

Module I: Introduction

20.109 Lecture 1
4 February, 2010

Module Overview

- **Introduction to:**
 - Fundamental concepts and techniques in molecular biology
 - A powerful and accessible strategy (**SELEX**) for identifying nucleic acids (**aptamers**) having desirable binding properties for a defined target

Module Overview

- **Lectures:**
 - Conceptual and practical considerations for successful SELEX
 - Become comfortable with the concept of a nucleic acid library
 - Broadly consider the practical applications of aptamers
 - Biology
 - Technology

Module Overview

Day	Lecture	Lab
1	Introduction	DNA library synthesis (PCR)
2	SELEX I: Building a Library	DNA library purification (agarose gel electrophoresis)
3	SELEX II: Selecting RNA with target functionality	RNA library synthesis (<i>In vitro</i> transcription = IVT)
4	SELEX III: Technical advances & problem-solving	RNA purification and heme affinity selection
5	Characterizing aptamers	RNA to DNA by RT-PCR
6	Introduction to porphyrins: chemistry & biology	Post-selection IVT Journal Club 1
7	Aptamer applications in biology & technology	Aptamer binding assay
8	Aptamers as therapeutics	Journal Club 2

Today's Objectives

- Provide a context for appreciating RNA as a macromolecule capable of specific interactions
 - Small molecules
 - Proteins
- Appreciate that:
 - Atomic level interactions underlie these binding events
 - Binding reactions occur in 3-dimensional space

The Central Dogma

DNA

Information storage

- Double stranded
- Helical structure
- Encodes genes



transcription

RNA

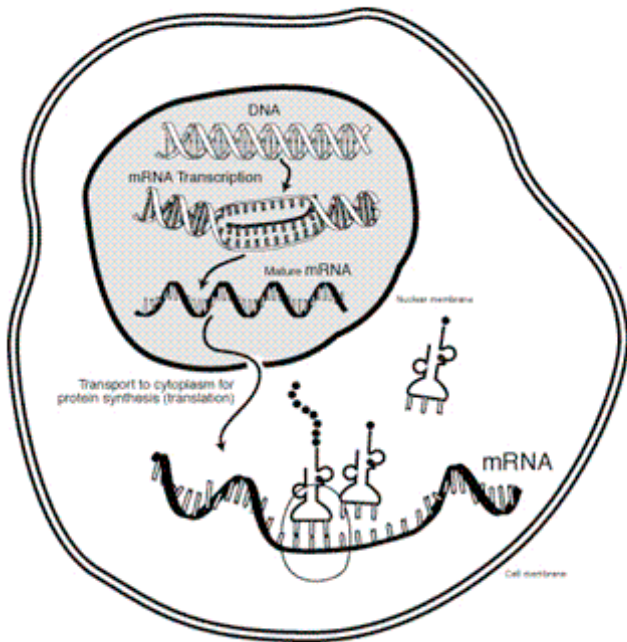
What is RNA good for?

translation

Protein

Diverse Functions

- Enzymes
 - *DNA replication*
 - *Energy production*
- Transport
 - *Cell membrane*
- *Motility*
 - *Actin, myosin*



RNA has diverse functions, too!

How is mRNA decoding take place during translation?

