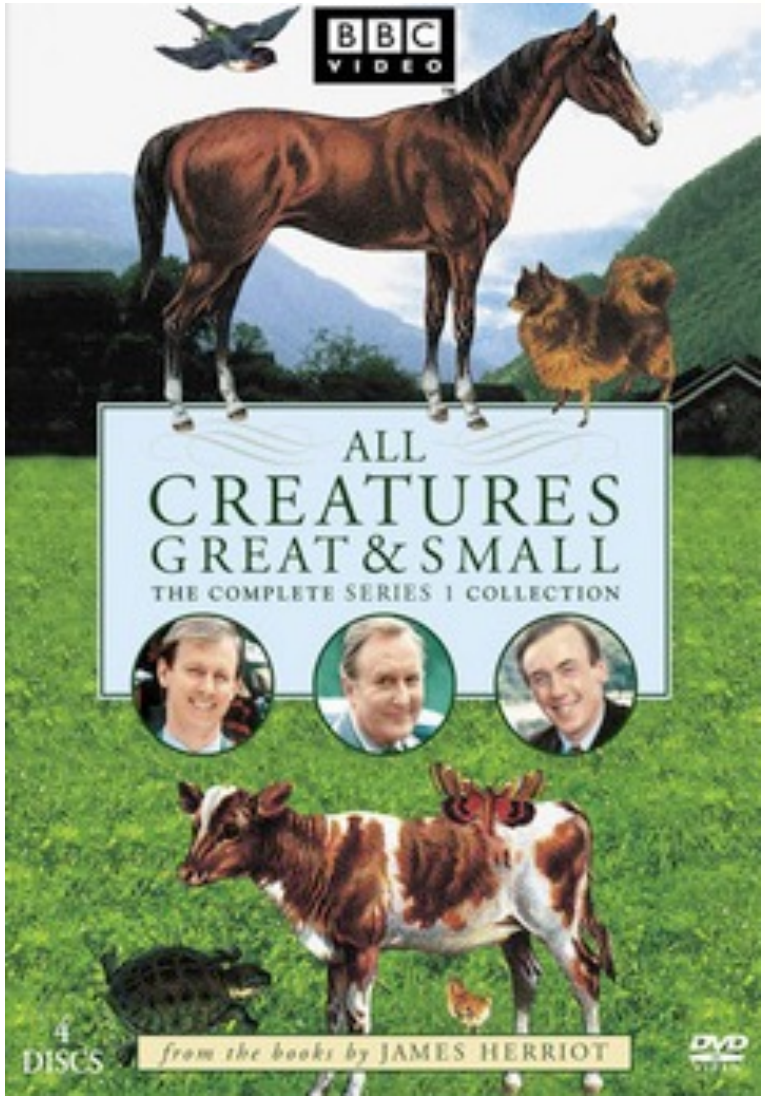
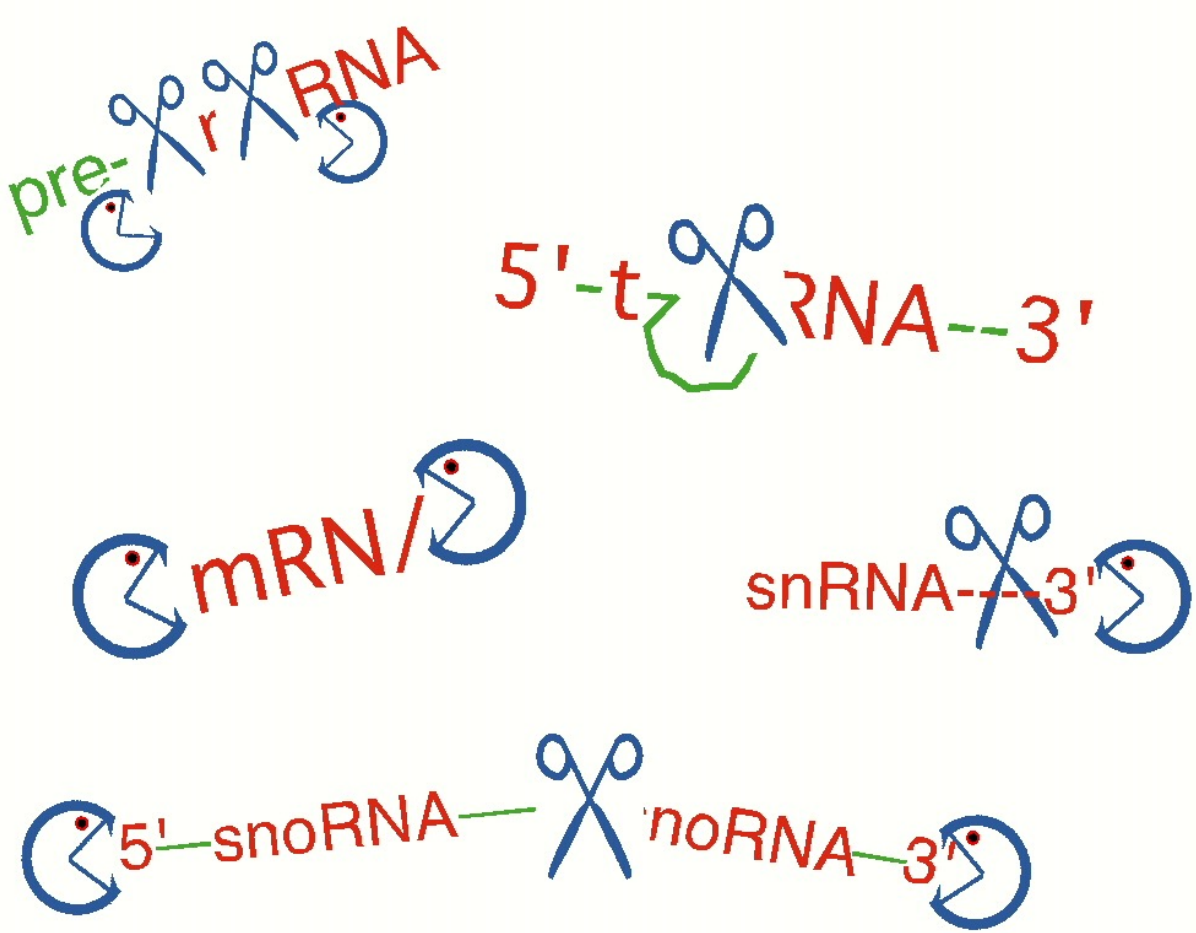
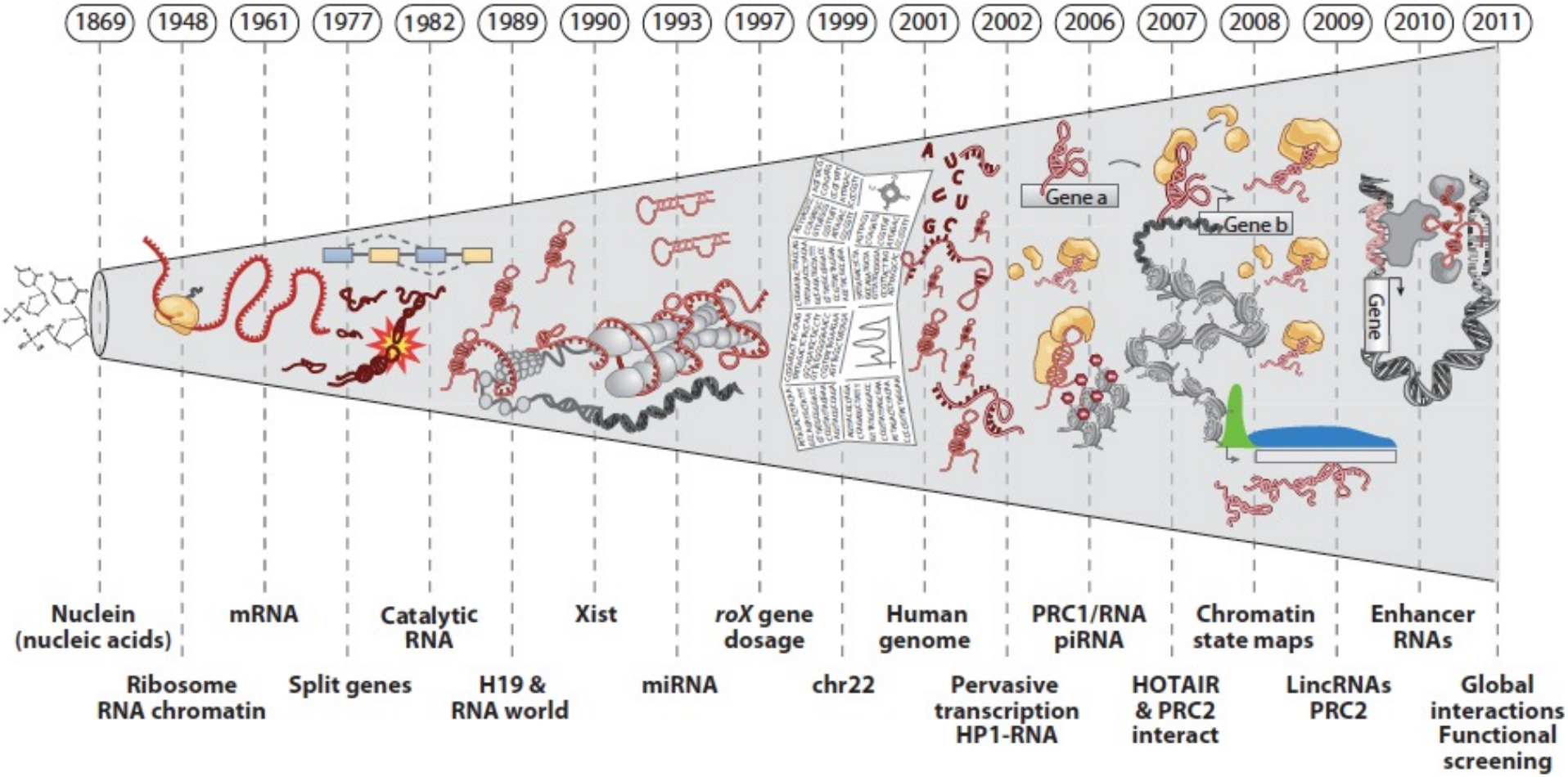
All RNAs great and small





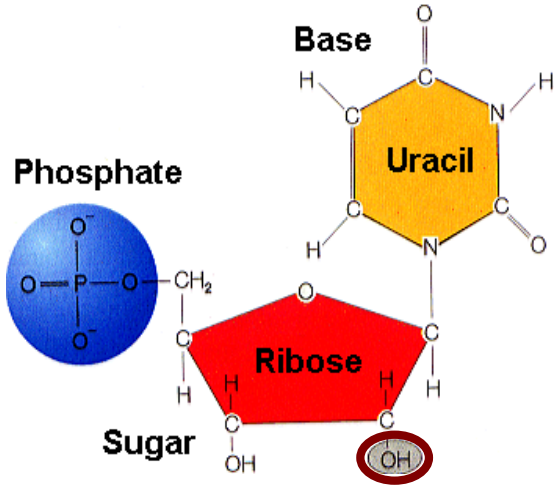
Institute of Genetics and Biotechnology
University of Warsaw

