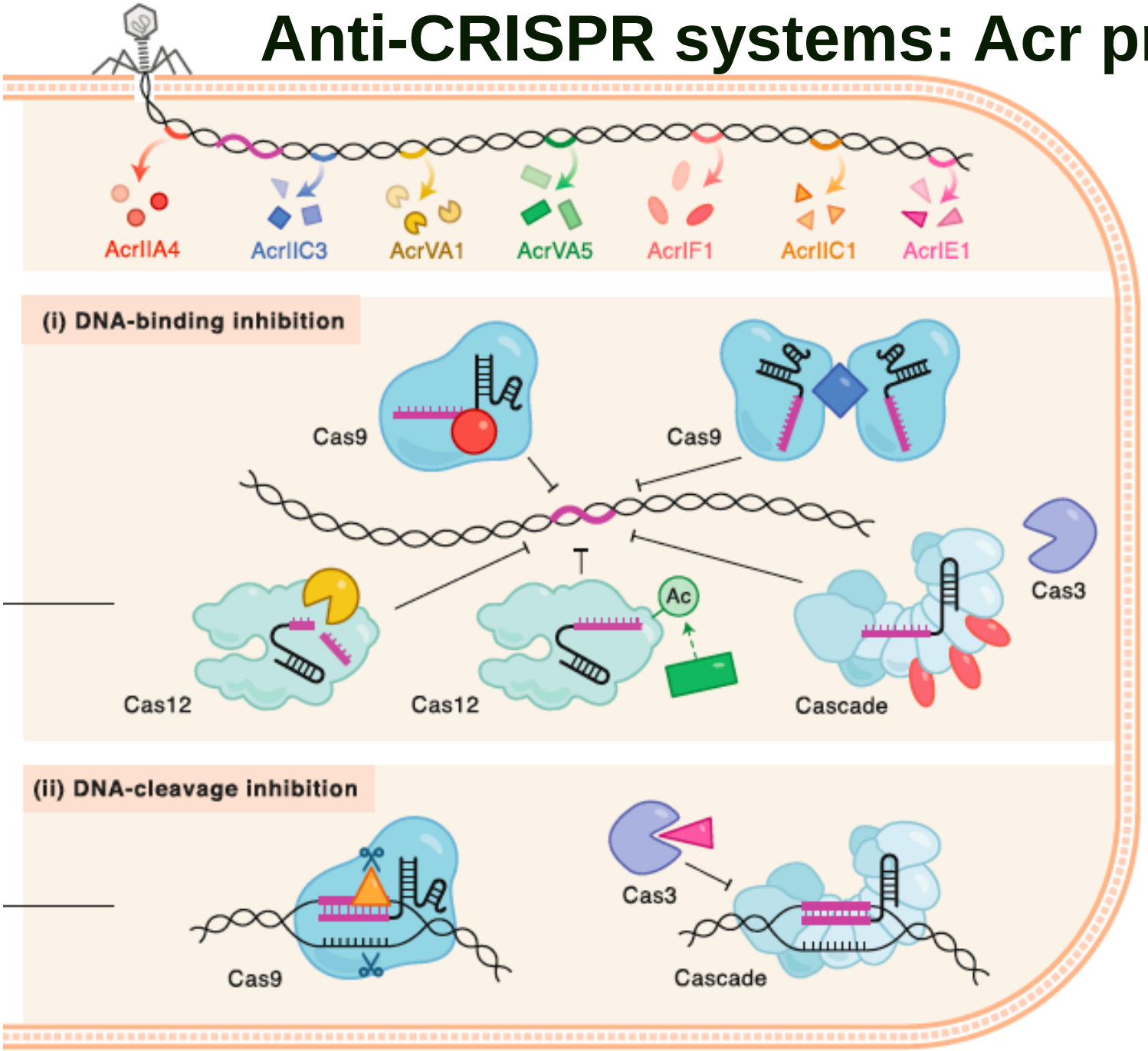
# Main CRISPR/Cas gene editing tools




*Pickar-Oliver and Gersbach, Nat Rev Mol Cell Biol, 2019*

https://www.youtube.com/watch?v=k99bMtg4zRk&fbclid=IwAR0xLX2v80gQlJQWoOOS6FkWX--XeIyYhksegRMuotAVOHySouTcGTIY

# Anti-CRISPR systems: Acr proteins




*al, Nature Methods, 2020*