– Two particularly critical players depend on RNA for proper function

- Ribosome
- tRNA

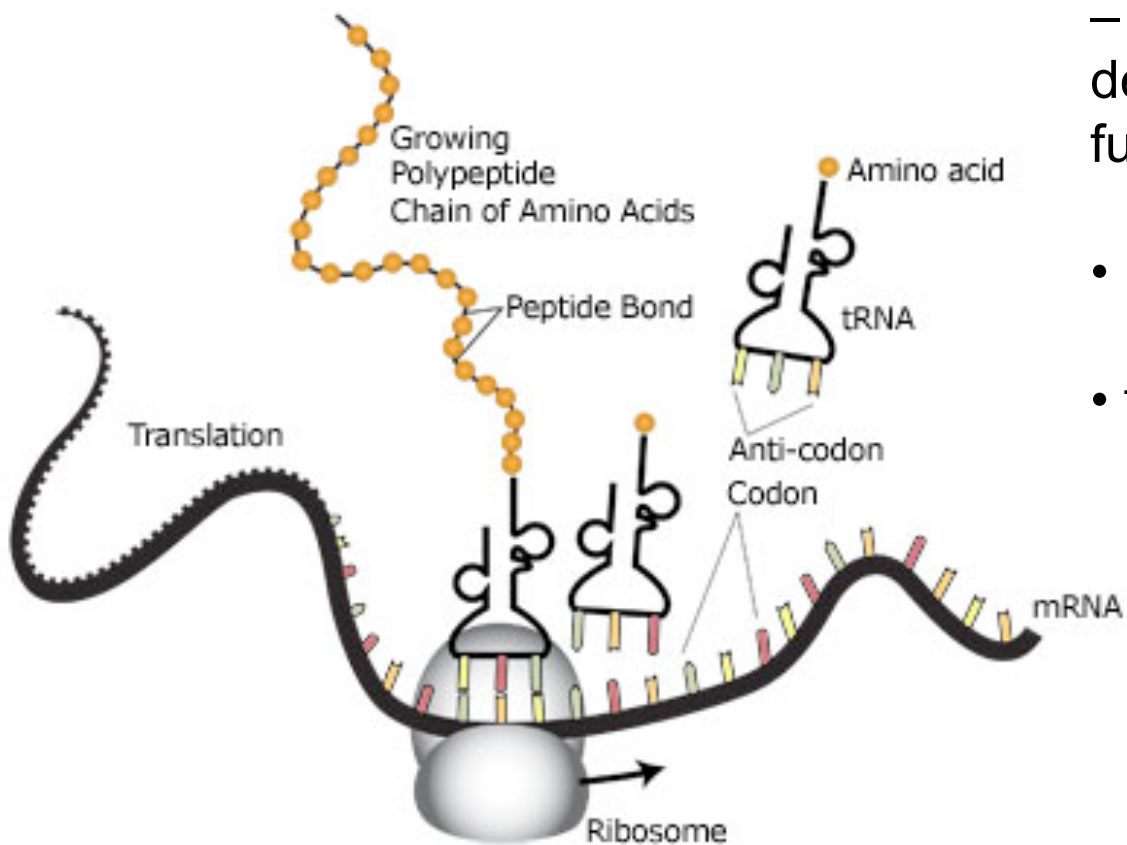
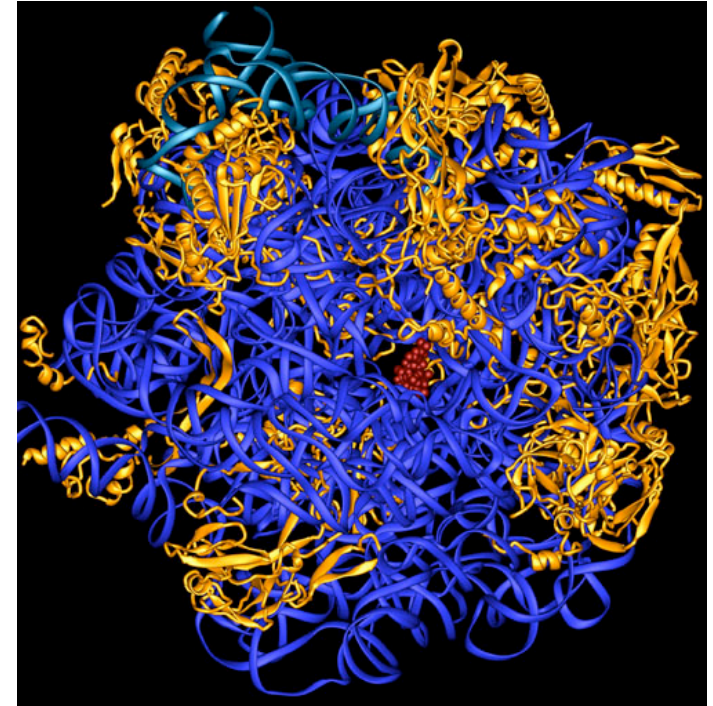


Image adapted from: National Human Genome Research Institute.

Ribosome composition and structure

- Two subunits
 - Large
 - 50S in prokaryotes/ 60S in eukaryotes
 - Small
 - 30S in prokaryotes/ 40S in eukaryotes
- Composition
 - 60% RNA!
 - 5S rRNA (LSU)
 - 23S rRNA (LSU)
 - 16S rRNA (SSU)