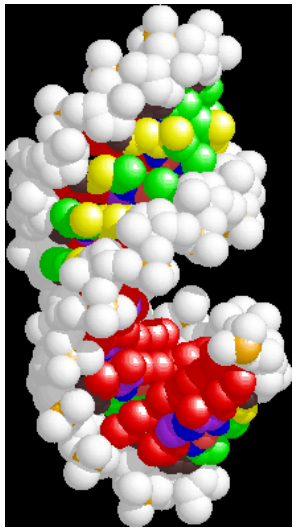
HISTORY OF RNA

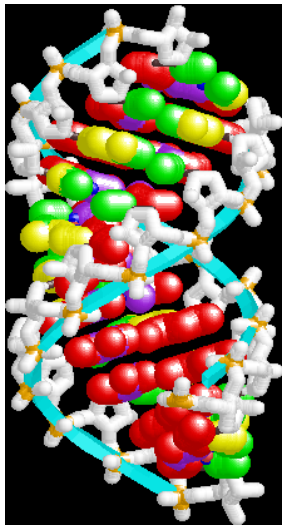


Rinn and Chang, Ann. Rev. Biochem, 2012

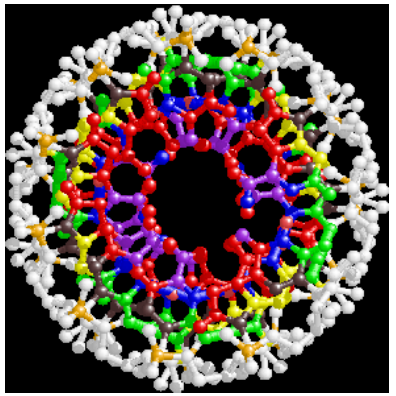
RNA – *aka* My Favorite Molecule







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)

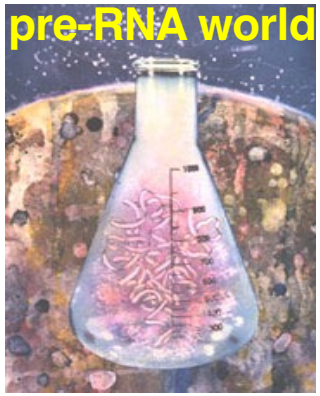
- create complex 3D structures

- specific and unspecific interactions with proteins and other RNAs

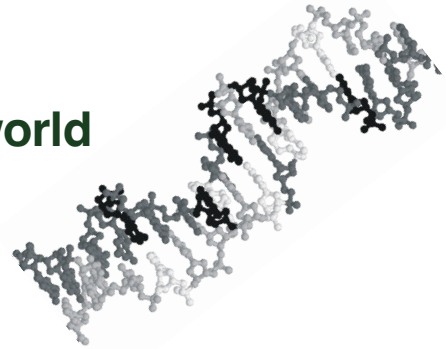
„*THE RNA WORLD*” hypothesis

RNA World proposed in the ‘60 by Carl Woese, Francis Crick and Leslie Orgel
The term used first in 1986 by Walter Gilbert, popularized by Manfred Eigen

„primordial soup”
„prebiotic soup”



RNA world

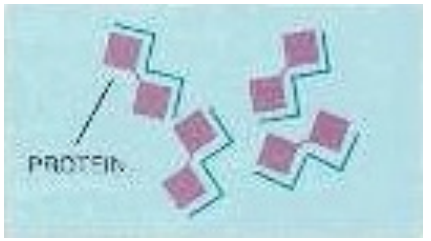


RNA+proteins

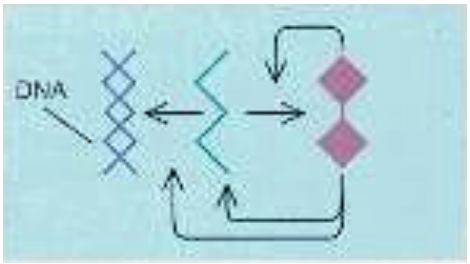
RNA+DNA+ proteins

RNA made via condensation from ribose and organic substances

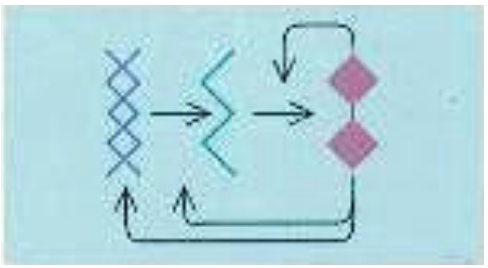
RNA evolution- molecules learns to replicate



RNA adds aminoacids, and synthesises polypeptides, proteins



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

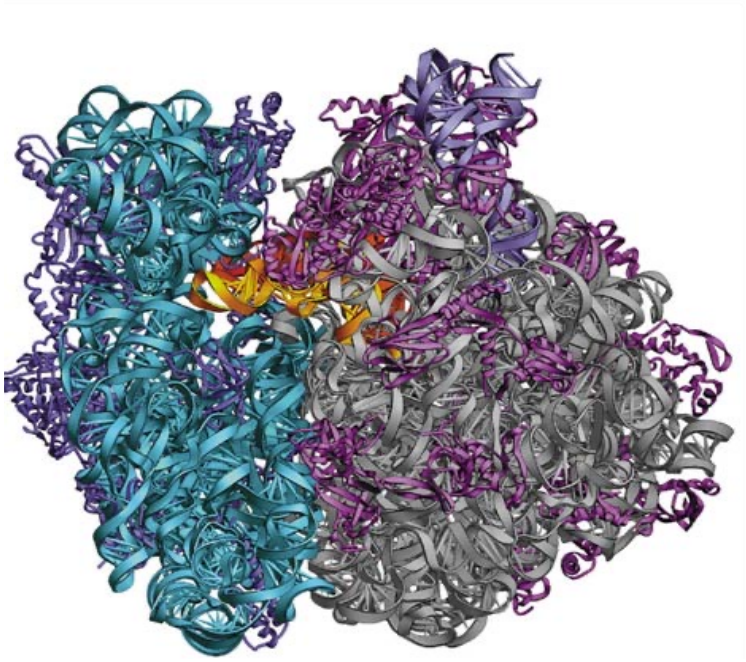


DNA and proteins take over major roles as genetic information and enzymes

MODERN RNA WORLD

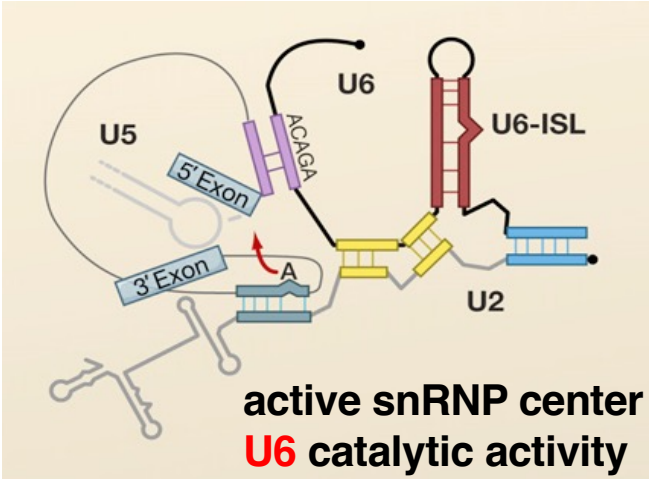
RNA vestiges- catalytic RNAs with active centres made of RNA

