

University of Warsaw



Rinn and Chang, Ann. Rev. Biochem, 2012

RNA – aka My Favorite Molecule





- 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



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*

5 snRNAs U6-ISL U5 U1, U2, U4, U5, **U6** U2 active snRNP center **U6** catalytic activity Brr2 Prp1 Lea1 Ms J2 Sm Cwc25 Active site Prp19 module Cwc22 Snu114 U6 snRN J5 Sm C complex, Cryo EM Galej et al, Nature, 2016

SPLICEOSOME - pre-mRNA splicing



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





Mechanism: nucleophilic attack of the ribose -OH group (H₂O, Me²⁺) on the phosphate

RNase P RNA – a true enzyme

tRNA processing, multiple turnover



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



mRNA SPLICING Nobel 1993



Phil Sharp Richard Roberts



RNAi Nobel 2006



Andrew Fire Craig Mello

SPLICEOSOME: pre-mRNA SPLICING



SPLICEOSOME – a ribozyme

ribonucleoprotein complex (RNP) organised around snRNAs

GENE SILENCING – RNAi



siRNAs/miRNAs:

- double stranded small noncoding RNAs
- complementary to mRNA targets
- participate in gene silencing
- mediate:
- TRANSCRIPTIONAL GENE SILENCING (TGS)
- transcription inhibition

<u>POST-TRANSCRIPTIONAL</u> <u>GENE SILENCING</u> (PTGS)

- mRNA cleavage or
- translation inhibition or
- translation activation

RNAs – STRUCTURE AND FUNCTION Nobel 2009



Elizabeth Blackburn Jack Szostak Carol Greider



Telomerase – **W** maintaing chromosome ends



Venkatraman Ramakrishnan Ada Yonath Thomas Steitz



Crystal structure of the ribosome



RNPs - STRUCTURE/METHODOLOGY



Jacques Dubochet



Joachim Frank



Nobel 2017

CRYO-EM



C for each particle: find best match
E for each particle: find best match

Nogales and Scheres, Mol Cell 2015

Richard Henderson 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. Sharma³*, Karine Gonzales², Yanjie Chao^{3,4}, Zaid A. Pirzada², Maria R. Eckert², 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 Charpentier⁴‡

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

Main CRISPR/Cas gene editing tools



mRNA vaccine



mRNA vaccine



mRNA vaccine



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