BI 8 LECTURE 18

OTHER RNAS AS REGULATORS AND RNAS AS ENZYMES

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3 March 2016

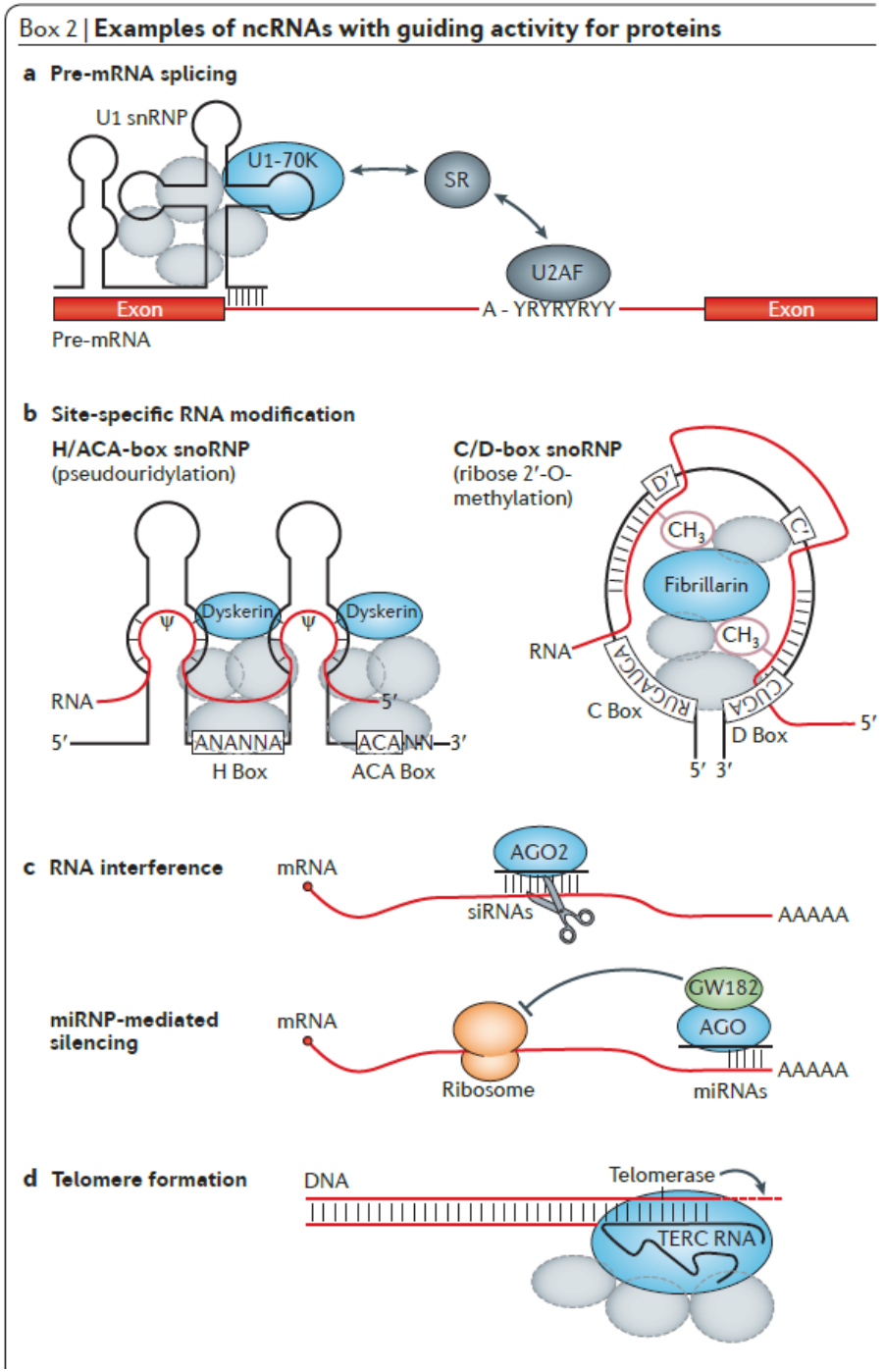
Read: Alberts et al.: Chapter 6, pp. 317-324, 346-347, 362-366

RNA is more than a protein coding molecule

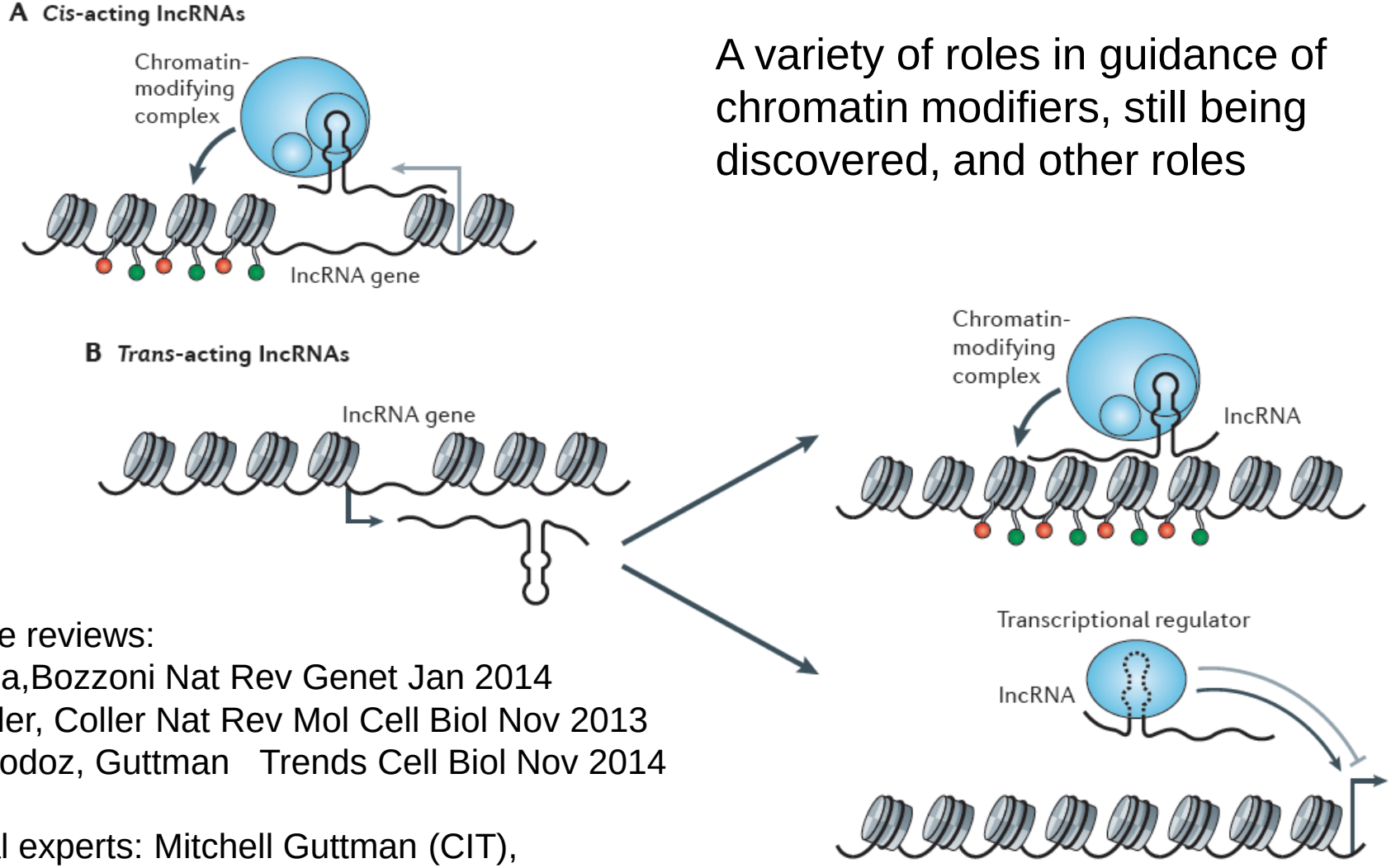
- RNA as regulator of other mRNAs' translation and lifespans (miRNA and artificial versions)
- RNA as a local orientation device to recruit chromatin modifier function to specific sites (supplementing action of transcription factors)
- RNA as an enzyme

Multiple regulatory modes exist for RNA molecules

Guidance for protein-based enzymes: using RNA complementarity for target specificity



Long noncoding RNAs: from the dark matter of the genome



A variety of roles in guidance of chromatin modifiers, still being discovered, and other roles

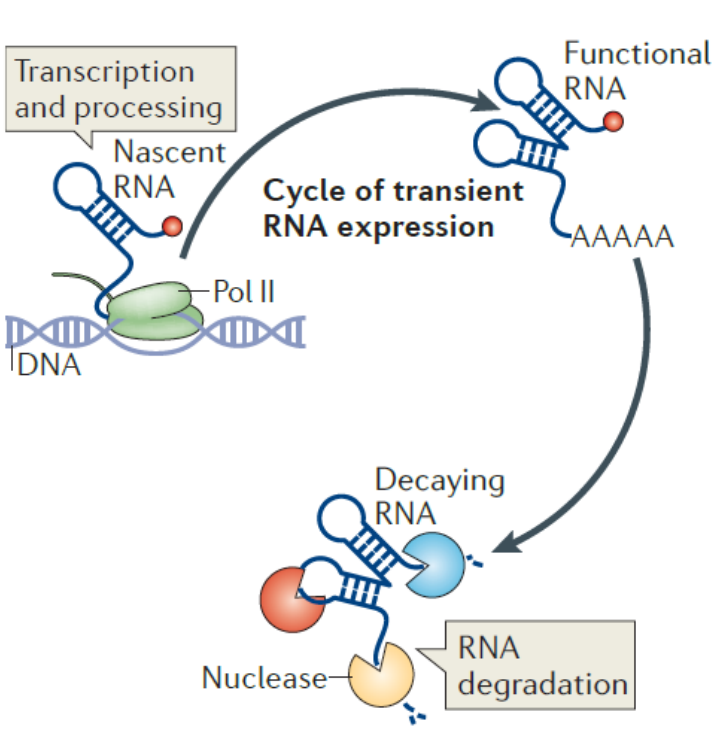
Some reviews:
Fatica,Bozzoni Nat Rev Genet Jan 2014
Geisler, Coller Nat Rev Mol Cell Biol Nov 2013
Quinodoz, Guttman Trends Cell Biol Nov 2014