RIBOSOME - protein synthesis

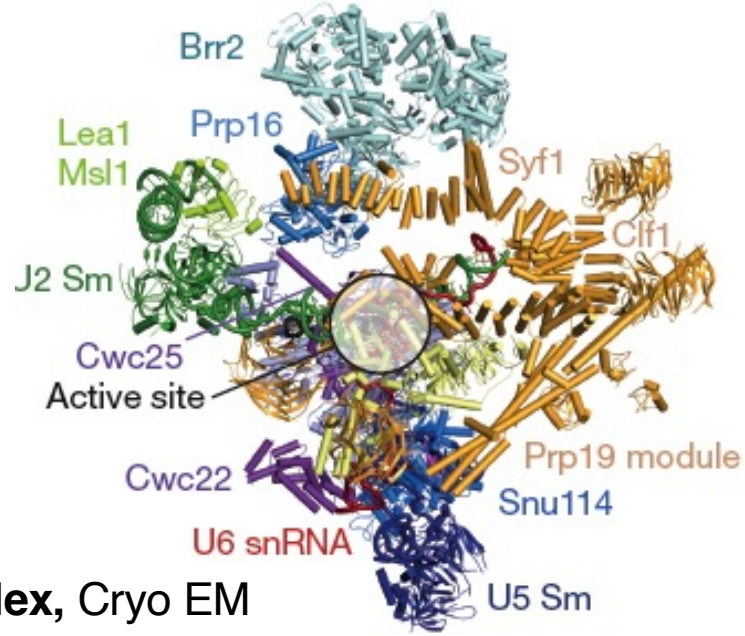


Ribosome, crystal structure
Cryo EM
Ditlev Brodersen, Venki Ramakrishnan

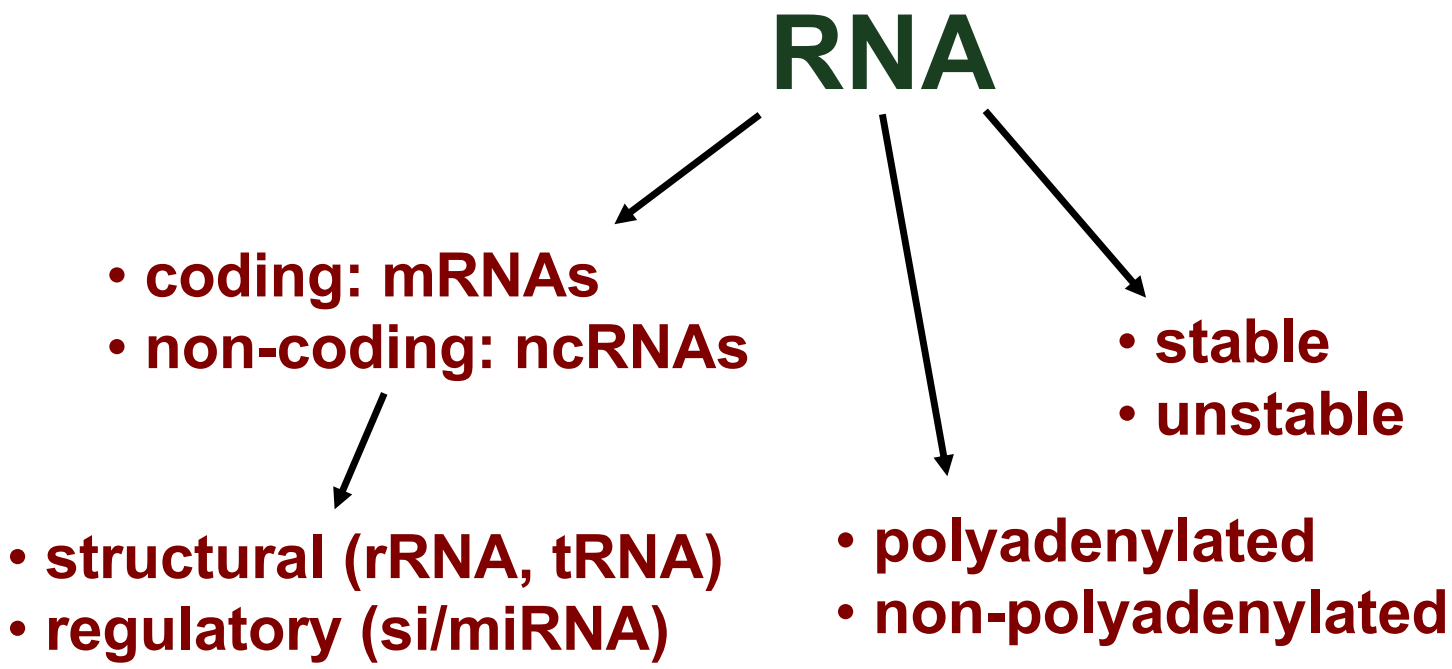
SPLICEOSOME - pre-mRNA splicing



5 snRNAs
U1, U2, U4, U5, U6



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



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

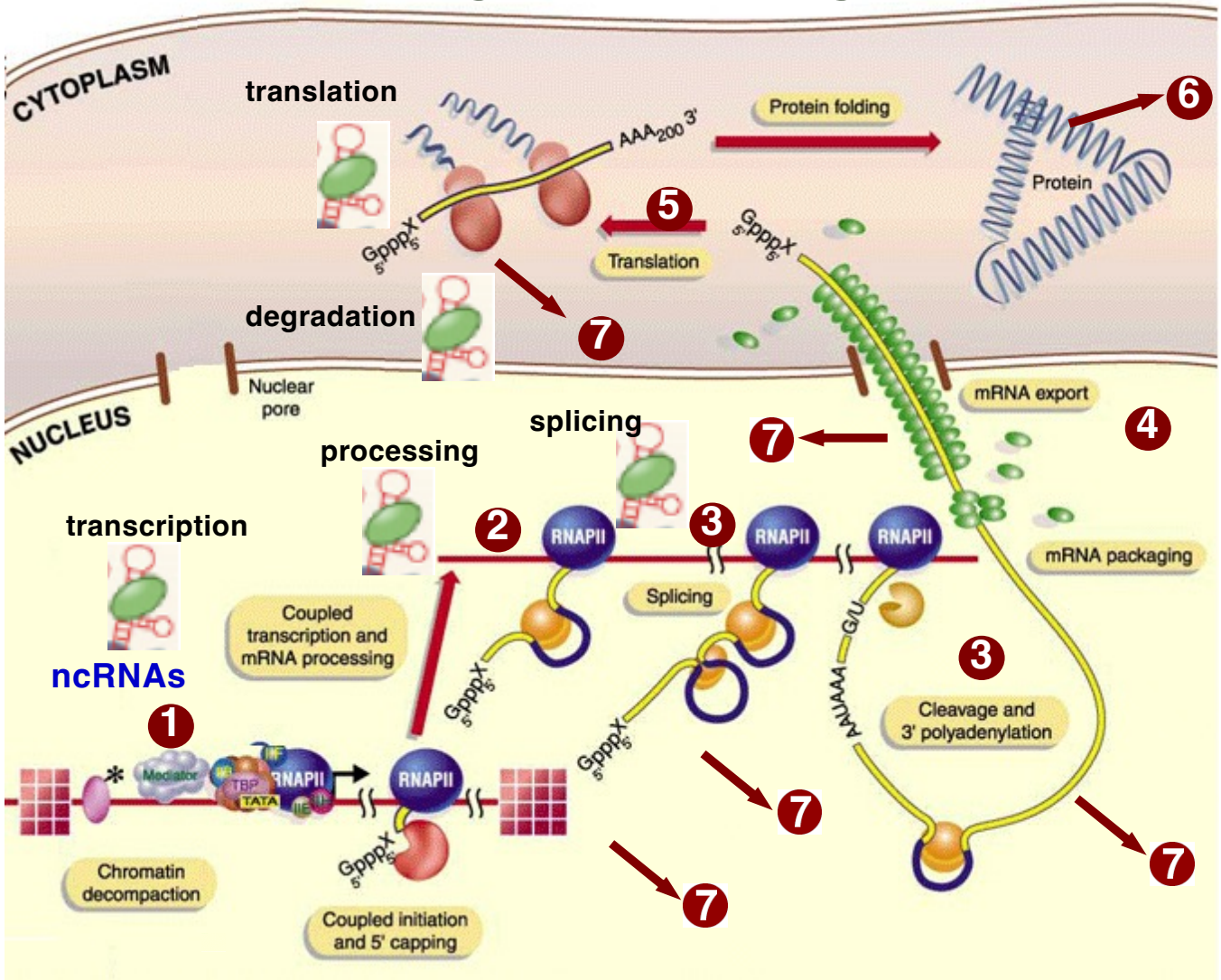
Regulation of RNA biogenesis involves alternative processes:

aTSS, aTIS, AS, APA

Lecture on ncRNAs by Monika Zakrzewska-Płaczek

RNA FLUX

Regulation of gene expression



1) chromatin

2) transcription

3) RNA processing and modification

4) RNA export

5) translation (mRNA)

6) protein stability

7) RNA degradation

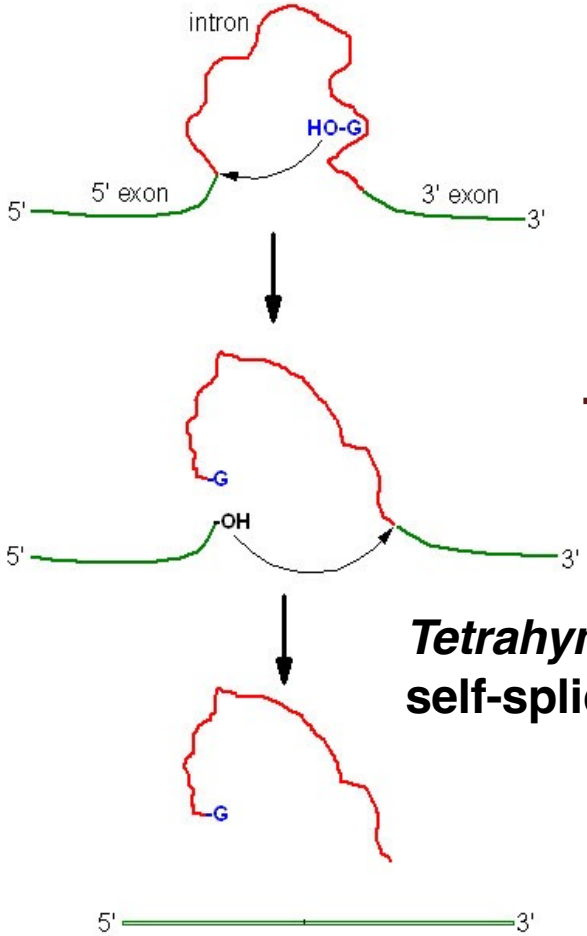
RNA capacity - CATALYTIC RNAs

Nobel 1989

RNA enzymes – **RIBOZYMES**

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

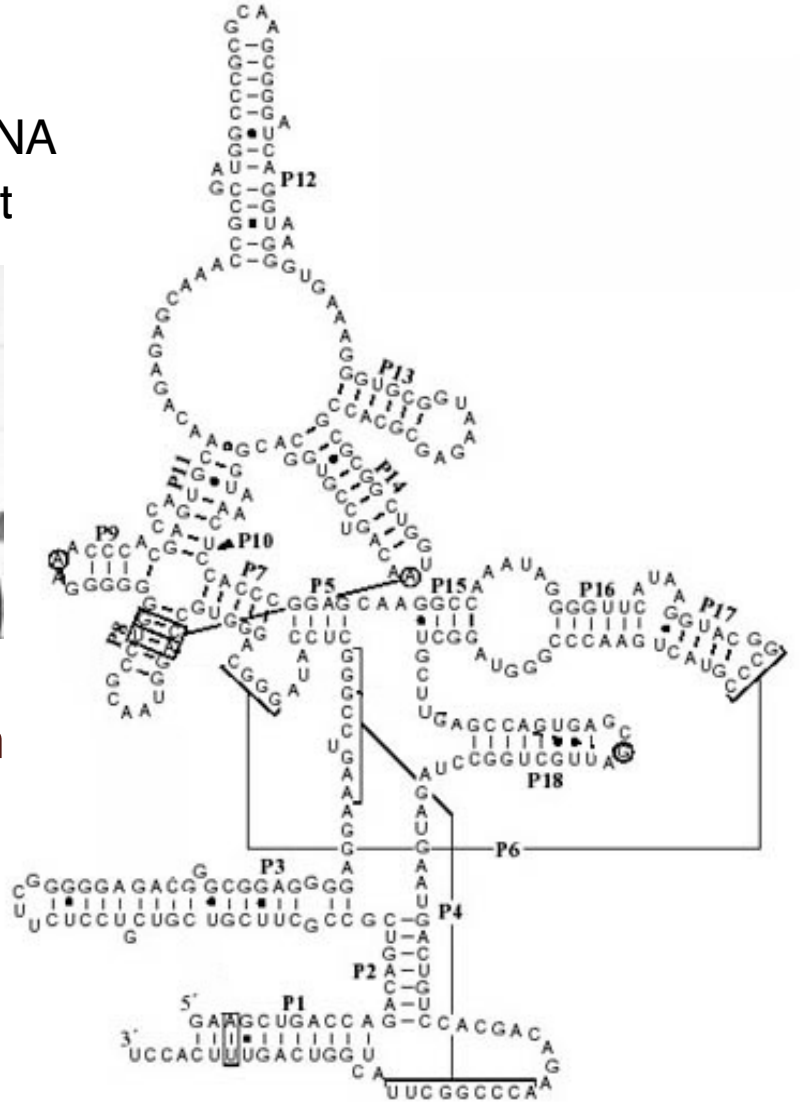
-1982 Sidney Altman - bacterial RNaseP RNA subunit



Thomas Cech

Sidney Altman

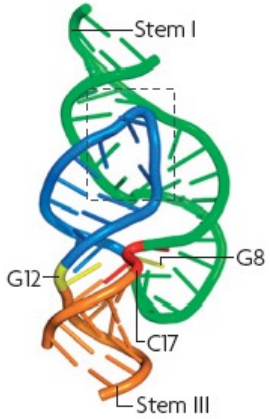
***Tetrahymena* group I self-splicing intron**