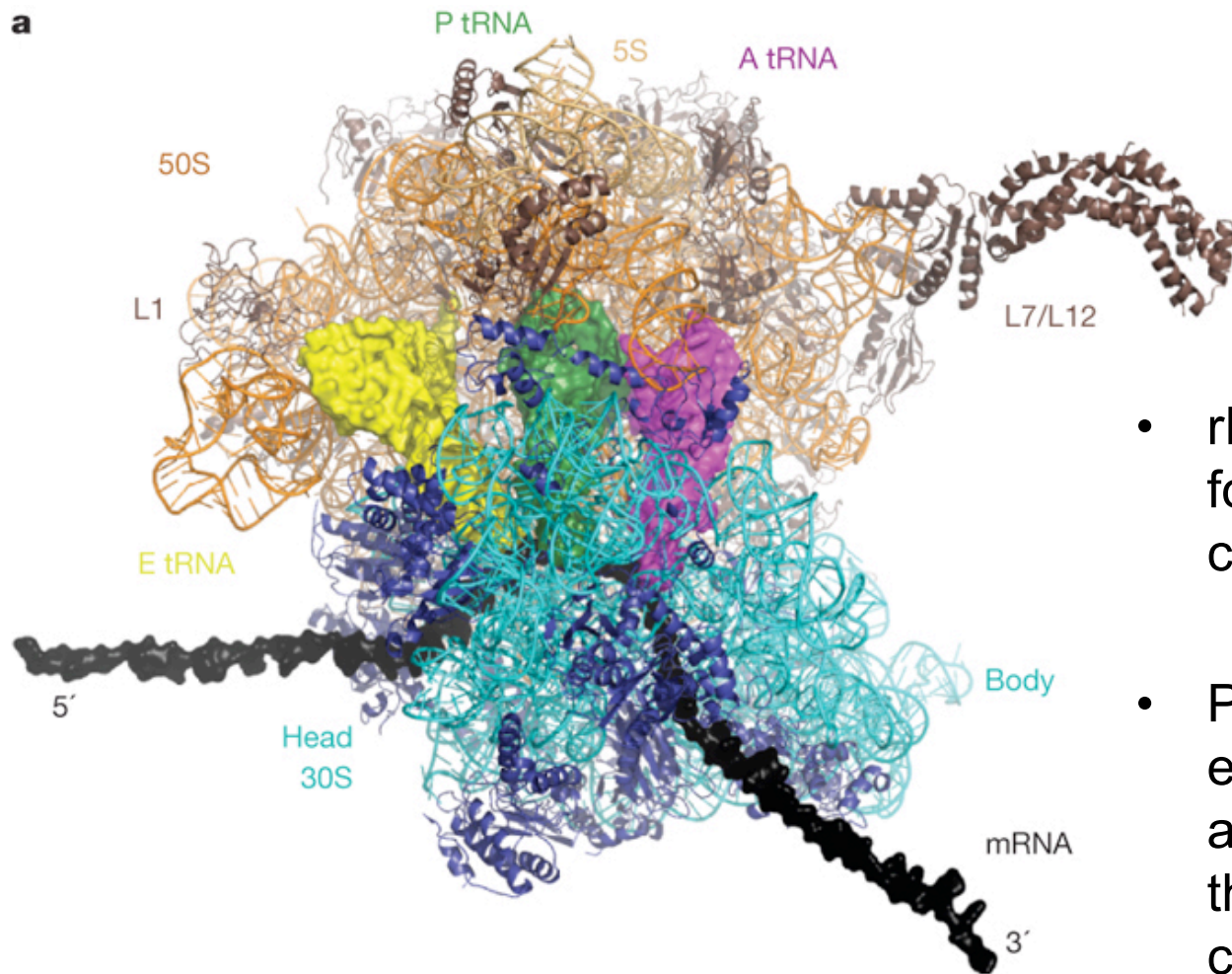
50S ribosomal subunit (*D. radiodurans*)

Blue = ribosomal RNA (rRNA)
Gold = protein

Ribosomal RNA functions

- *Structural*

- Organize the protein components in the ribosome



- rRNA serves as a scaffold for the associated protein components
- Proper assembly requires establishing very specific atomic contacts between the RNA and protein components

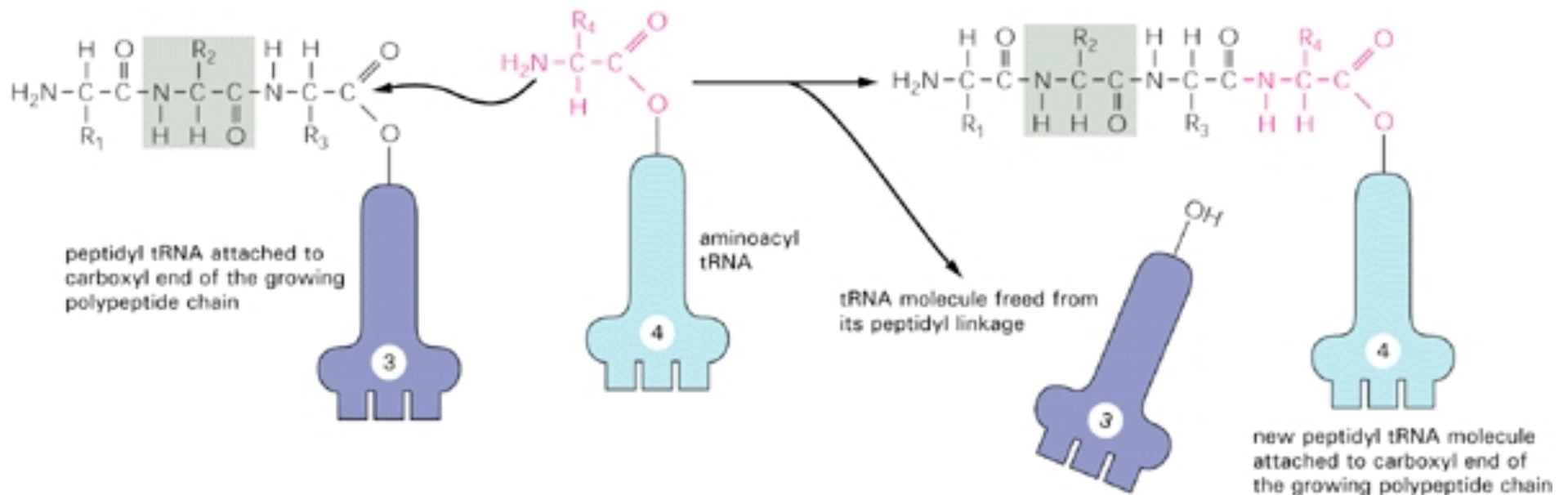
Ribosomal RNA functions

- *Enzymatic*

- 23S rRNA involved in catalyzes the peptidyl transfer reaction step during translation