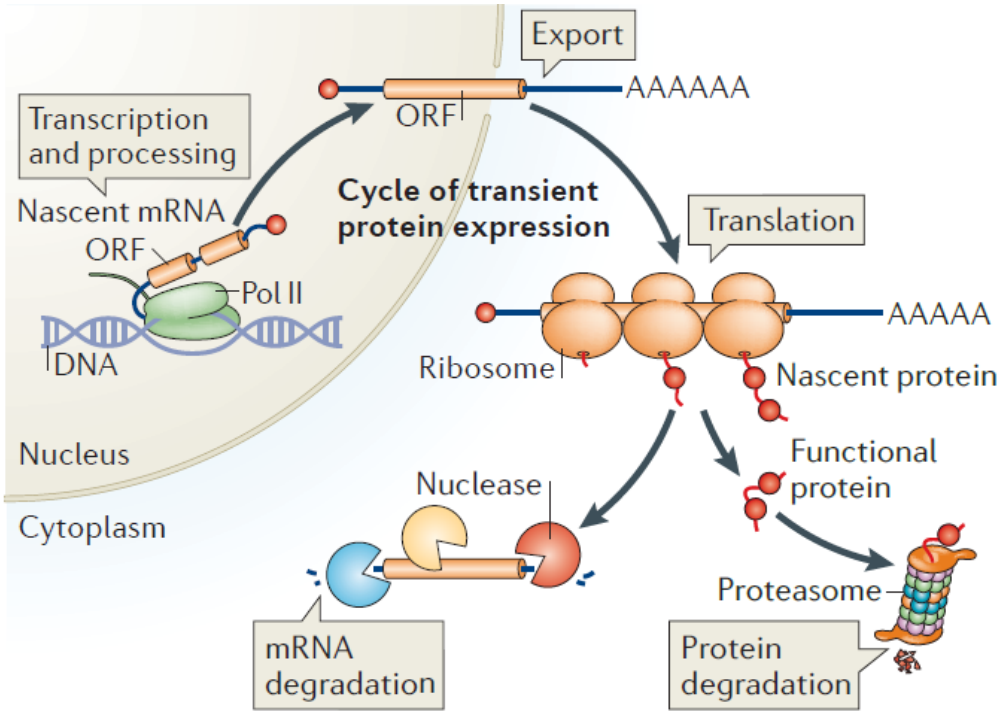
Local experts: Mitchell Guttman (CIT), Howard Chang (Stanford)

Regulatory RNA as a scaffold

- Long non-coding RNAs probably use base pairing to interact with other nascent RNAs
- Long non-coding RNAs use secondary structure to recruit multiple proteins
- Often long non-coding RNAs recruit chromatin modifying enzymes, including histone deacetylases (repression)
- Local binding, local action, maybe helping to organize domains of different gene regulation activities in cell nucleus

Functional noncoding RNA: synthesis & turnover kinetics make it a quick-change artist





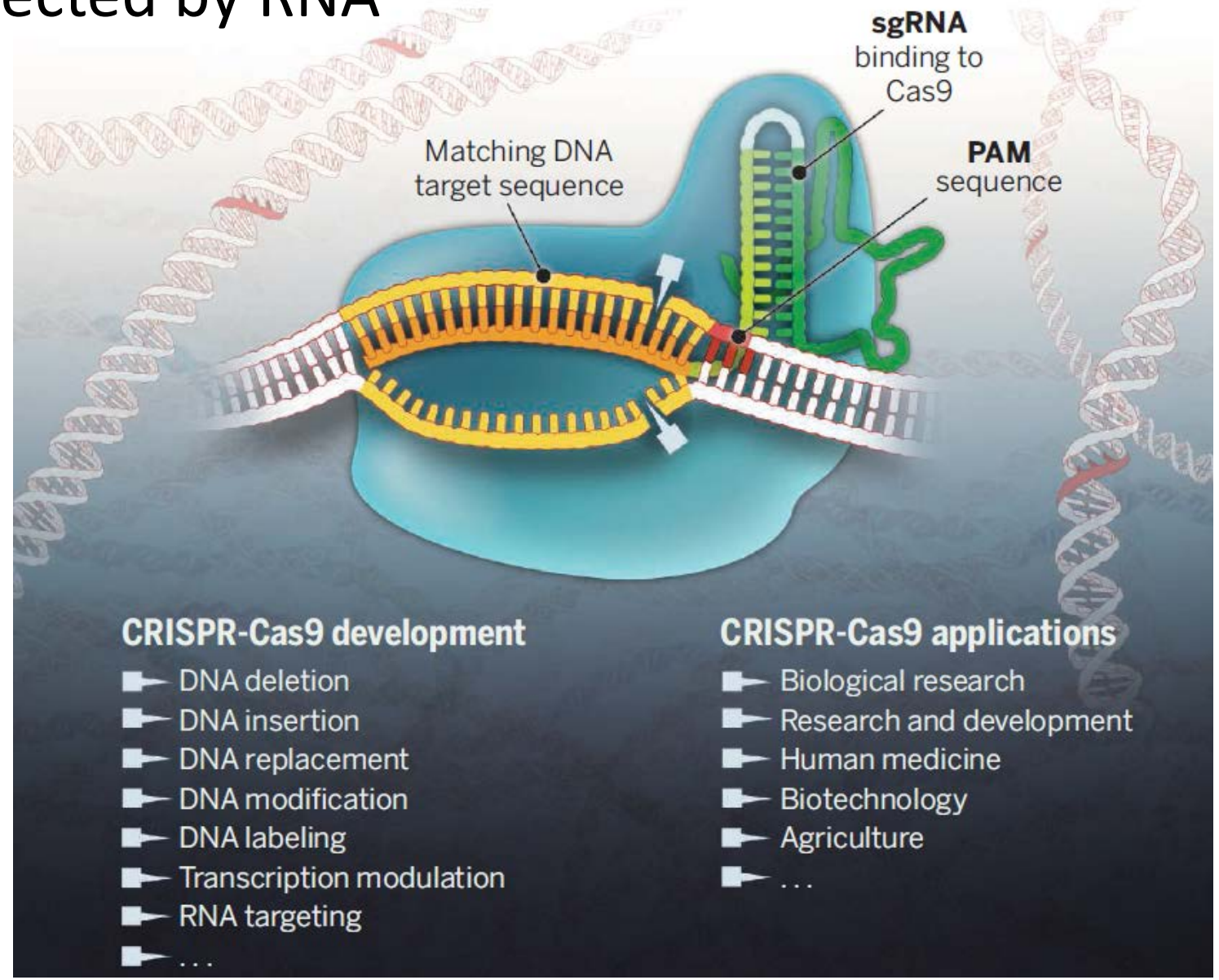
Many extra steps needed to make, destroy protein

From Geisler & Coller, 2013

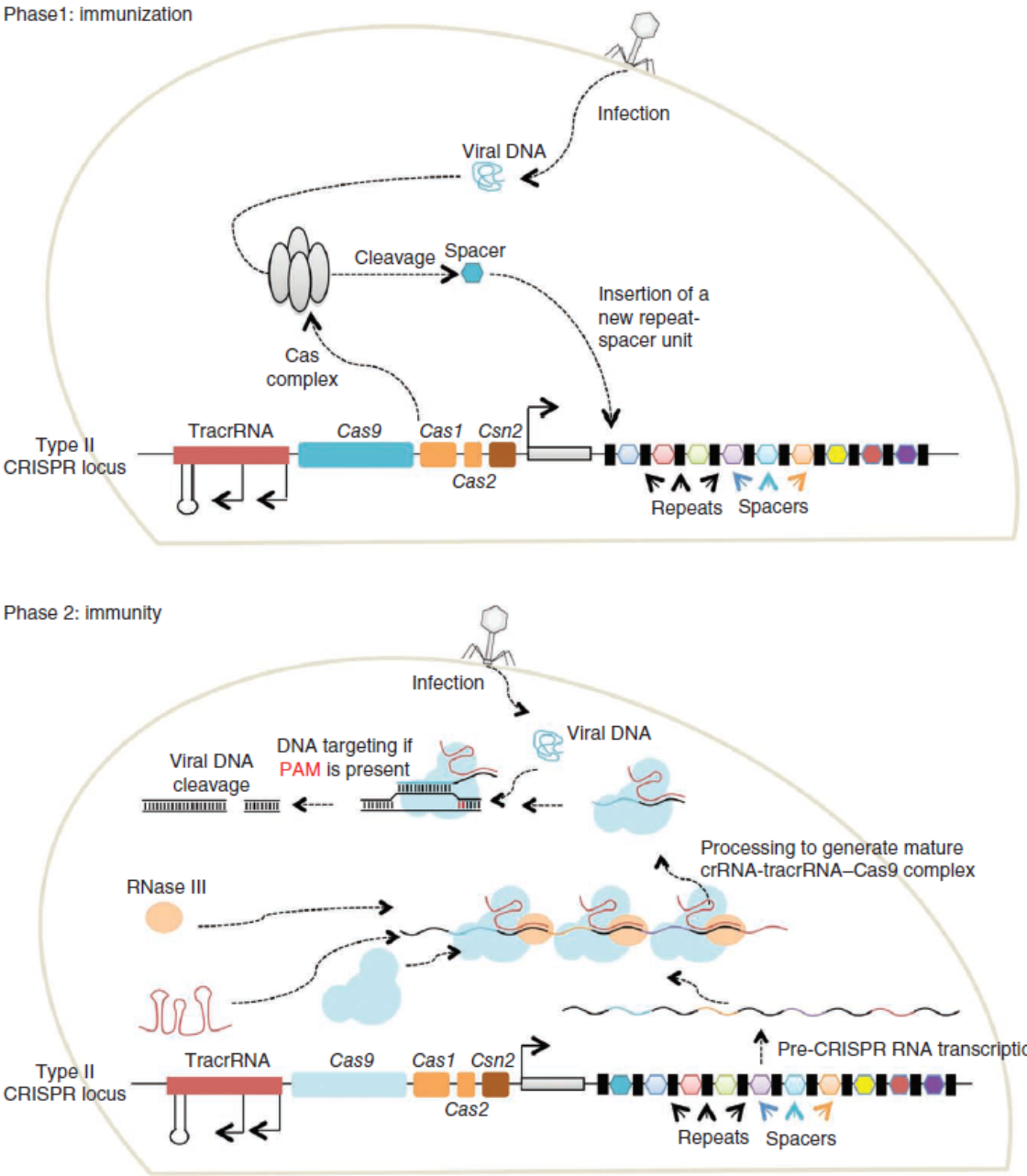
Ways that RNA contributes to enzymatic activity