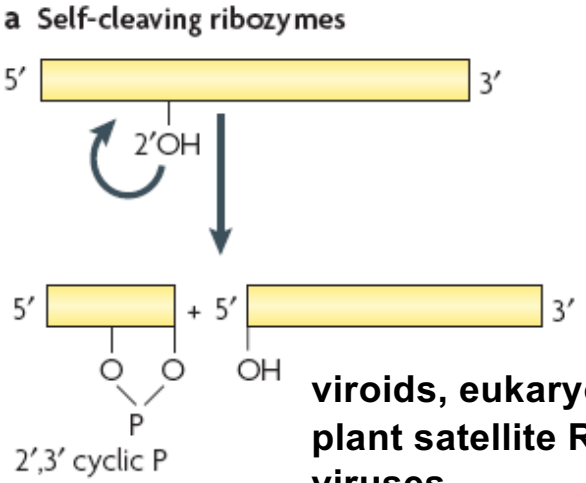


***Escherichia coli* RNaseP RNA**

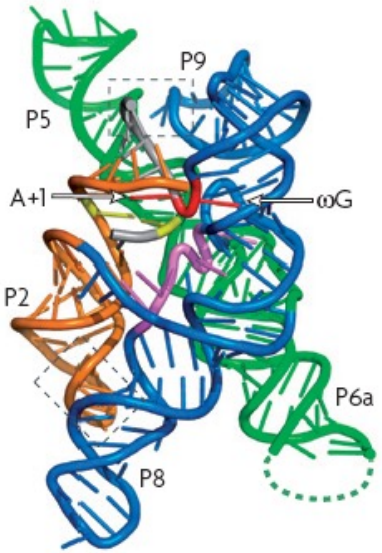
RIBOZYMES

Hammerhead, Hairpin, HDV

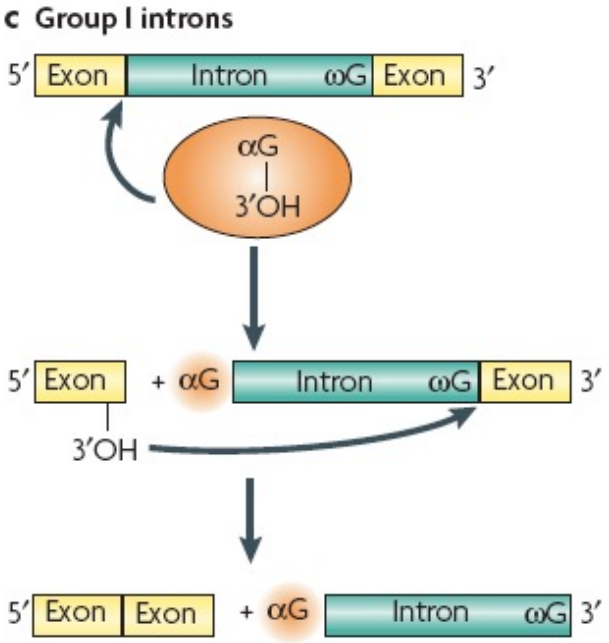


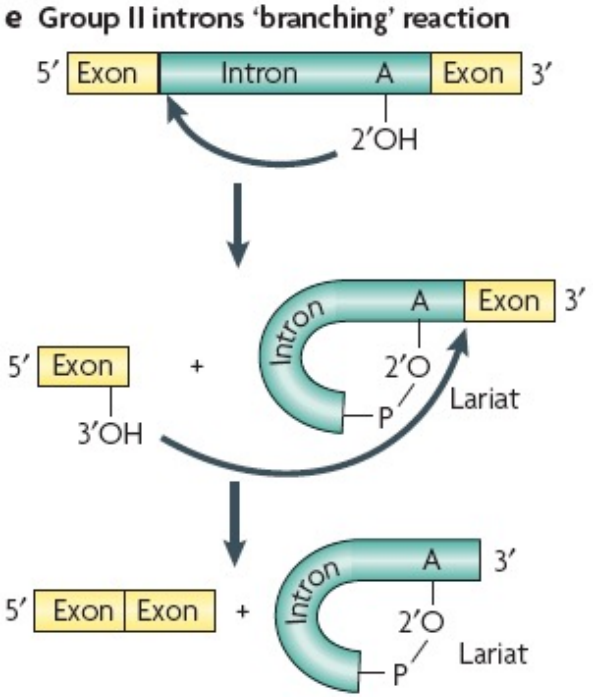


viroids, eukaryotes plant satellite RNA, viruses

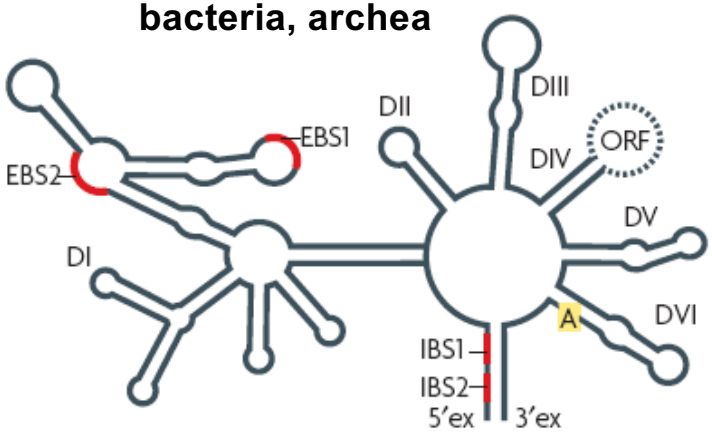


organelles (fungi, plants), bacteria, mitochondria (animals)





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



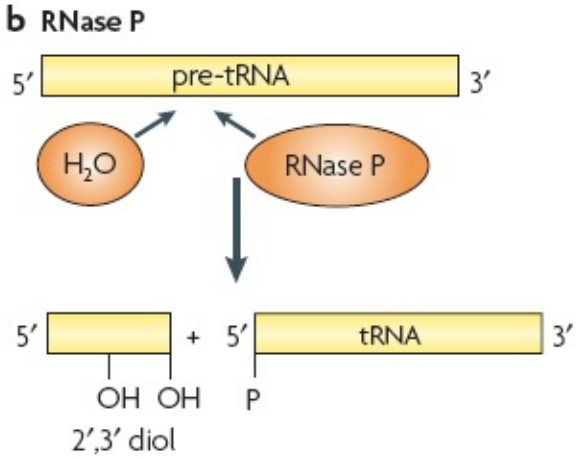
Serganov and Patel, *Nat. Rev. Genet.*, 2007

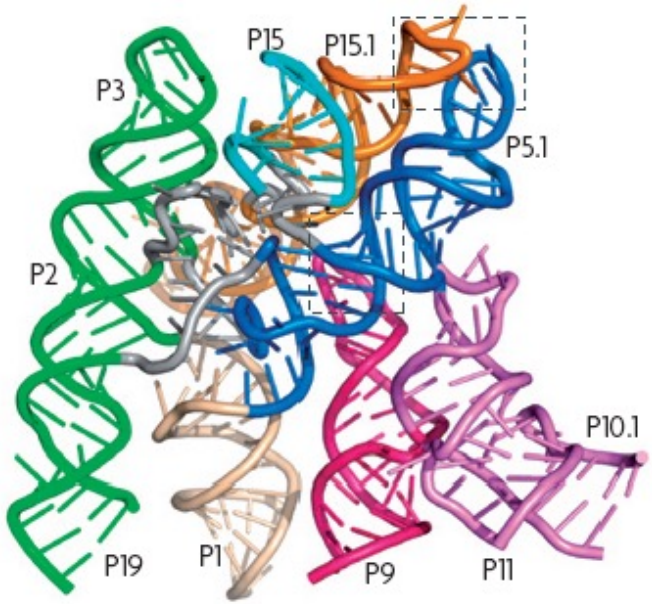
Mechanism: nucleophilic attack of the ribose -OH group (H_2O, Me^{2+}) on the phosphate