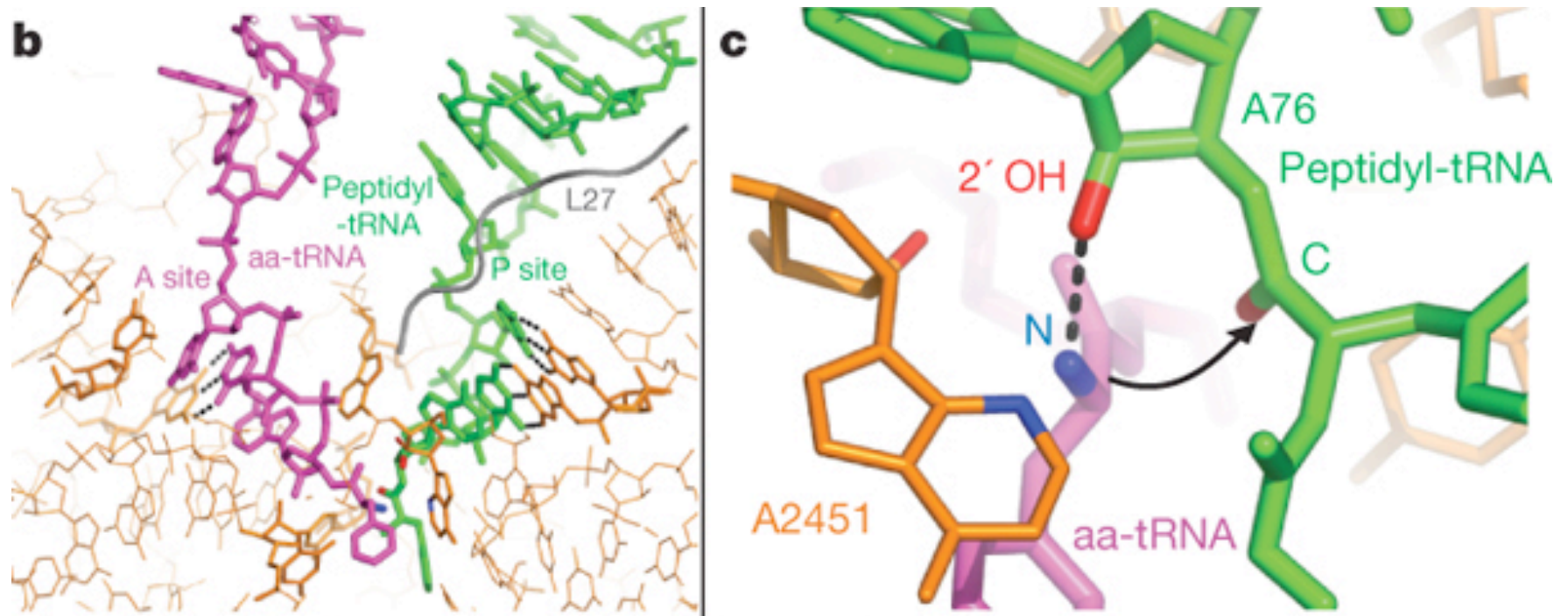
- Binding site for the aminoacyl tRNA is located in the 23S rRNA

- Rate enhancement over non-enzymatic reaction: $\sim 10^5!$



Ribosomal RNA functions

- *Specific atomic contacts are made between rRNA residues and the amino acyl-tRNA*



rRNA enzymatic activity as a drug target

- *Antibiotics*

- Used to treat microbial pathogens
- Several different classes
- Target different cellular processes
- Selectively inhibit pathogen but not host pathways