- RNA as guide, by base pairing with substrate
- RNA as catalyst
  - stabilizing transition state &/or
  - creating it through attack on substrate
- These are primary roles of multiple classes of RNAs

CRISPr—Cas9: a revolution in genome editing, directed by RNA



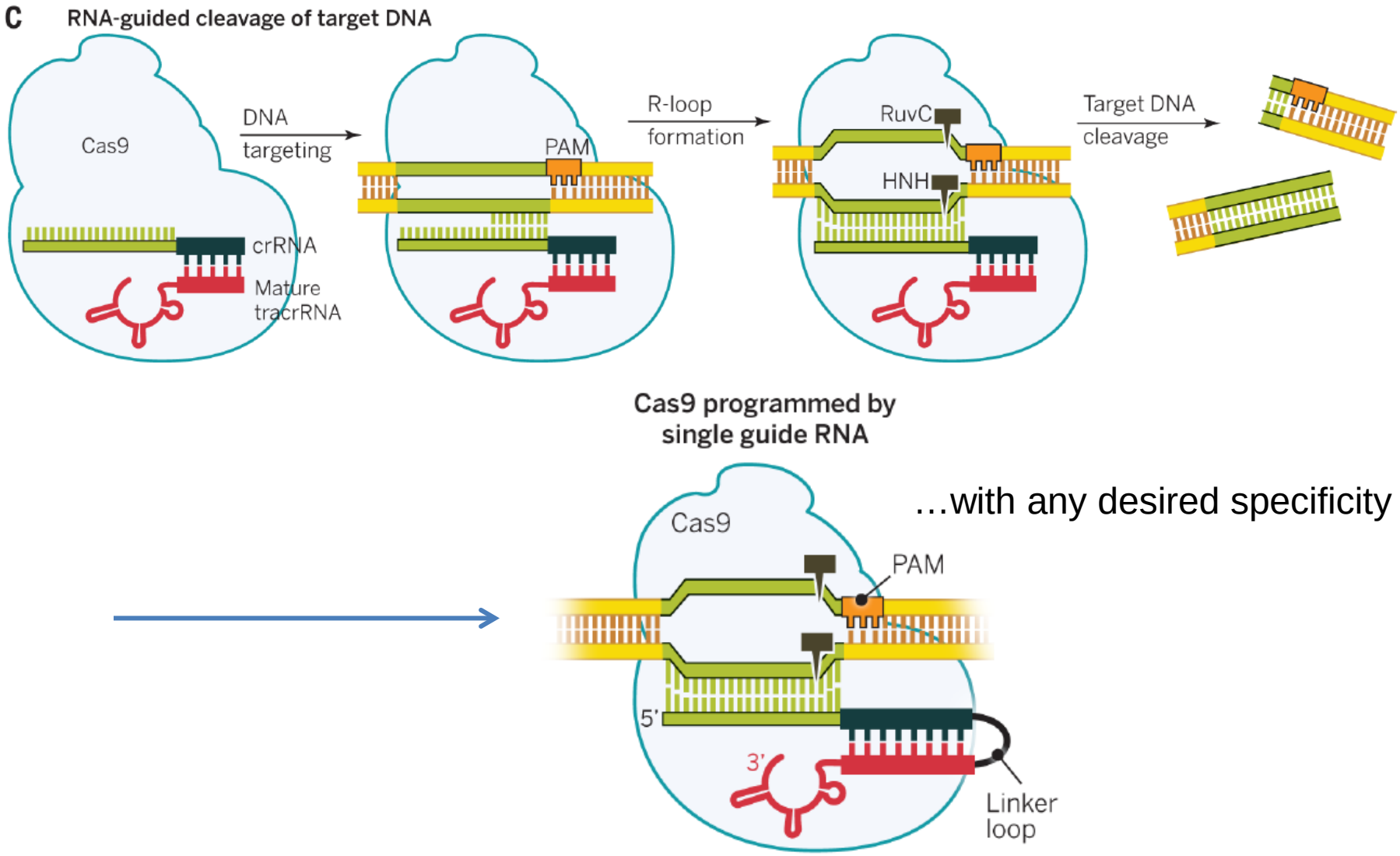
Evolution of a defense system: know your enemy, really really well



Invading phage DNA is integrated into an array of DNA repeats:
a common part of a template for RNA synthesis that will be used to guide Cas9 nuclease vs. future infections!

(Mali, Esvelt, Church 2013)

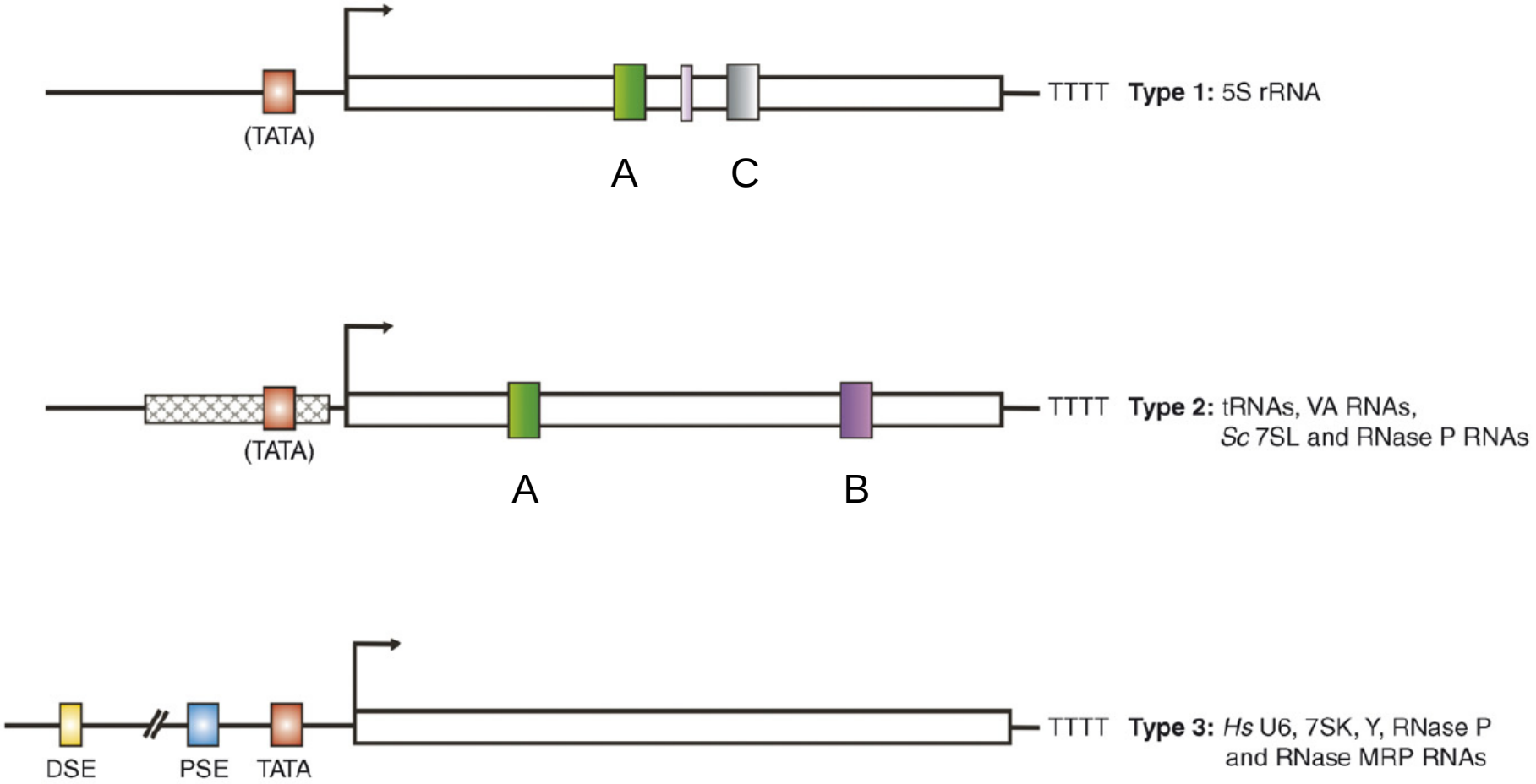
Tracer RNA replaced by “Guide RNA”: a bacterial mechanism for “immunity” can be made into a tool



…with any desired specificity

Many catalytic-like small RNAs are synthesized by RNA polymerase III rather than I or II

(contrast with miRNAs, synthesized by RNA pol II)



Colored boxes: special cis-regulatory elements for pol III; also oligoT termination signals

(Dieci, ….& Pagano, Trends Genet., 2007)

The benefits of 2’-OH reactivity:

RNA can also catalyze enzymatic reactions, not just find templates

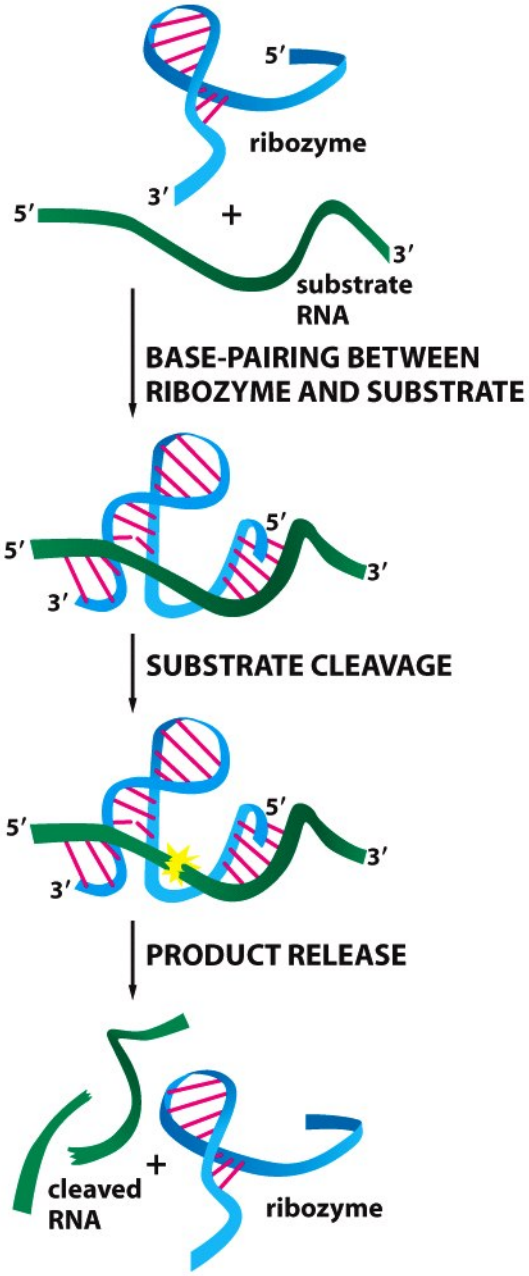
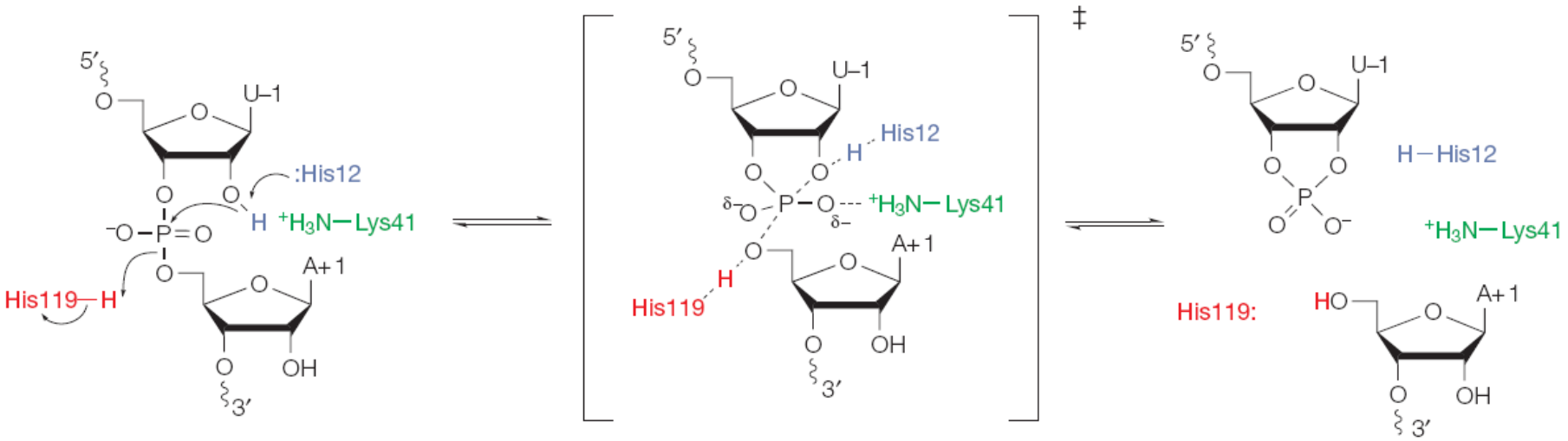


Figure 6-103 *Molecular Biology of the Cell* (© Garland Science 2008)

Table 6–5 Some Biochemical Reactions That Can Be Catalyzed by Ribozymes

ACTIVITY	RIBOZYMES
Peptide bond formation in protein synthesis	ribosomal RNA
RNA cleavage, RNA ligation	self-splicing RNAs; RNase P; also *in vitro* selected RNA
DNA cleavage	self-splicing RNAs
RNA splicing	self-splicing RNAs, perhaps RNAs of the spliceosome
RNA polymerizaton	*in vitro* selected RNA
RNA and DNA phosphorylation	*in vitro* selected RNA
RNA aminoacylation	*in vitro* selected RNA
RNA alkylation	*in vitro* selected RNA
Amide bond formation	*in vitro* selected RNA
Glycosidic bond formation	*in vitro* selected RNA
Oxidation/reduction reactions	*in vitro* selected RNA
Carbon–carbon bond formation	*in vitro* selected RNA
Phosphoamide bond formation	*in vitro* selected RNA
Disulfide exchange	*in vitro* selected RNA

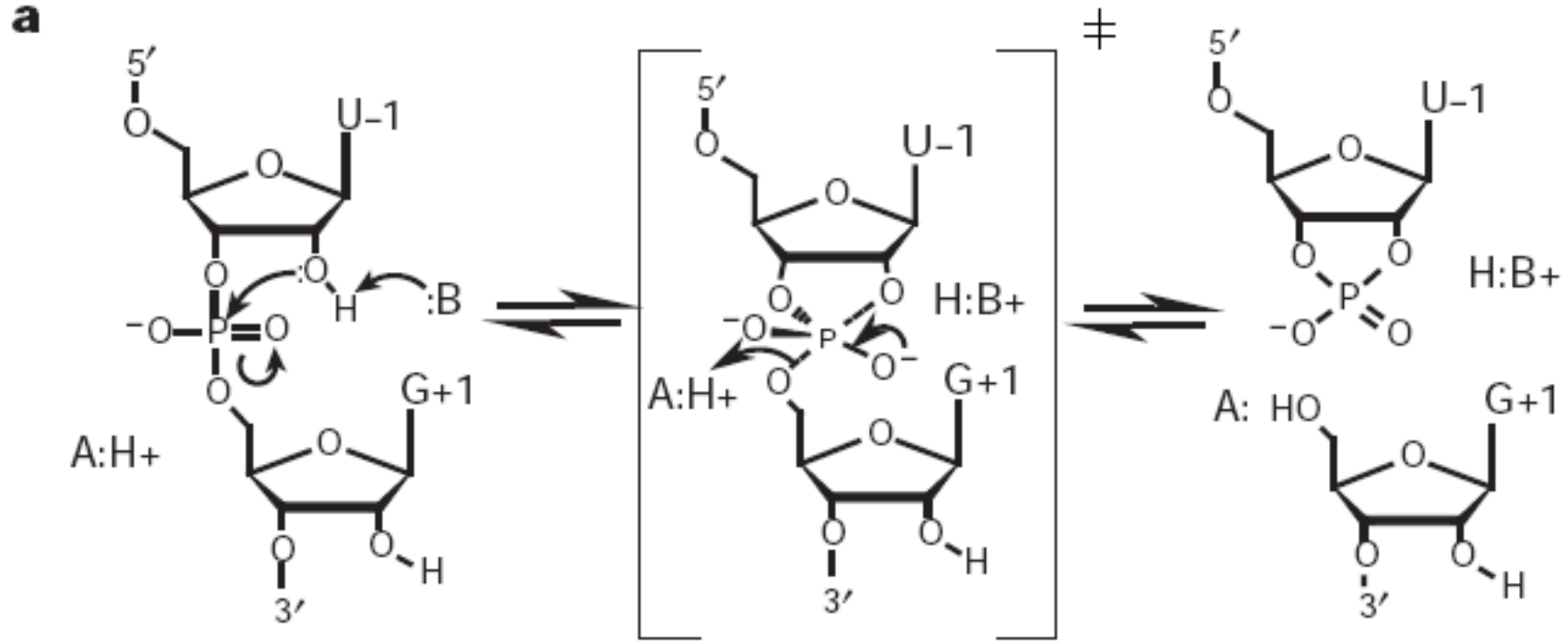
How a protein catalyzes RNA cleavage (RNase A)



Concerted acid-base catalysis: (1) His12 abstracts proton to create oxyanion for attack; (2) protonated His 12, unprotonated His119 and protonated Lys41 stabilize the transition state; (3) cleaved polynucleotide is released; later (not shown) His12 “gives back”

(Fedor, Williamson Nat Rev Mol Cell Bio 2005)