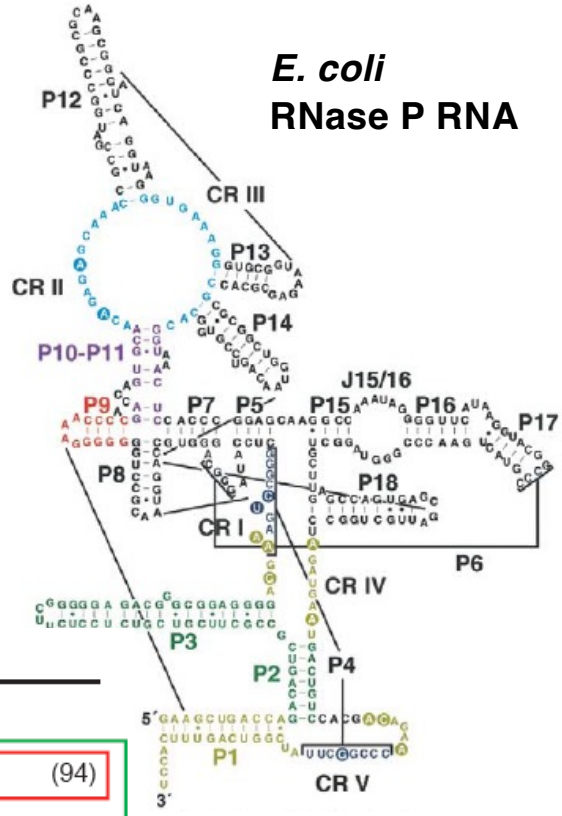
RNase P RNA – a true enzyme

tRNA processing, multiple turnover

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

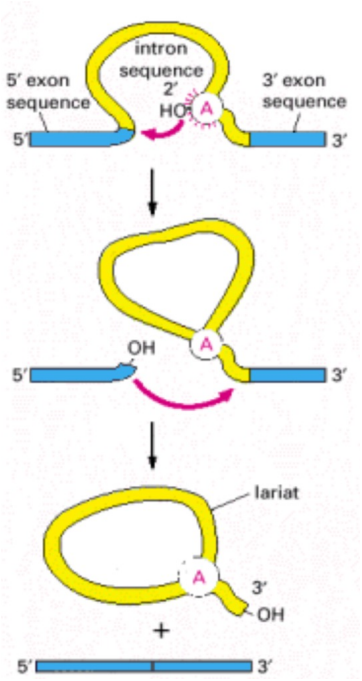






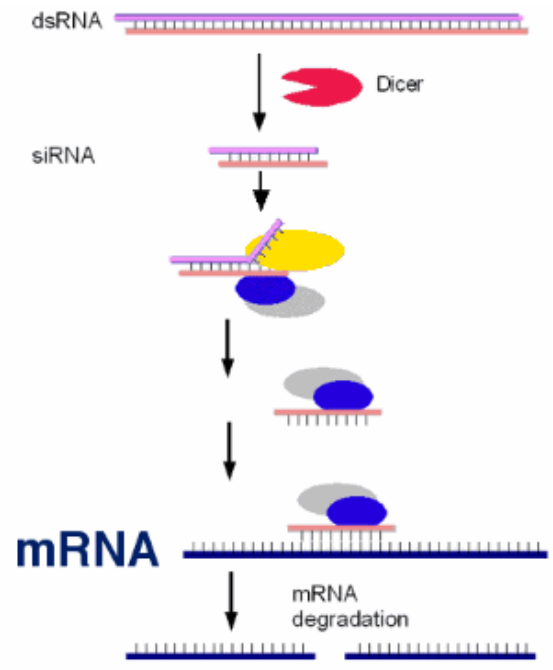
E. coli
RNase P RNA

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

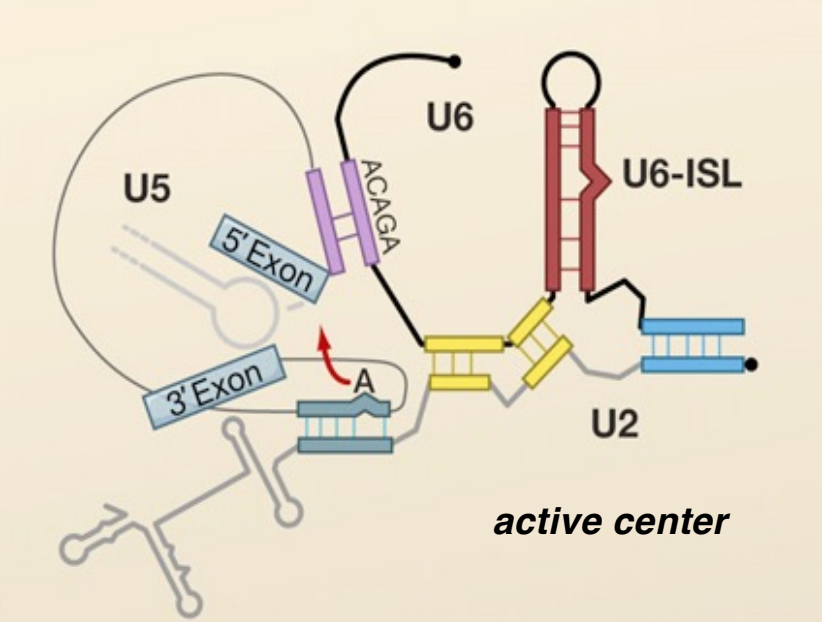
Phil Sharp
Richard Roberts



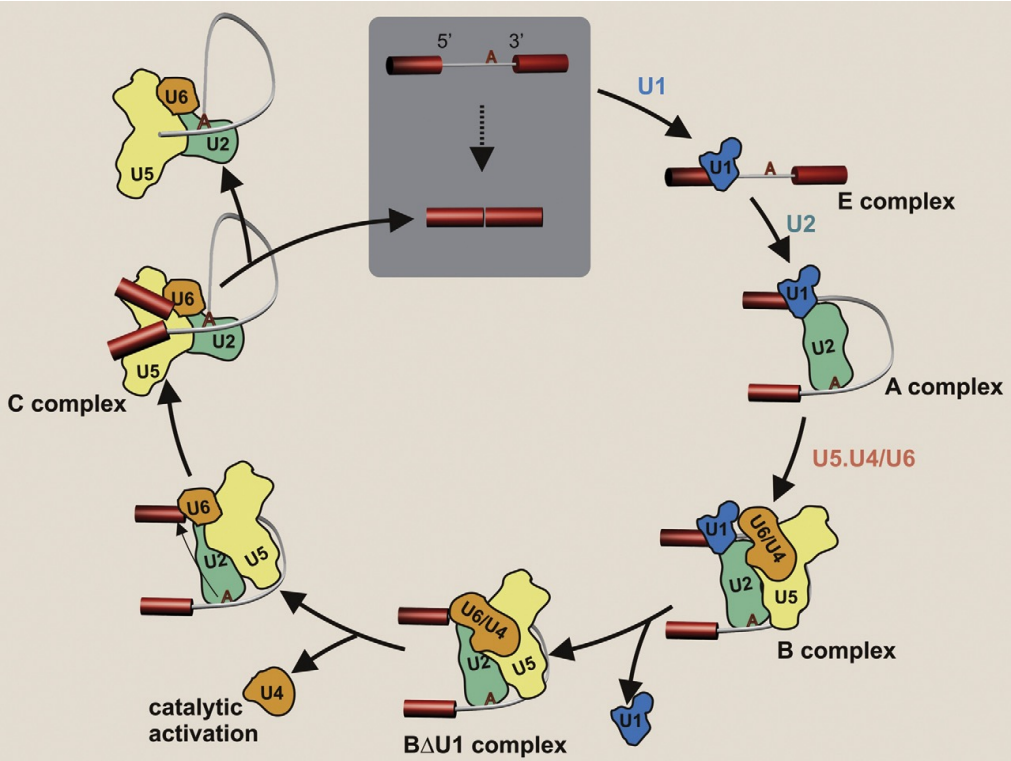
RNAi Nobel 2006

Andrew Fire
Craig Mello

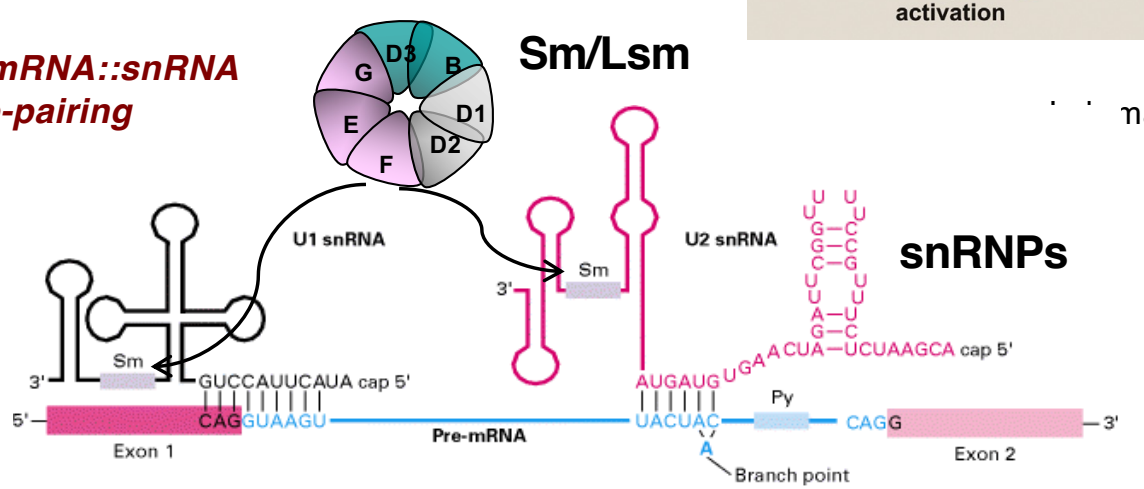
SPLICEOSOME: pre-mRNA SPLICING



U6 snRNA is a catalytic molecule



pre-mRNA::snRNA base-pairing



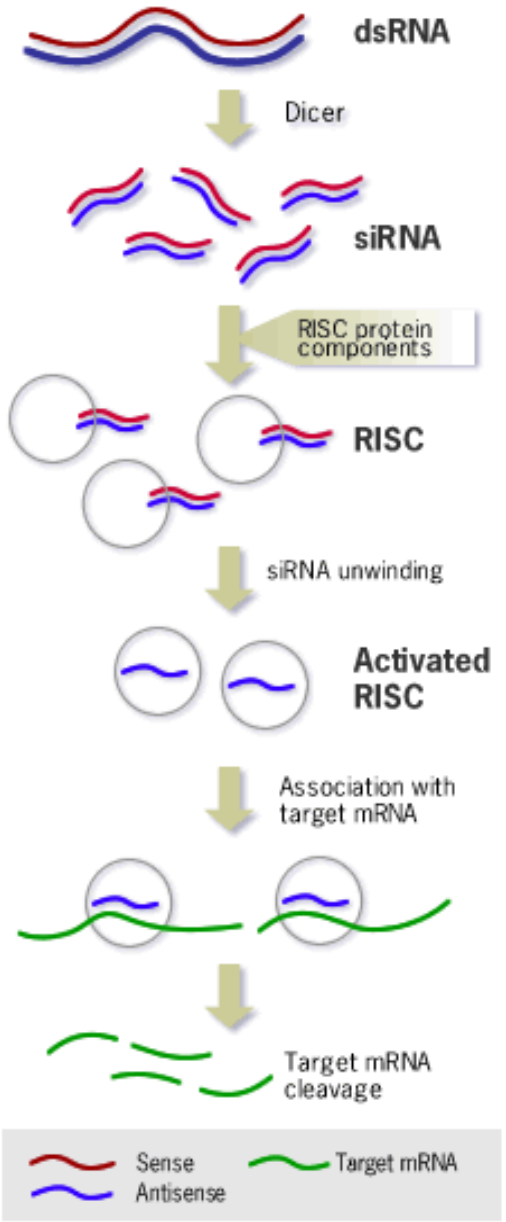
. . nann and Stark, *Curr. Op. Str. Biol.*, 2009

SPLICEOSOME – a ribozyme

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)

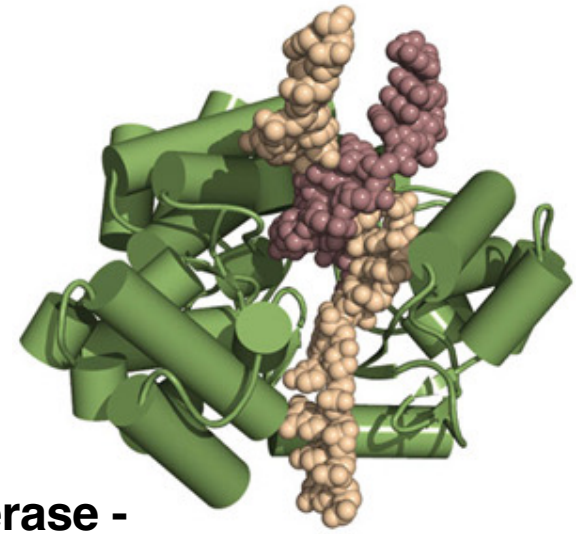
- **mRNA cleavage** *or*
- **translation inhibition** *or*
- **translation activation**