- Chloramphenicol

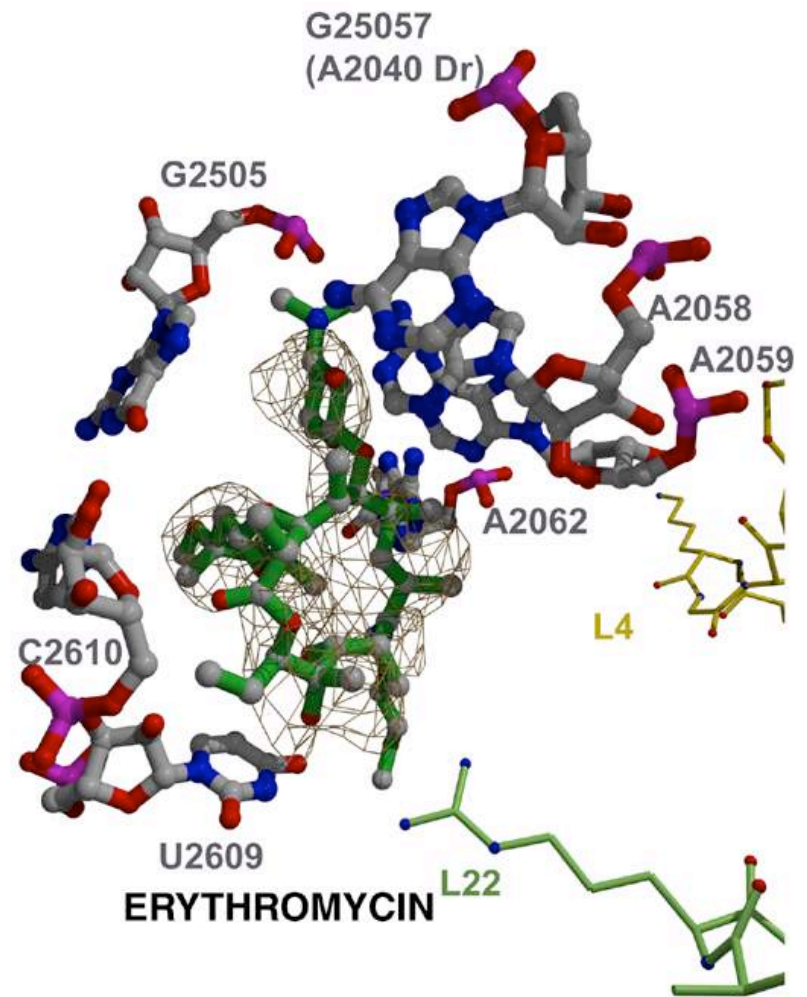
- ***Inhibit ribosomal peptidyl transferase activity!***

- Macrolides

- E.g. erythromycin, clarithromycin
- ***Inhibit nascent peptide transfer away from the peptidyl transferase site***

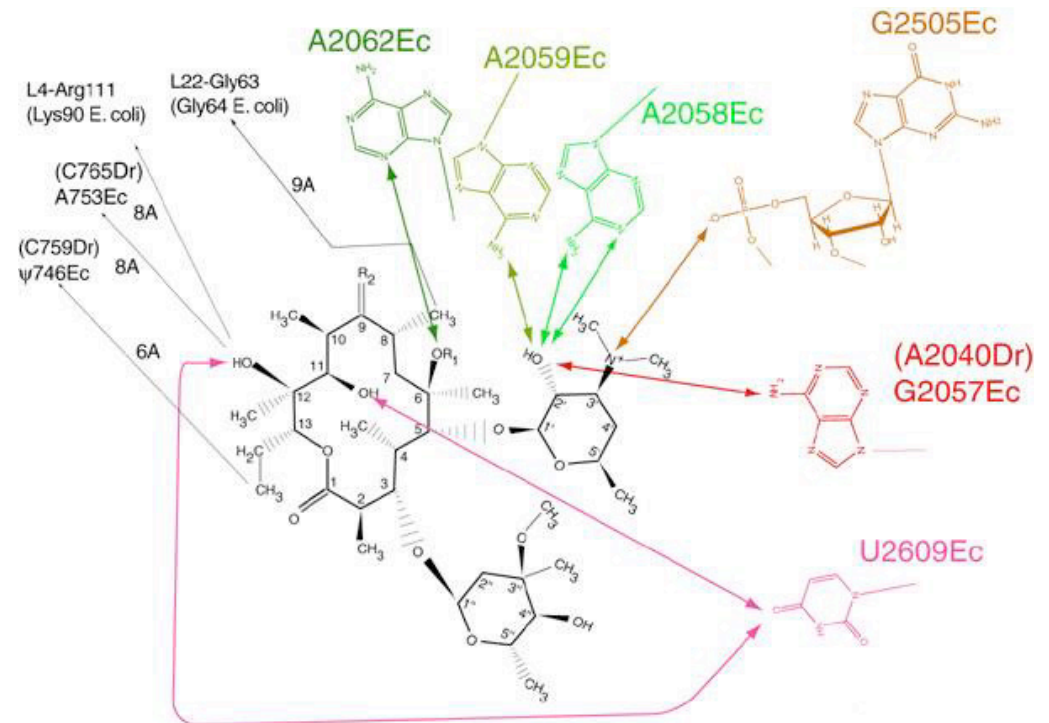
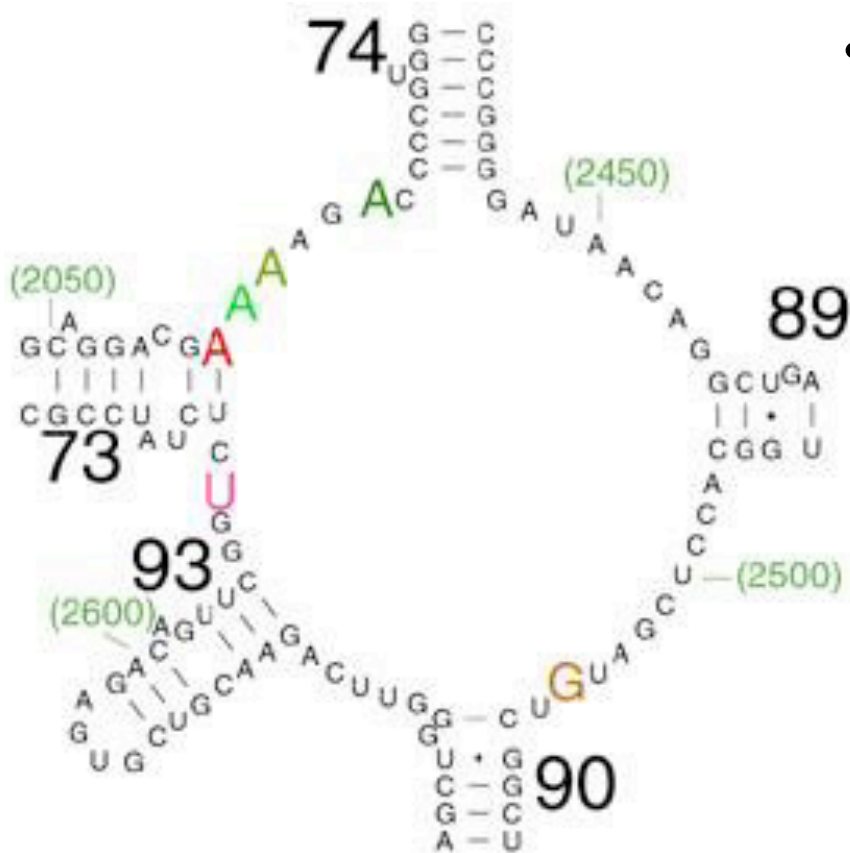
Exploiting rRNA enzymatic activity