RNA self-cleavage:
the easiest job for an RNA



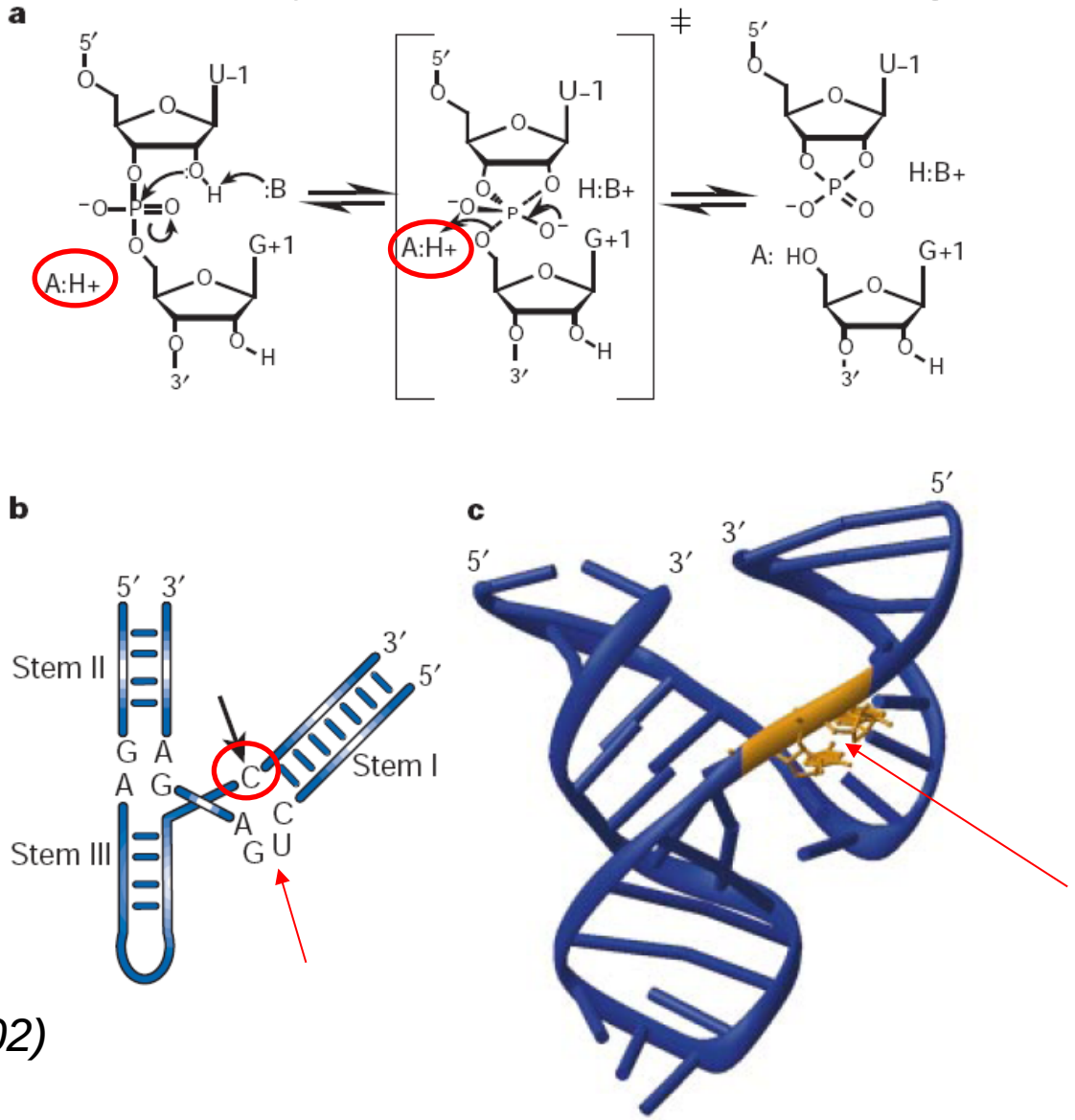
Extremely similar to RNase cleavage… especially if acid and base moieties are there for transition state stabilization

(Doudna, Cech Nature 2002)

∴ STRUCTURE CAN HELP!

“Hammerhead” ribozyme: a naturally occurring RNA enzyme that *catalyzes* its own cleavage

Highly structured “hammerhead” motif allows an RNA plant virus to clip out genome-length RNA pieces from a long concatenated replication product



(Doudna, Cech Nature 2002)

RNA-dependent catalysis can also stabilize transition states by *complementary base pairing*

This plays a huge role in splicing: every eukaryote's need for precise RNA cleavage

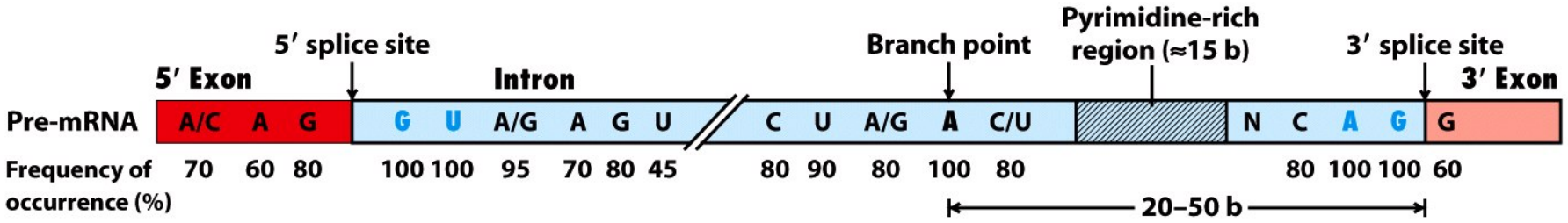


Figure 8-7
Molecular Cell Biology, Sixth Edition
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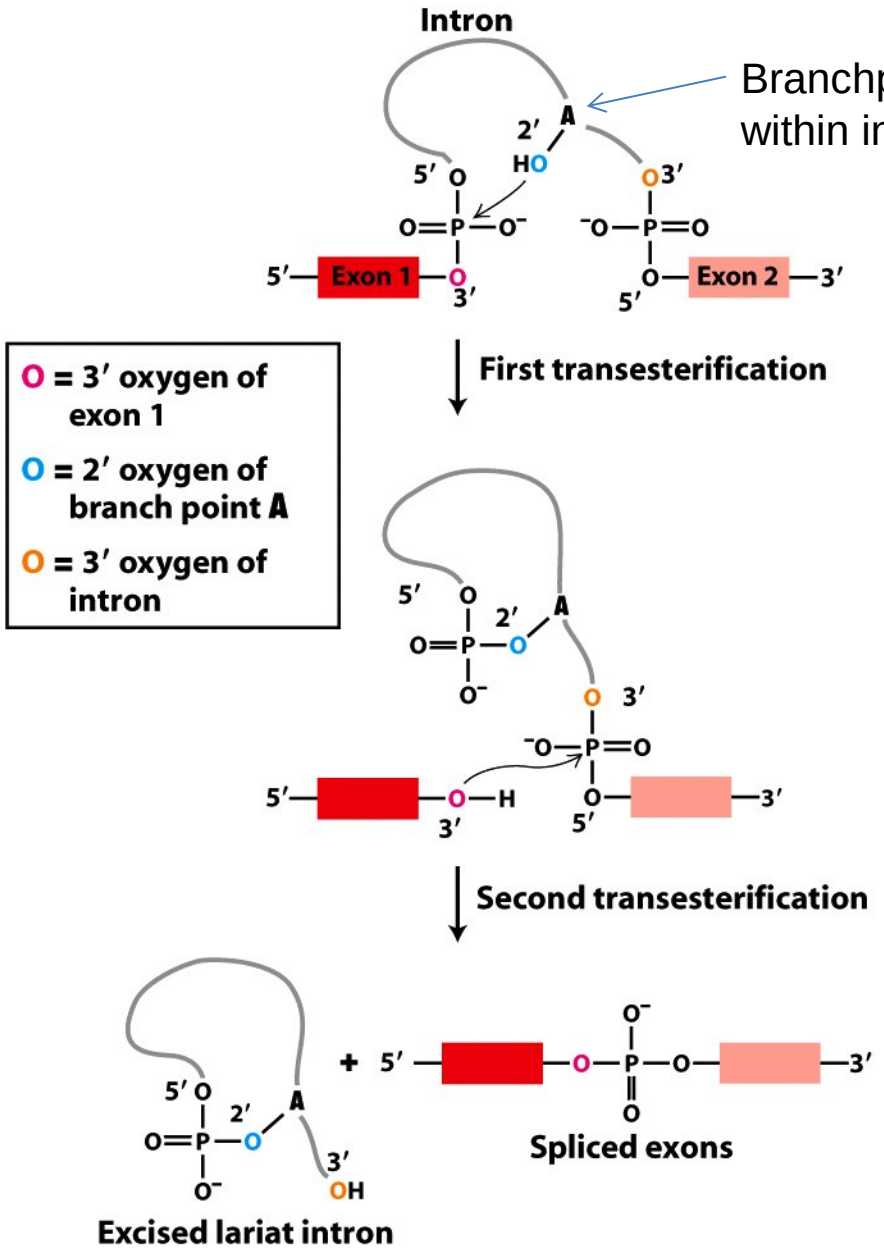
not completely deterministic, but guided by key sequences in substrate RNA… These are targets for base pairing

Reminder of what happens during splicing

Chemistry is permissive but a big problem to find the right phosphodiester bonds to be attacked



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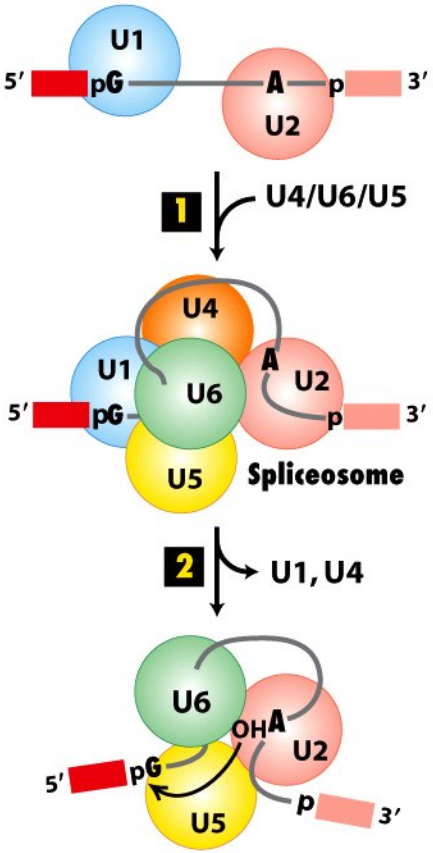
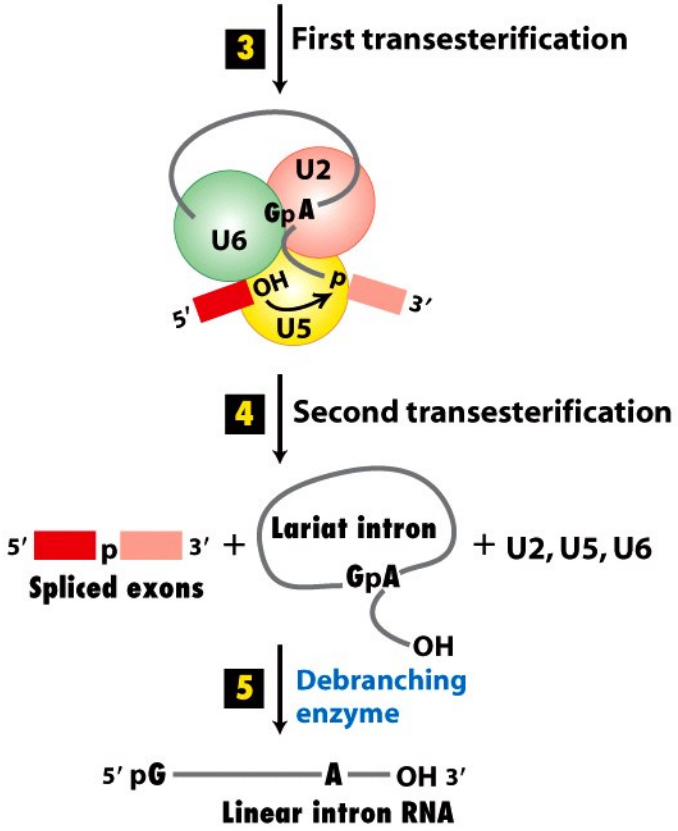
Splicing normally gets a lot of help from ribonucleoprotein "spliceosomes": key players, snRNA-Protein complexes





snRNP: small nuclear ribonucleoproteins, complexes centered on snRNAs

Main snRNPs:
U1: 5' for assembly
U2: at 3' branch site
U5: for second attack
U6: 5' for all catalytic events