RNAs – STRUCTURE AND FUNCTION

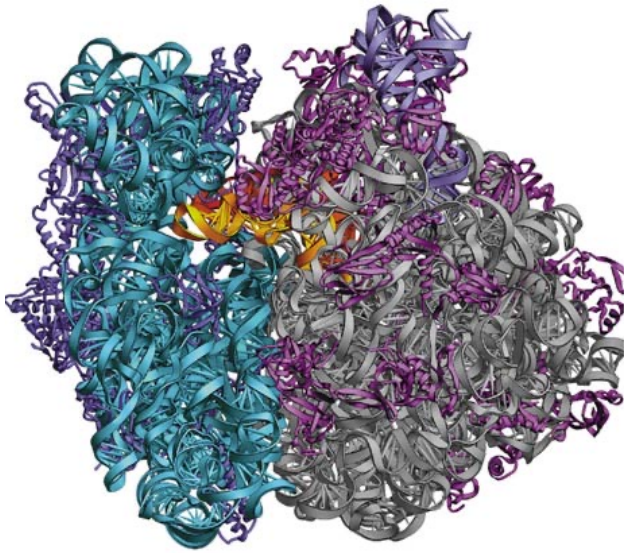
Nobel 2009

Elizabeth Blackburn
Jack Szostak
Carol Greider

Venkatraman Ramakrishnan
Ada Yonath
Thomas Steitz

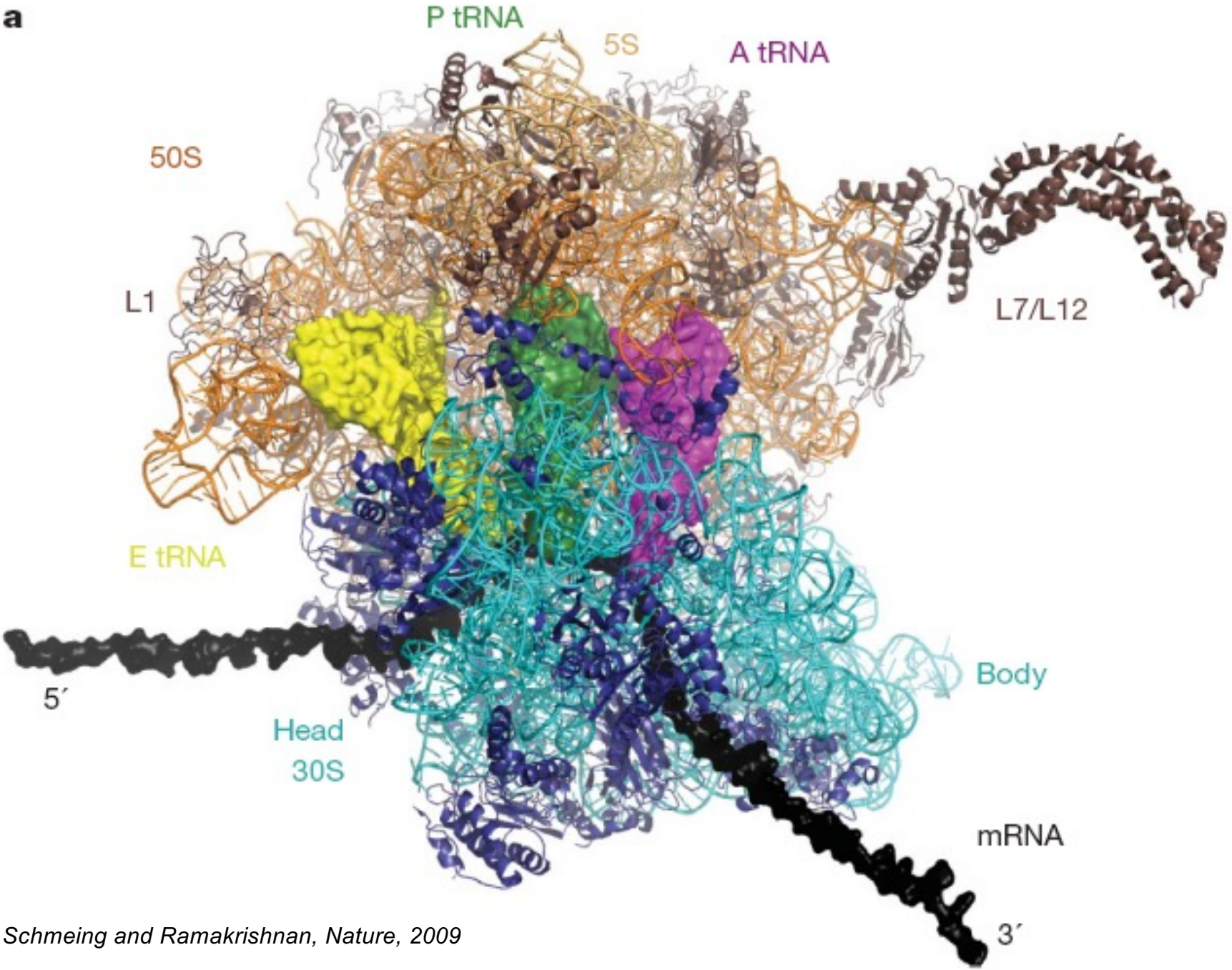


Telomerase -
maintaing chromosome ends



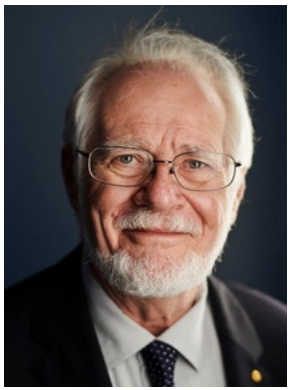
Crystal structure of the ribosome

THE RIBOSOME



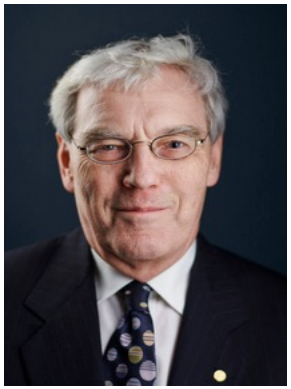
Schmeing and Ramakrishnan, Nature, 2009

RNPs - STRUCTURE/METHODOLOGY



Jacques Dubochet

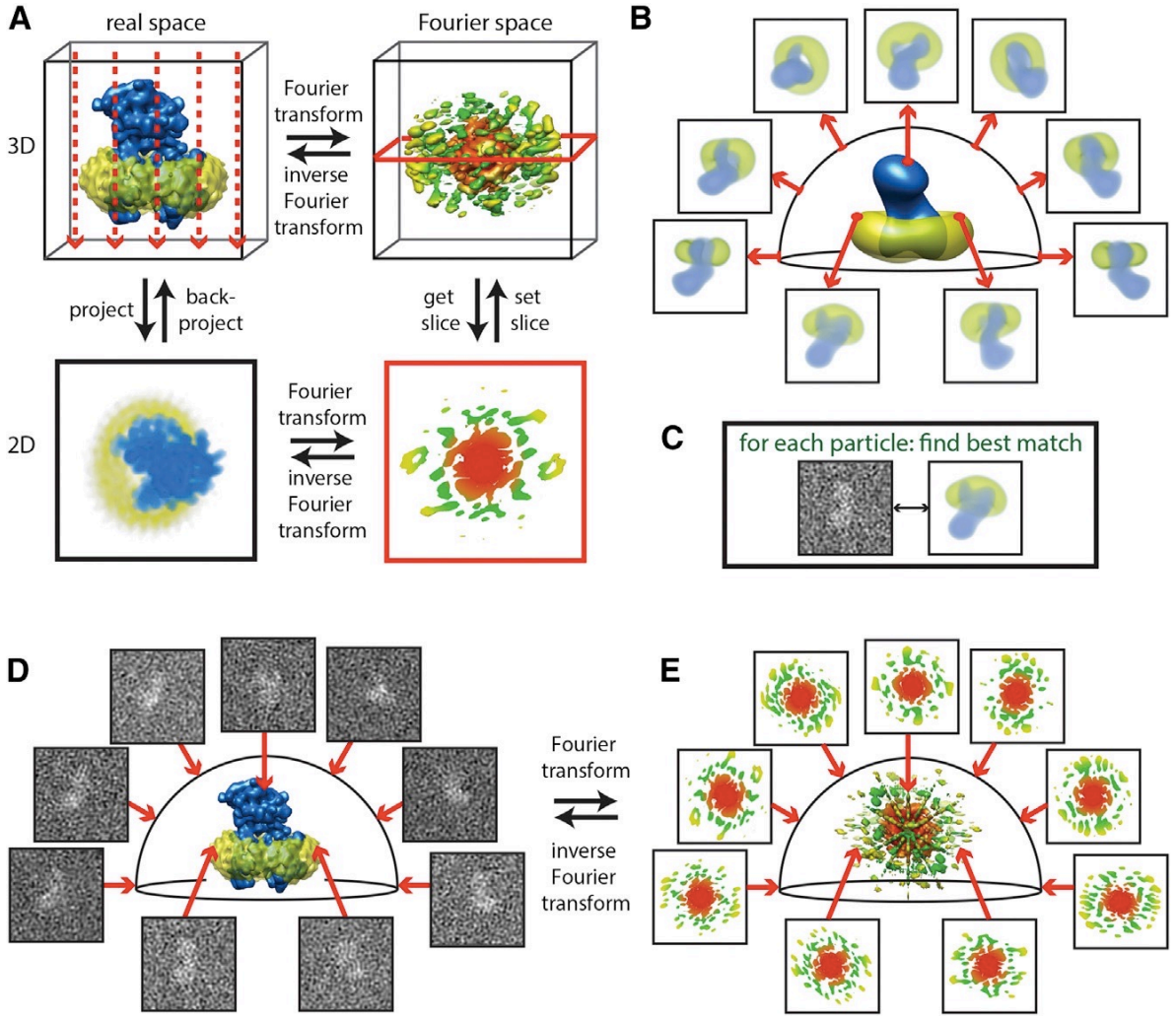
Joachim Frank



Richard Henderson

Nobel 2017

CRYO-EM



Nogales and Scheres, Mol Cell 2015

[Lecture on crystallography and CryoEM by Marcin Nowotny]

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

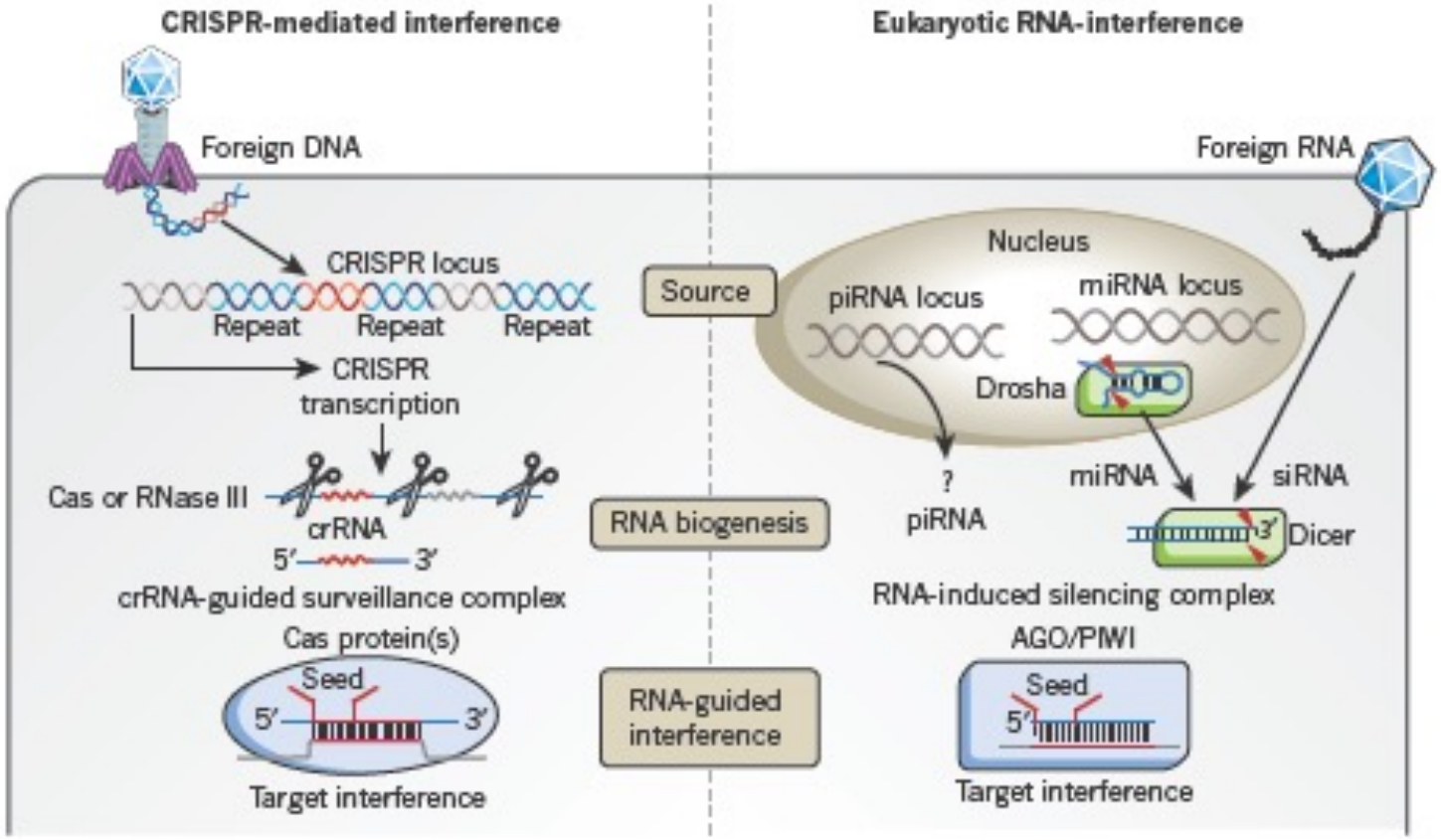
Elitza Deltcheva[1,2], Krzysztof Chylinski[1,2]*, Cynthia M. Sharma[3]*, Karine Gonzales[2], Yanjie Chao[3,4], Zaid A. Pirzada[2], Maria R. Eckert[2], Jörg Vogel[3,4] & Emmanuelle Charpentier[1,2]

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

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

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

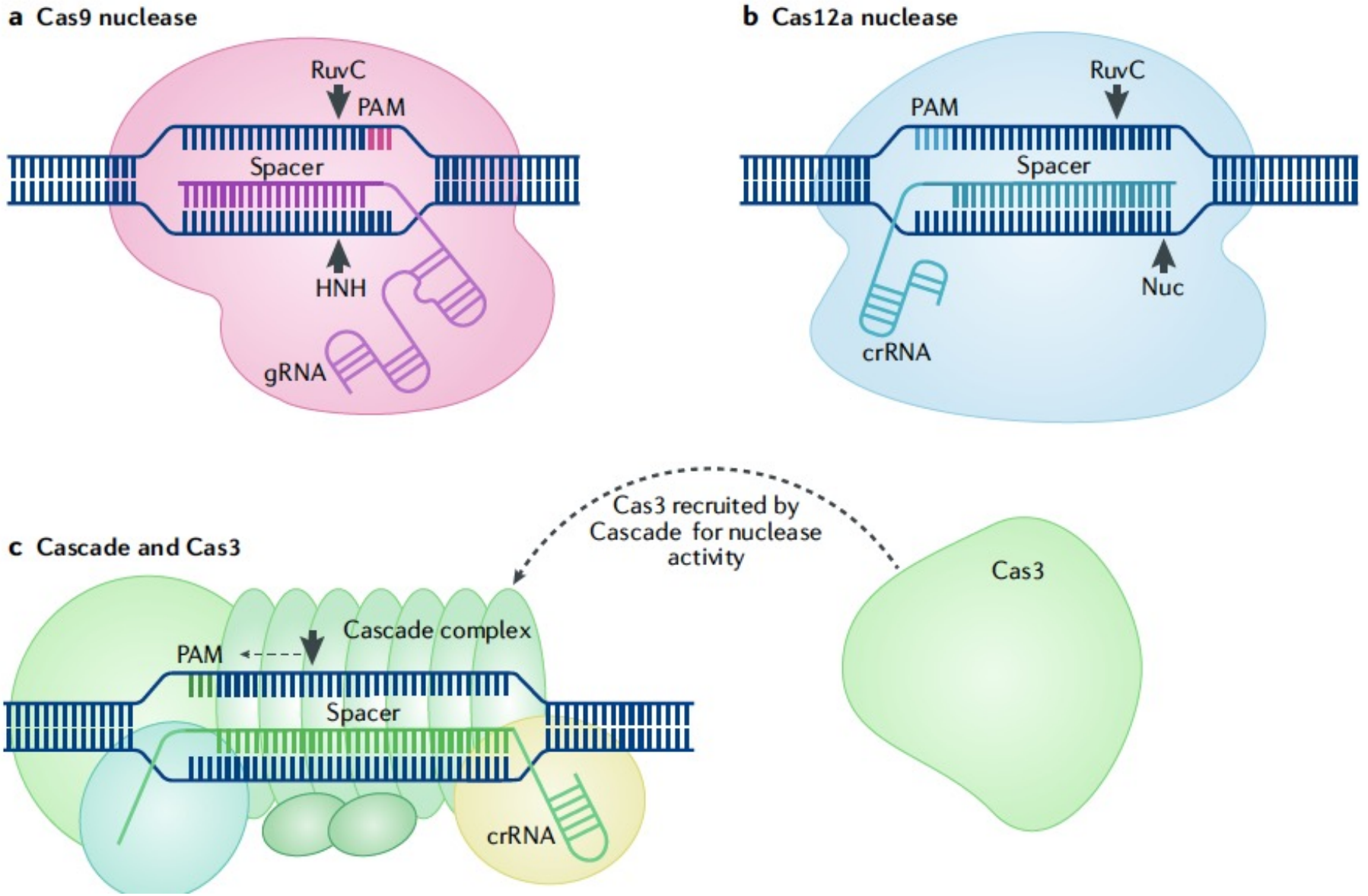
CRISPR Clustered Regularly Interspaced Short Palindromic Repeat
Cas- CRISPR associated