- *Erythromycin binds directly to the 50S ribosome*
 - Interacts exclusively with the 23S rRNA!
 - No contacts between drug and any ribosomal protein
 - Bind in the peptidyl transferase cavity
 - Interferes with nascent peptide channeling from the PT cavity
 - Specific rRNA-erythromycin atomic level interactions stabilize the complex
 - Inhibits protein synthesis



Exploiting rRNA enzymatic activity

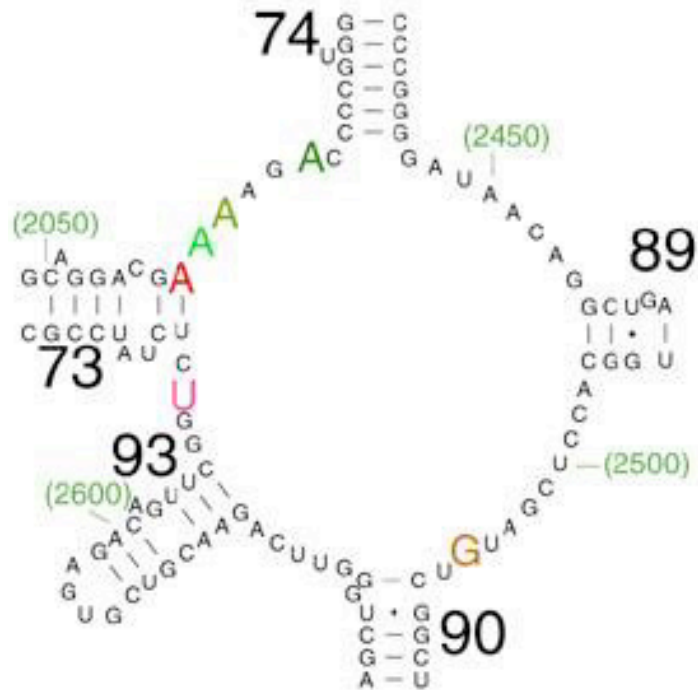
- Specific atomic level interactions underlie erythromycin interaction with the rRNA