Figure 8-11
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mRNA intron is base paired with U1, U6 snRNAs: handoff requires ATP hydrolysis

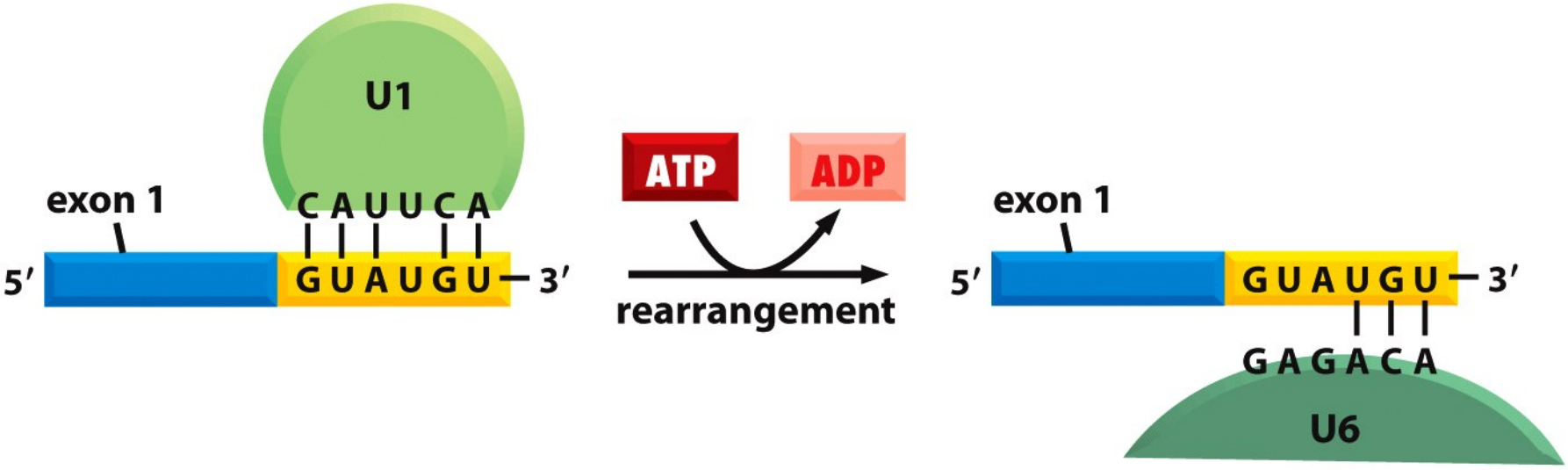

Figure 6-30a *Molecular Biology of the Cell* (© Garland Science 2008)

Displacement of original binding protein for 3’ end of intron by U2… also requires ATP hydrolysis

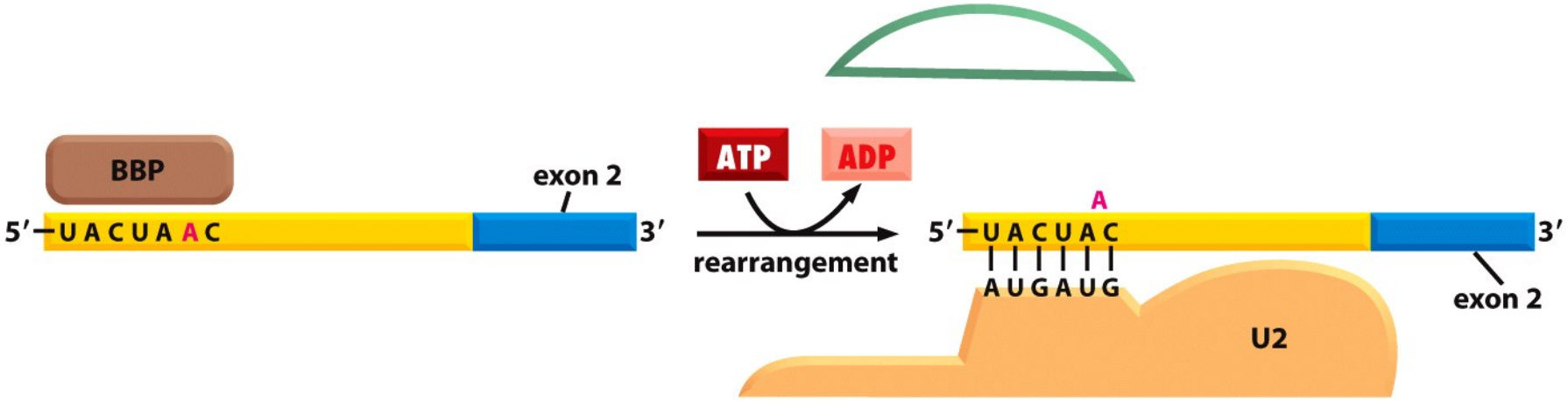

Figure 6-30b *Molecular Biology of the Cell* (© Garland Science 2008)

Base pairing is important for splicing efficiency

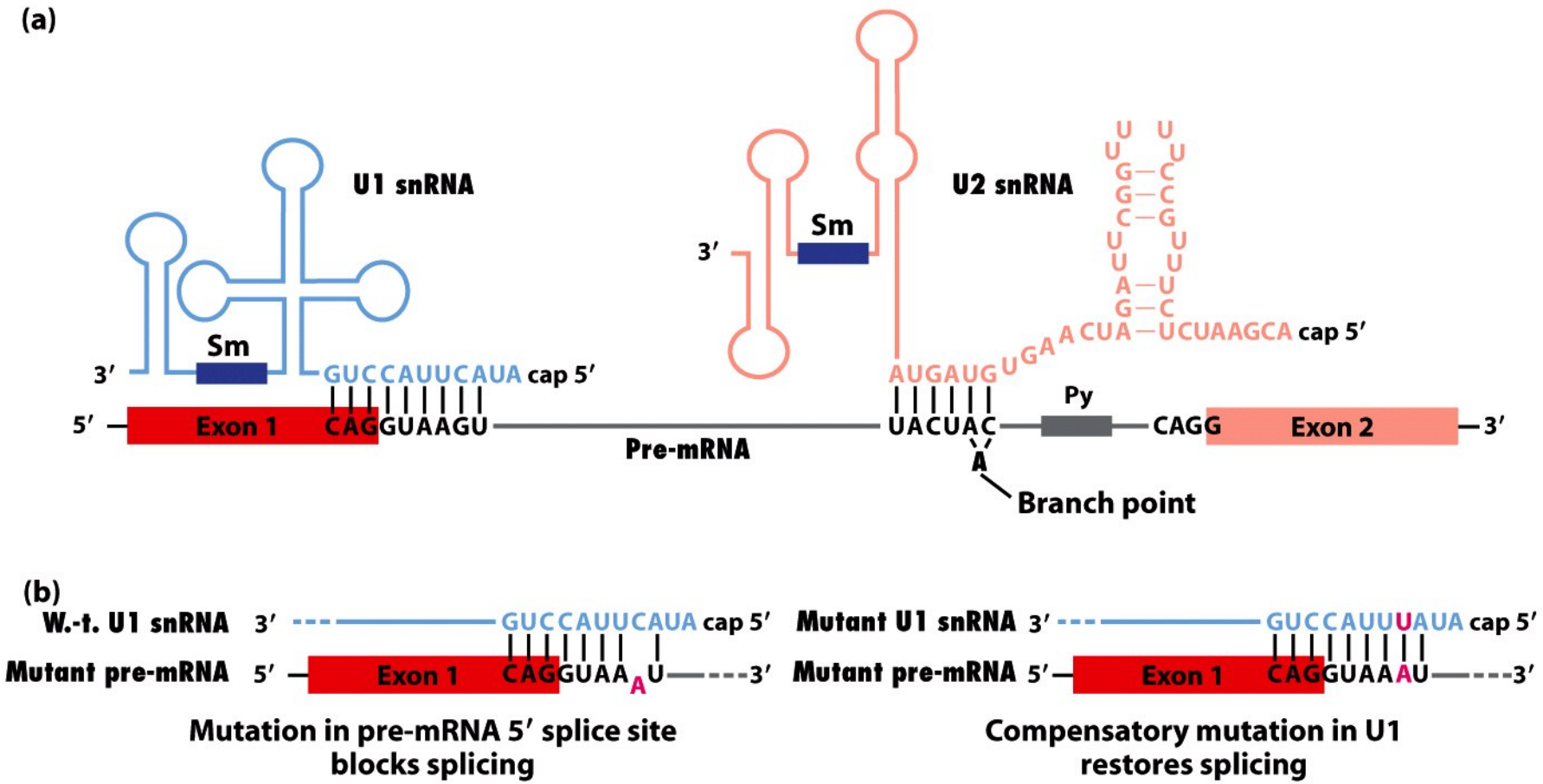

Figure 8-9
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U6/U2 machine carries out the splicing with help from U5 base pairing and more ATP hydrolysis