- **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

Main CRISPR/Cas gene editing tools



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

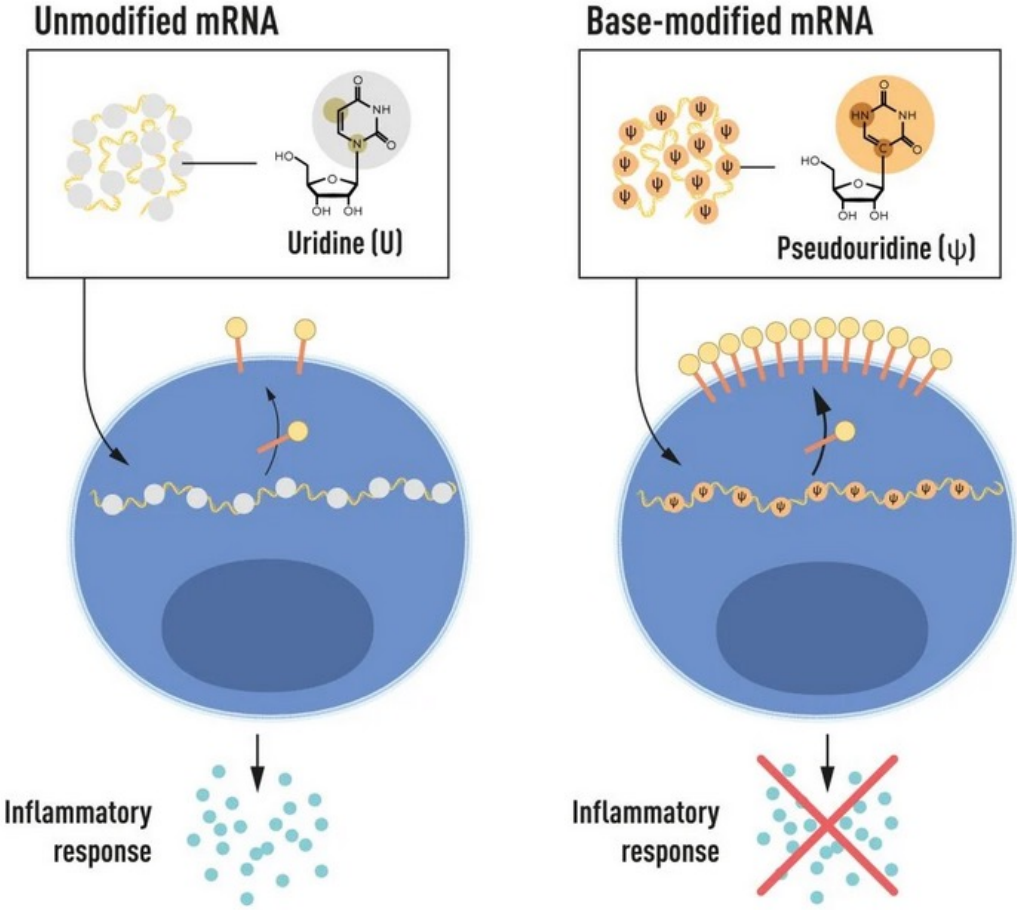
https://www.youtube.com/watch?v=k99bMtg4zRk&fbclid=IwAR2HcVxLX2v80gQlJQWoOOS6FkWX--XeIyYhksegRMuotAVOHySouTcGTIY

mRNA vaccine

Nobel 2023

Katalin Karikó Drew Weissman

”for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19”



https://www.nobelprize.org/prizes/medicine/2023/press-release/

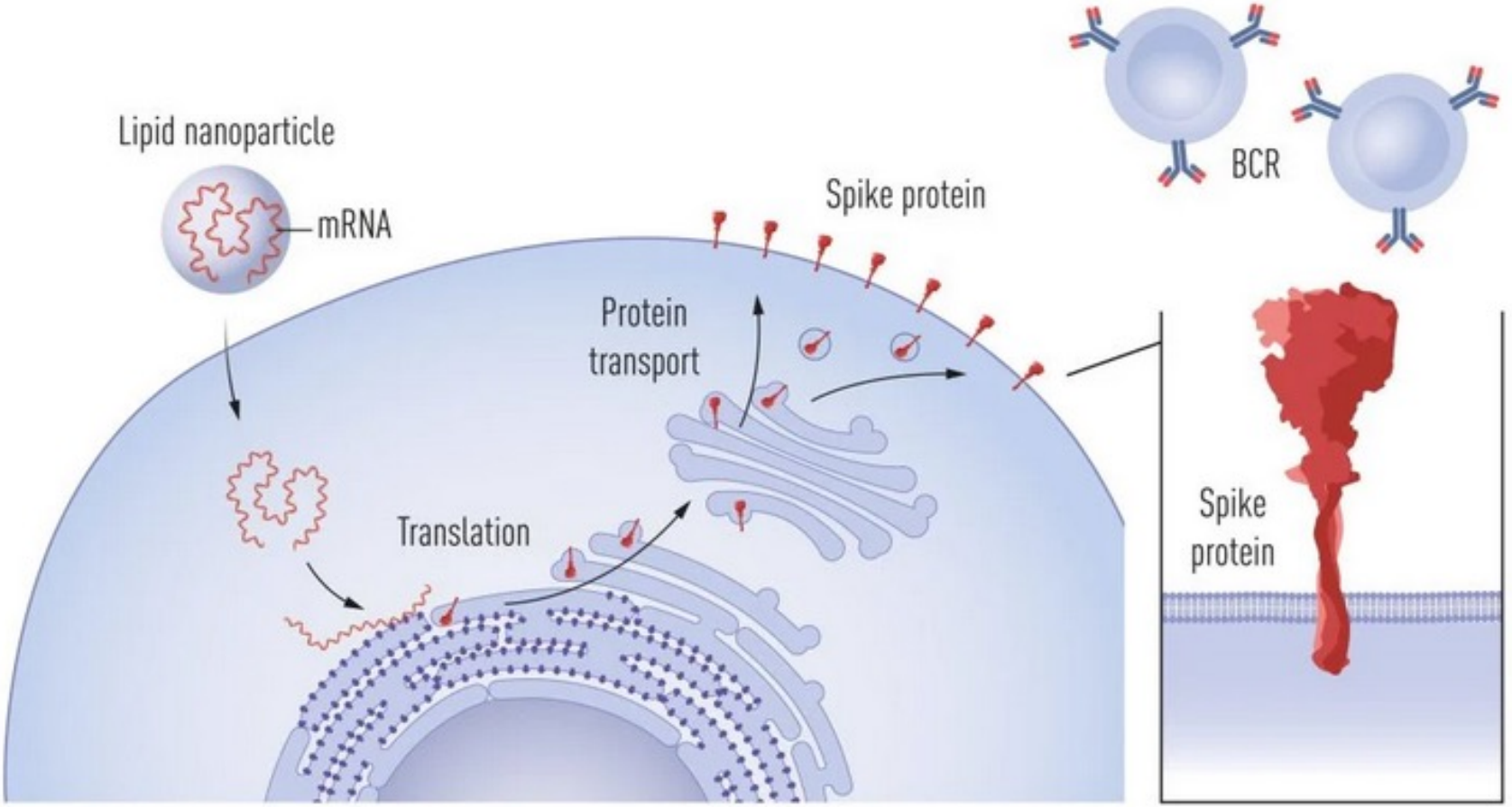
Incorporation of modified bases, N1-methylpseudo-uridine (m1ψ) alone or in combination with m^5C, evades undesired immune activation by *in vitro* transcribed mRNA

m1ψ-containing mRNA is more efficiently translated, resulting in higher protein production, when delivered into cells and into mice

dsRNA contaminations can be removed through HPLC purification

Karikó K, Buckstein M, Ni H, Weissman D. 2005 *Immunity*

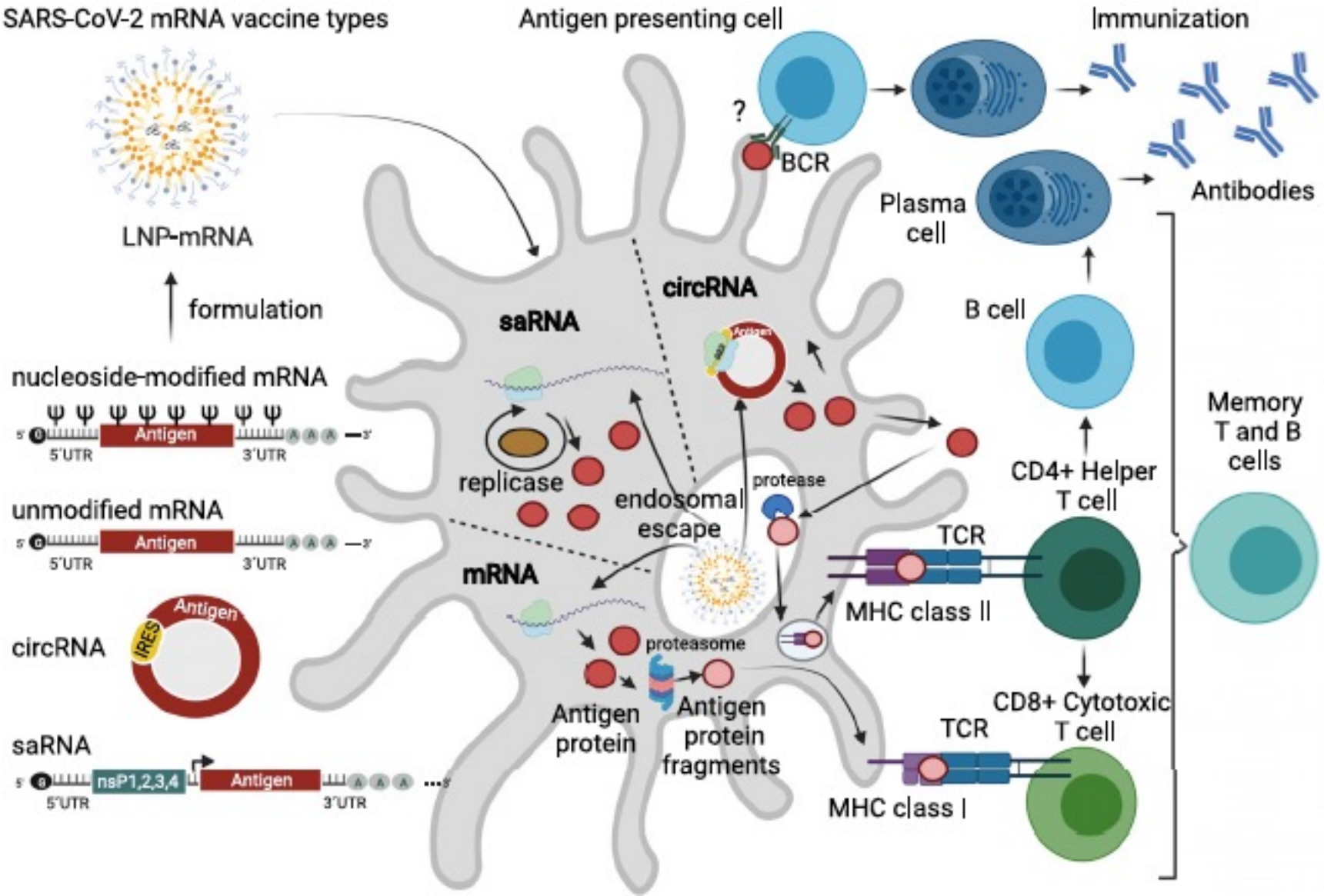
mRNA vaccine



Spike production following mRNA vaccination and recognition of spike by B cells.
Following uptake of mRNA into cells, facilitated by lipid nanoparticles, the mRNA acts as a template for spike protein production. Spike is then transiently expressed on the cell surface, where it is recognized by B cells via their B cell receptors (BCRs), stimulating the secretion of spike-specific antibodies.