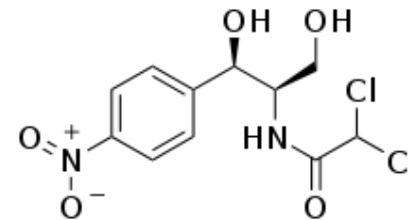
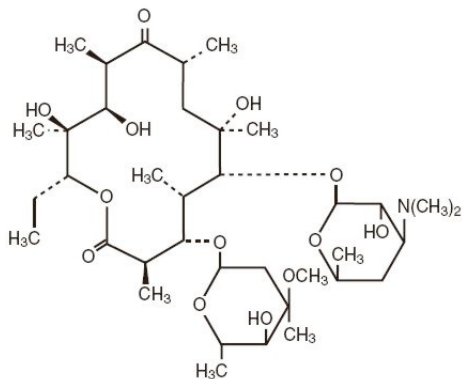
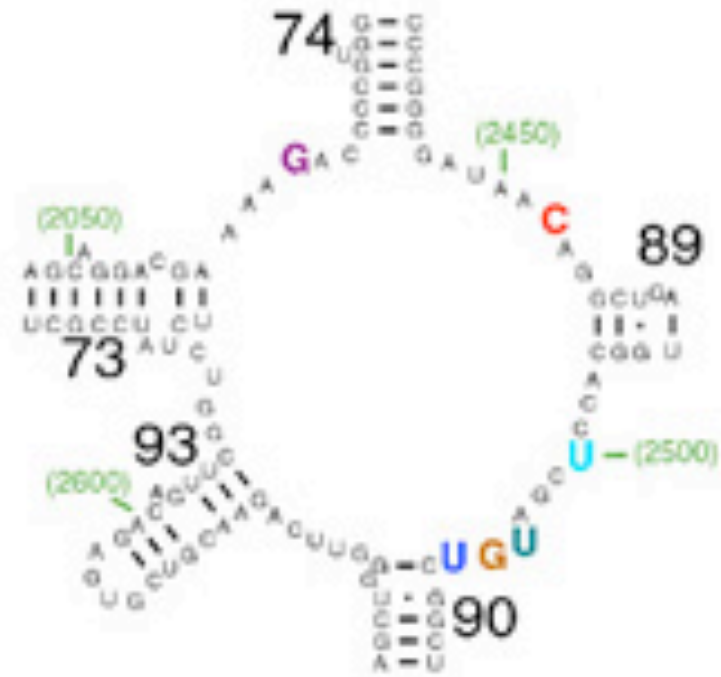
Erythromycin	R ₁ = H;	R ₂ =O
Clarithromycin	R ₁ = CH ₃ ;	R ₂ =O
Roxithromycin	R ₁ = H;	R ₂ = N-O-CH ₂ -O-CH ₂ -CH ₂ -O-CH ₃