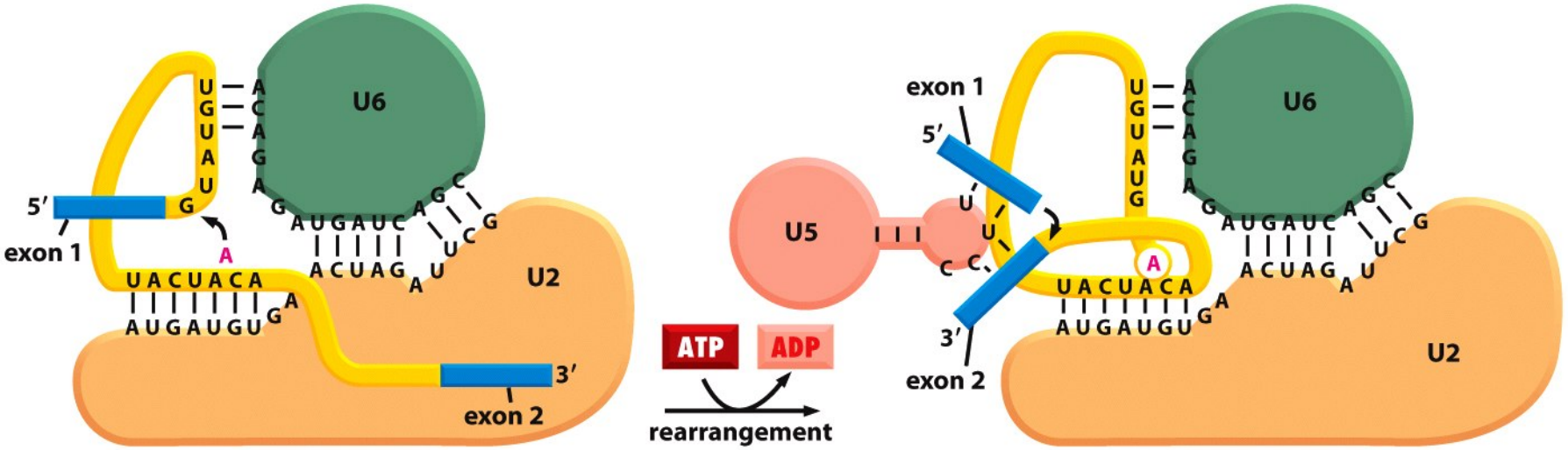
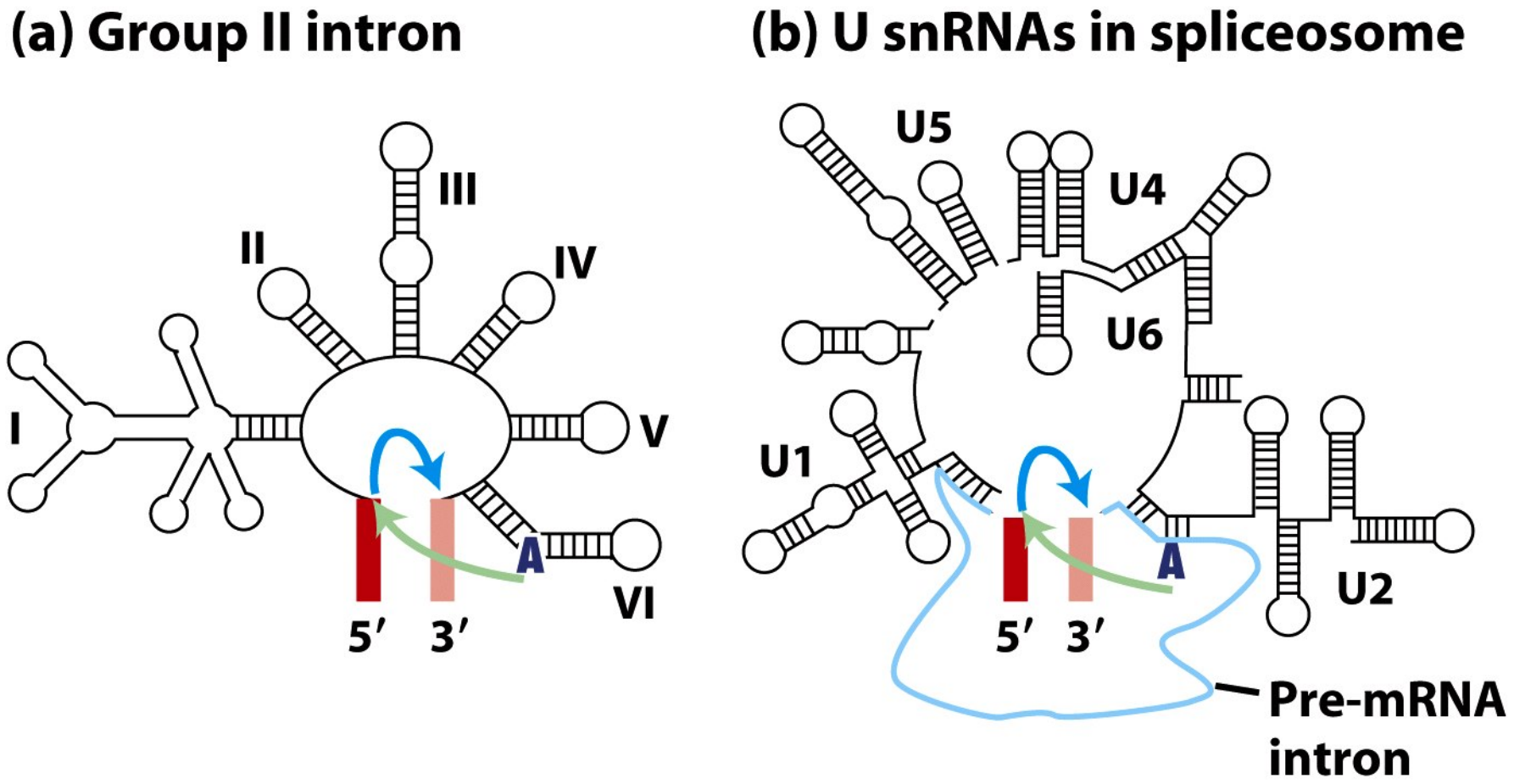


Figure 6-30c *Molecular Biology of the Cell* (© Garland Science 2008)

There are small introns in some microorganisms with such a highly specialized structure that they can splice out themselves….Possibly analogous folding to “spliceosomes”



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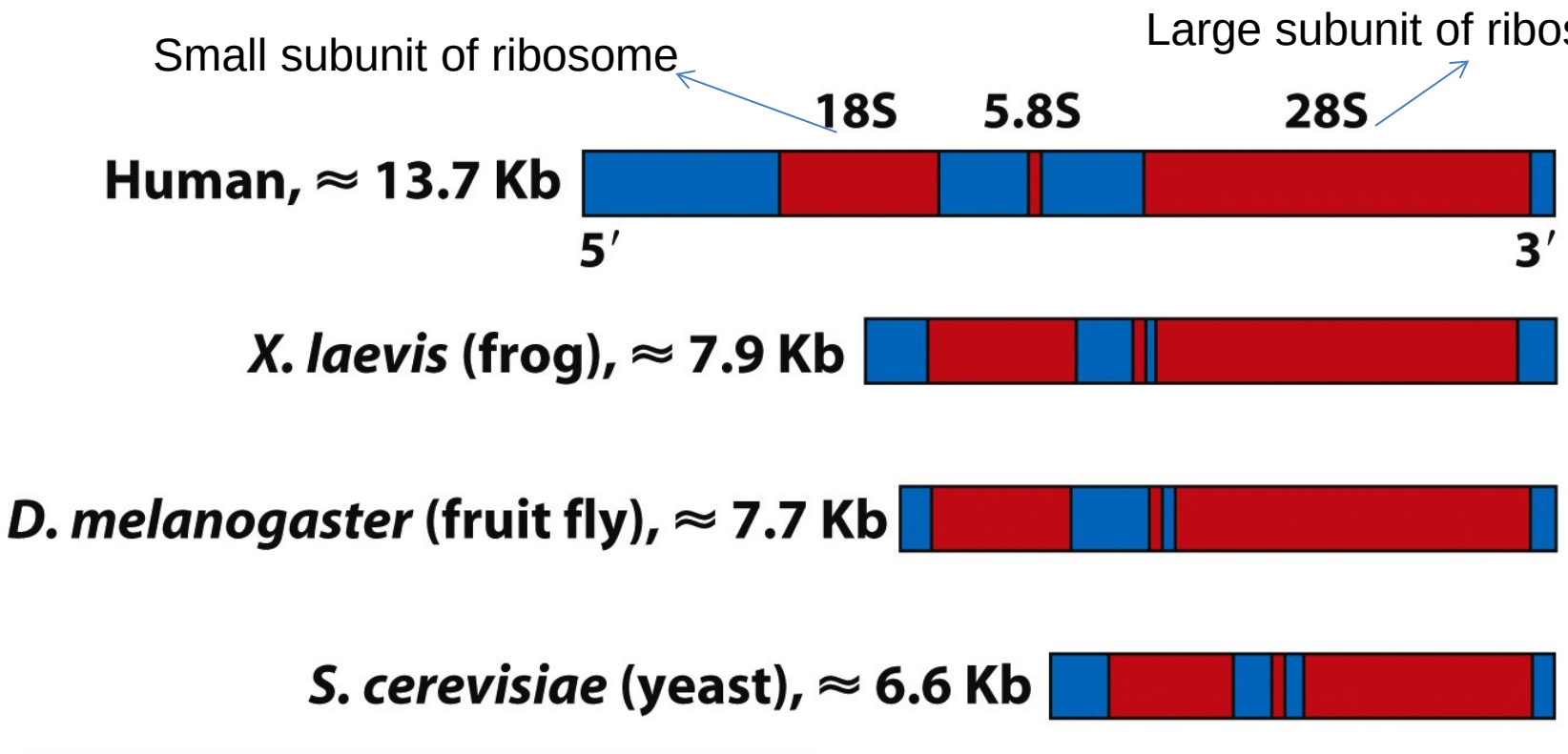
Translation in the ribosome itself may also depend on ribozyme-like activity of rRNAs