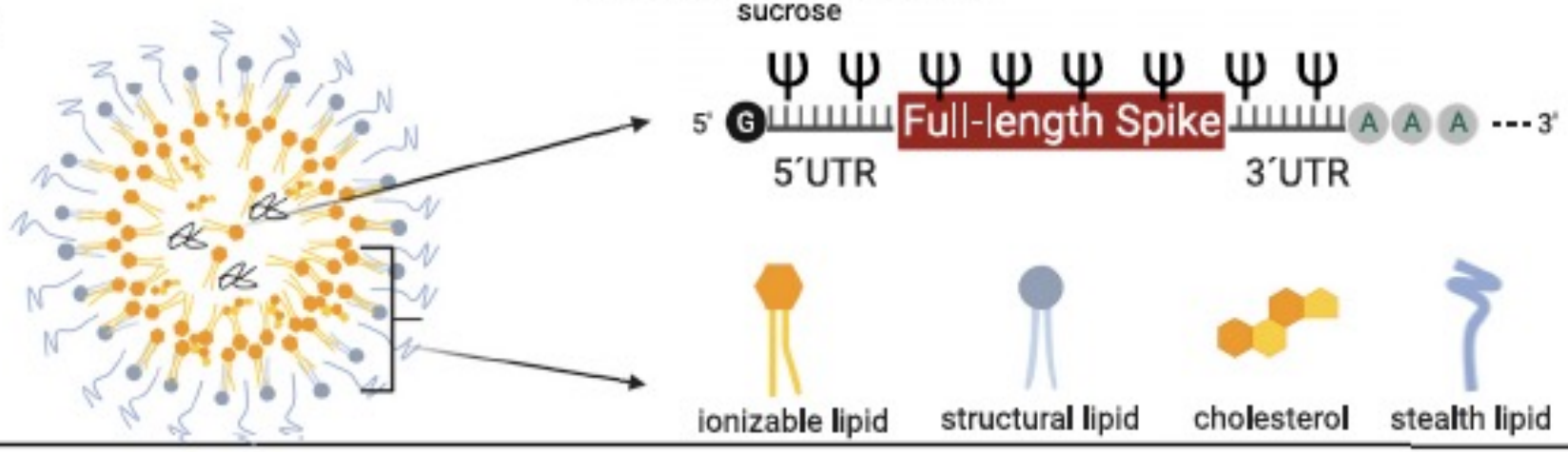
https://www.nobelprize.org/prizes/medicine/2023/press-release/

mRNA vaccine

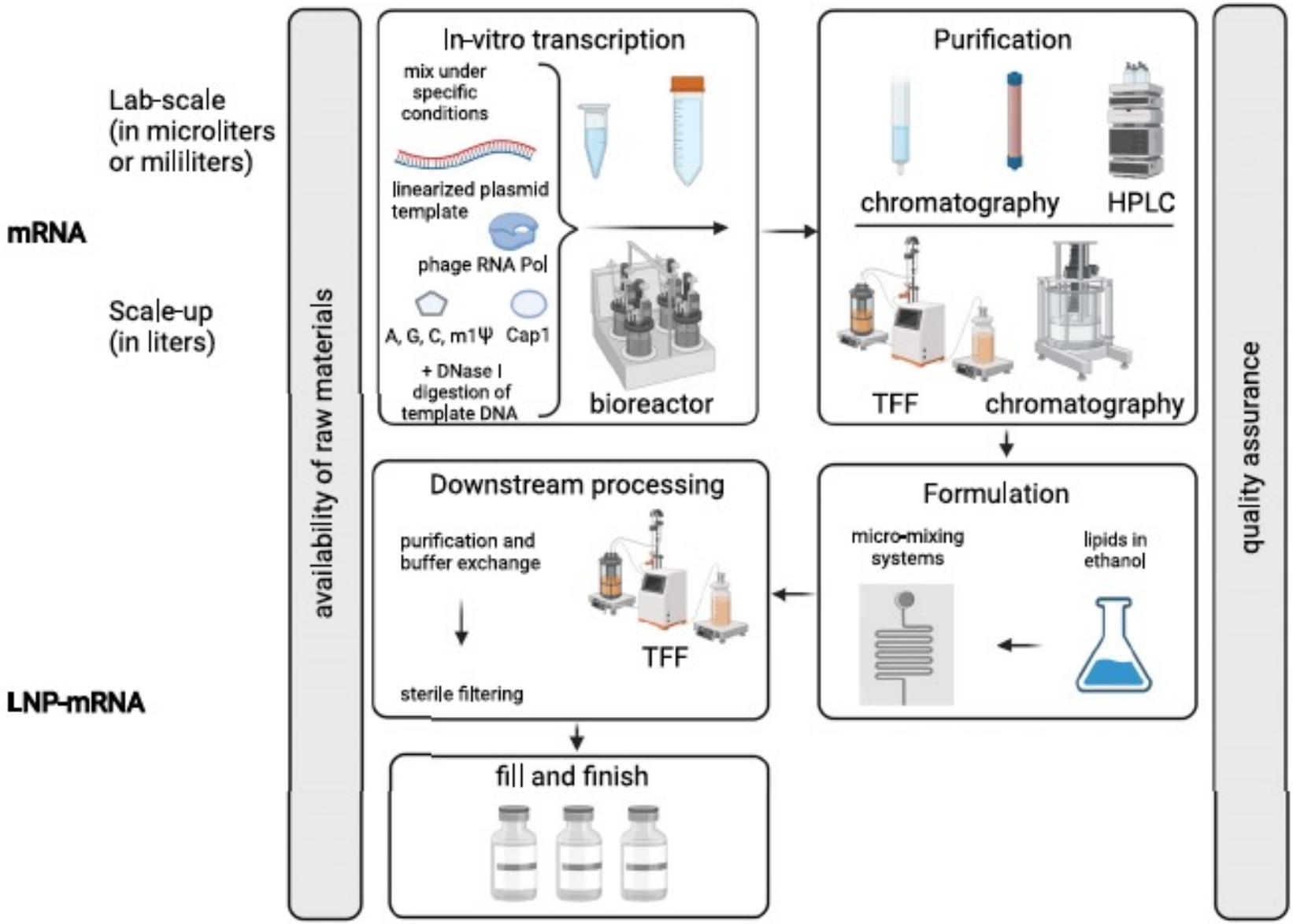


mRNA vaccine

	BioNTech / Pfizer (BNT162b2)	Moderna (mRNA-1273)
Common features		
mRNA	full-length Spike with K986P and V987P substitutions 1-methylpseudouridine (m1Ψ) replacement of 100% U sequence: codon optimized, GC-rich	
LNP other	helper lipids: DSPC, cholesterol sucrose	
Unique features		
mRNA	- co-transcriptional-capping with Cap1 analogue ($m2^{7,3'-O}$)Gppp($m^{2'-O}$)ApG) - 5´UTR: human α-globin RNA with optimized Kozak sequence: GCCACCATG - 3´UTR: AES and mtRNR1 3'-UTR motifs - stop codon: two stop codons - polyA: A30LA70 (linker [L]: GCAUAUGACU)	- enzymatical capping to obtain natural mammalian Cap 1 structure - 5´UTR: not disclosed, with GC-rich tract CCCCGGCGCC - 3´UTR: human β-globin gene - stop codon: three stop codons - polyA: not disclosed
LNP	- ionizable lipid: ALC-0315 - stealth lipid: ALC-0159 - % Molar ratio of Ion: Chol: Struc: PEG= 46.3:42.7:9.4:1.6	- ionizable lipid: SM-102 - stealth lipid: PEG2000-DMG - % Molar ratio of Ion: Chol: Struc: PEG= 50:38.5:10:1.5
other	- PBS buffer, salts: potassium chloride, monobasic potassium phosphate, sodium chloride, basic sodium phosphate dihydrate	- Tris buffer, salts: thromethamine, thomethamine hydrocholoride, acetic acid, sodium acetate



mRNA vaccine

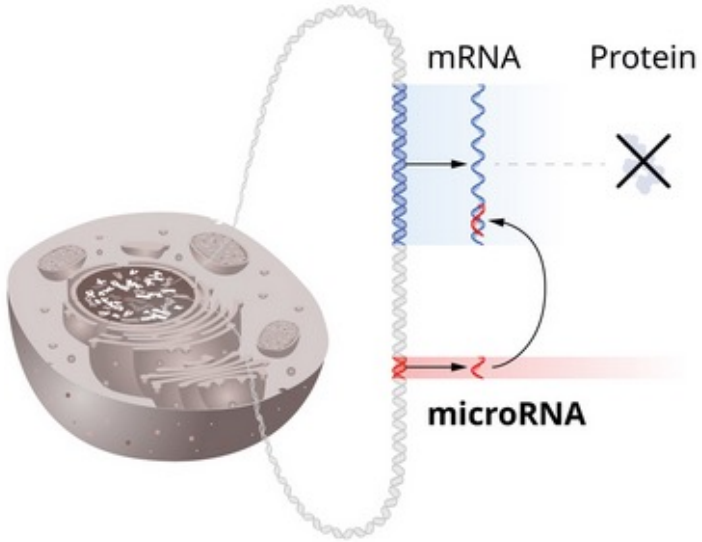


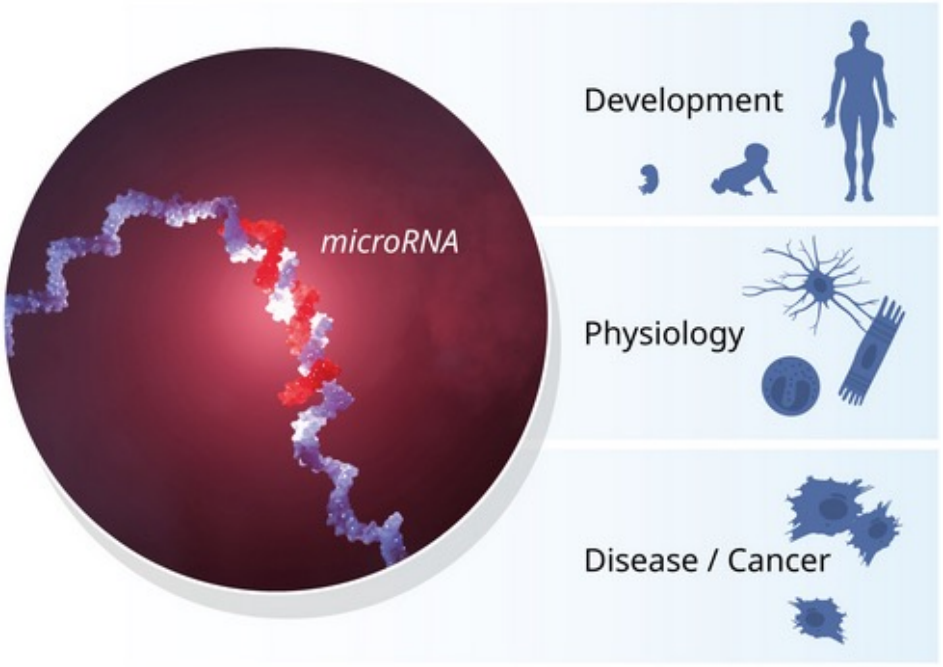
microRNA

Nobel 2024

Victor Ambros **Gary Ruvkun**

"for the discovery of microRNA and its role in post-transcriptional gene regulation"





Next lecture

RNA mechineries
Nascent transcripts
Co-transcriptional and post-transcriptional processess
Gene loops and Rloops
Splicing
3’ end formation
Translation cycle
RNA enzymes and complexes