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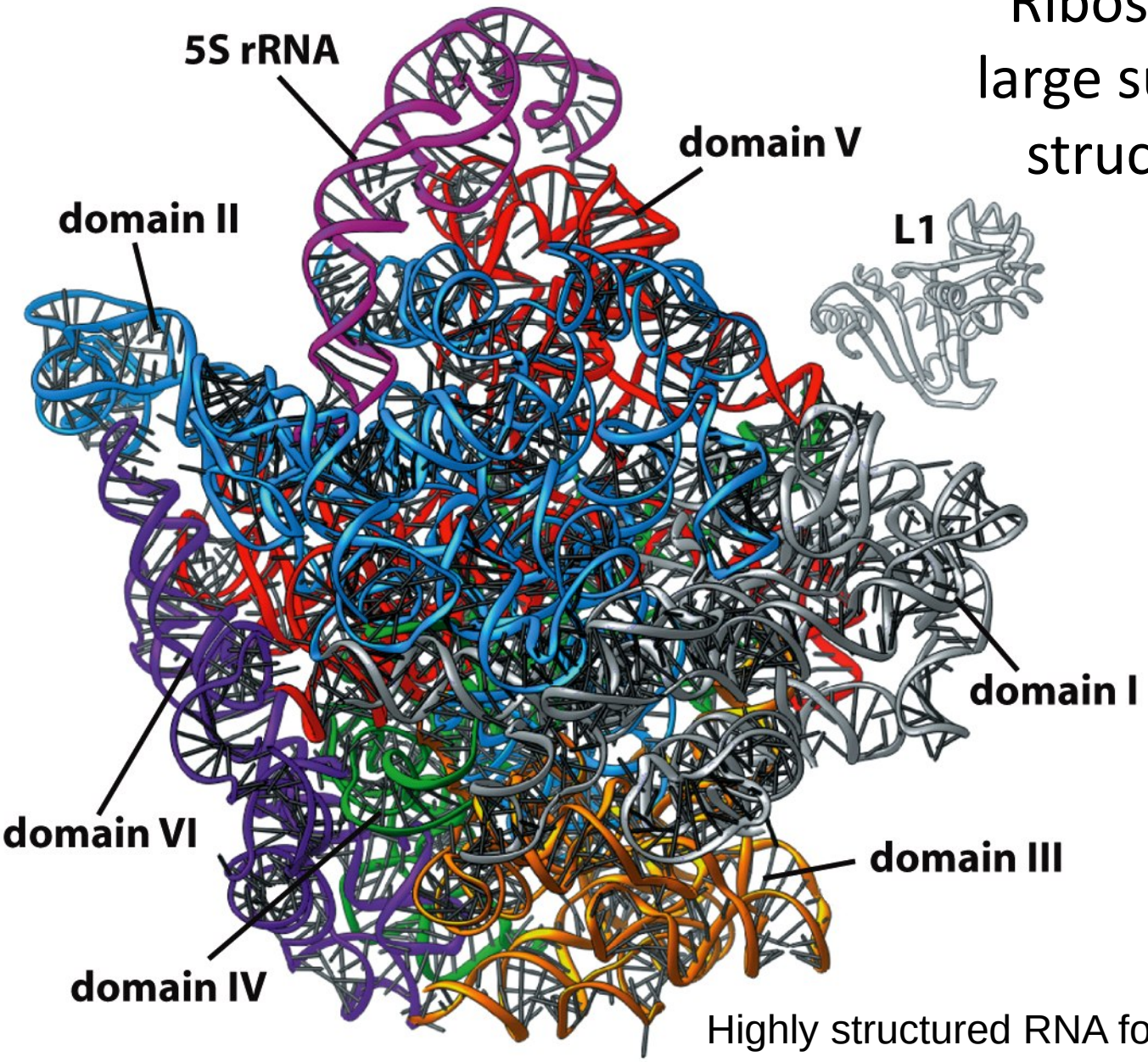
Ribosomal RNA primary transcripts are processed by cleavage into the separate rRNAs

18S: ~1.9kb 28S: ~4.7 kb

Ribosomal large subunit structure



Highly structured RNA forms whole shell of subunit

Figure 6-69a *Molecular Biology of the Cell* (© Garland Science 2008)

Within ribosome, complementarity to 16S (small rRNA) can define translational start site (Shine Dalgarno box in bacteria), but also…

acts as quality control for alignment/pairing of codon/ tRNA anticodon at each step of translation

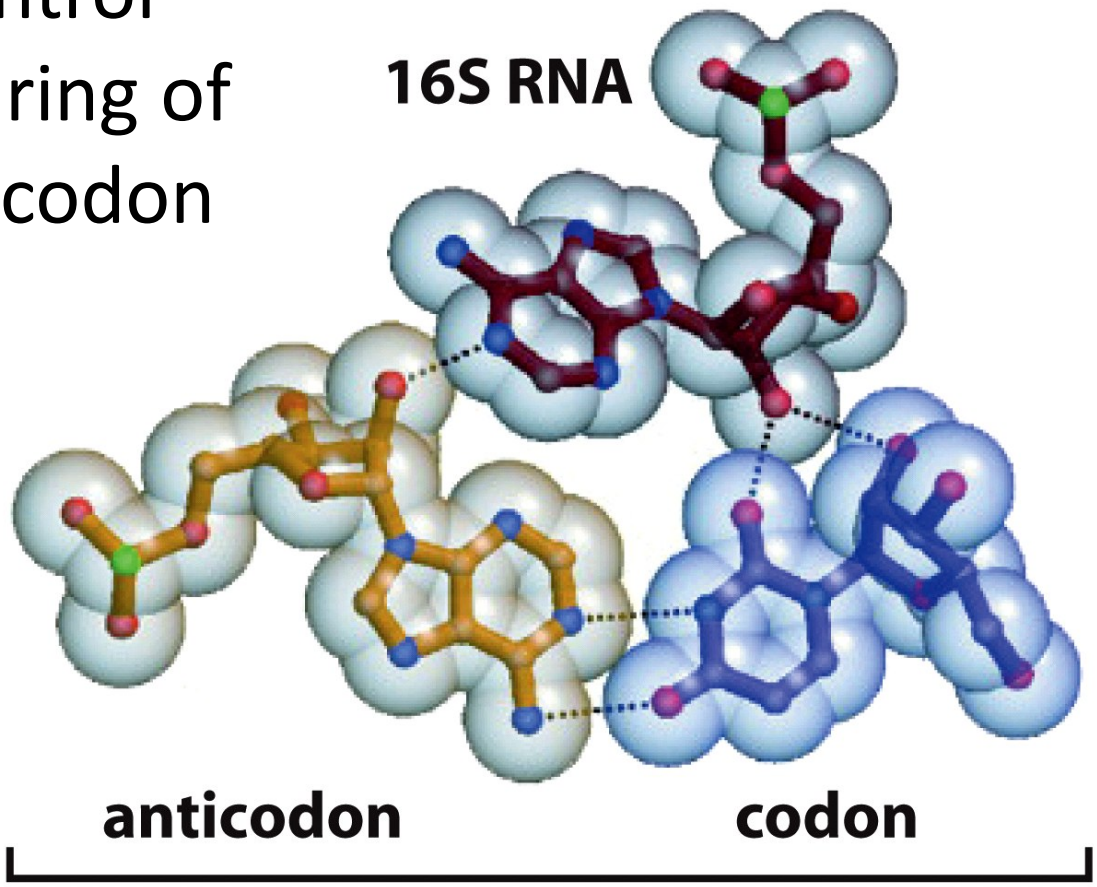
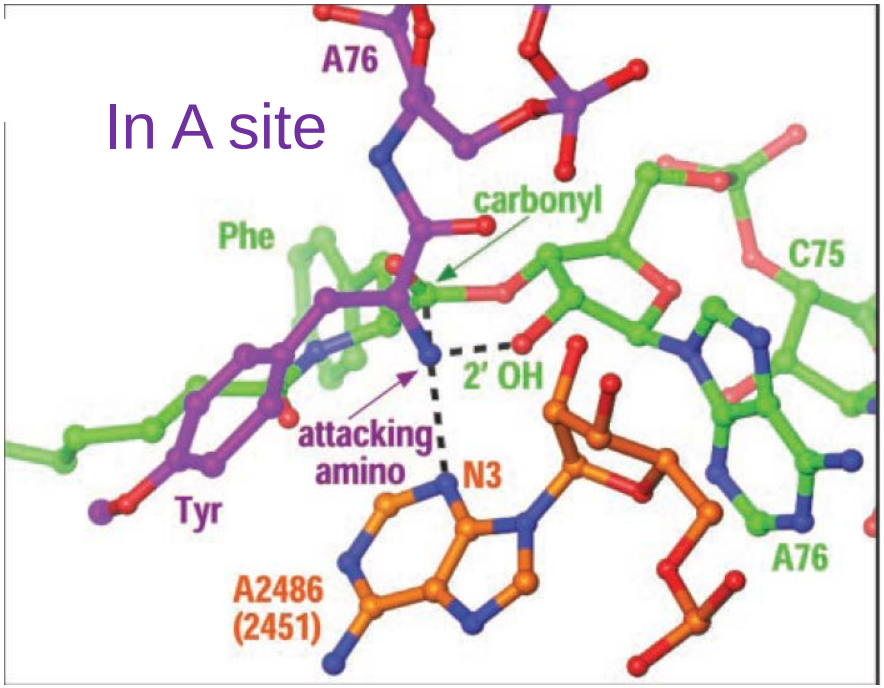


Figure 6-68 *Molecular Biology of the Cell* (© Garland Science 2008)

And a ribozyme-like catalyst lies at the heart of the large ribosomal subunit… crucial for peptidyl transferase reaction



In P site

Large rRNA

(Hansen, … Steitz PNAS 2002; Fedor, Williamson Nat Rev Mol Cell Bio 2005)

RNA as the jack of all trades of molecular biology

- Base pairing with other molecules for recognition and complex assembly
- Secondary structure for unique features
- Biochemical reactivity for enzyme activity
- Templating possible for replication

RNA world first?

- Versatility: genetic code and active agent
- Ease of synthesis and turnover, for flexibility
- Inheritance at heart of some of most crucial functions
- Ability of isolated RNAs to do many jobs, including self replication