Structurally distinct antibiotics can bind to the same rRNA

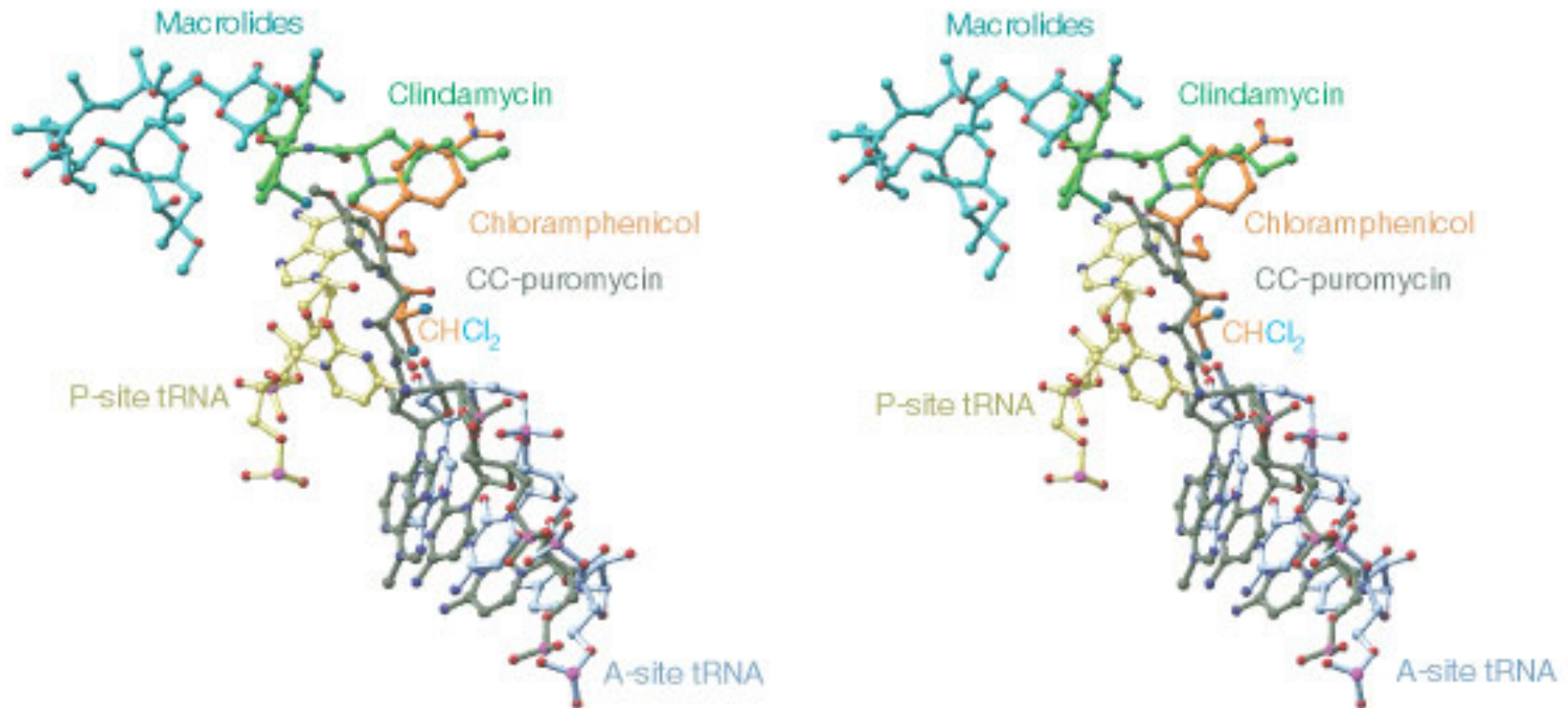
Erythromycin contacts



Chloramphenicol contacts



Structurally distinct antibiotics can bind to the same rRNA



- Different compounds can interact with a single RNA target
 - *Overlapping or distinct molecular contacts with the RNA*
- What are some potential resulting consequences when thinking of RNA as a binding reagent?

Now what about tRNA?

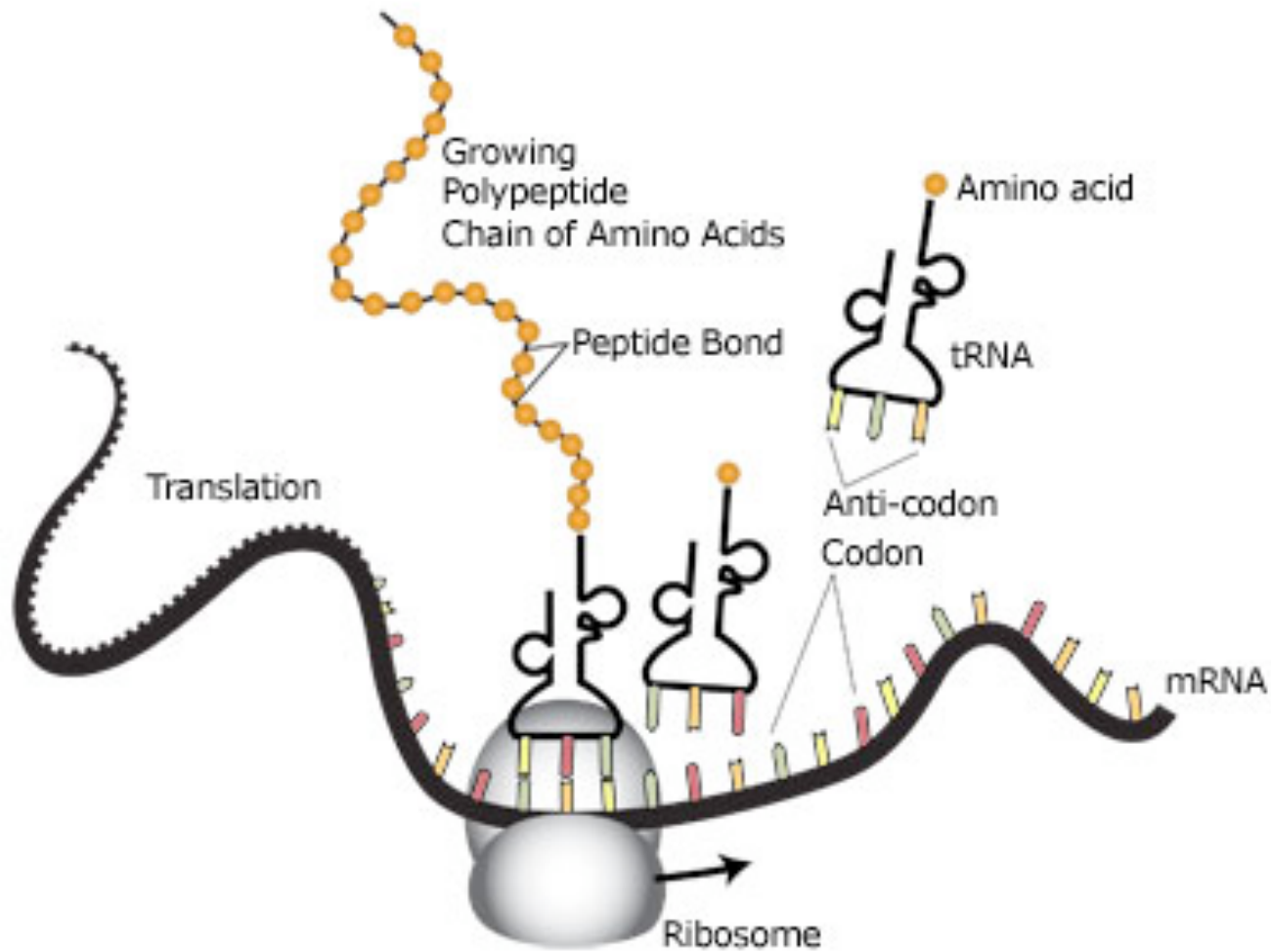
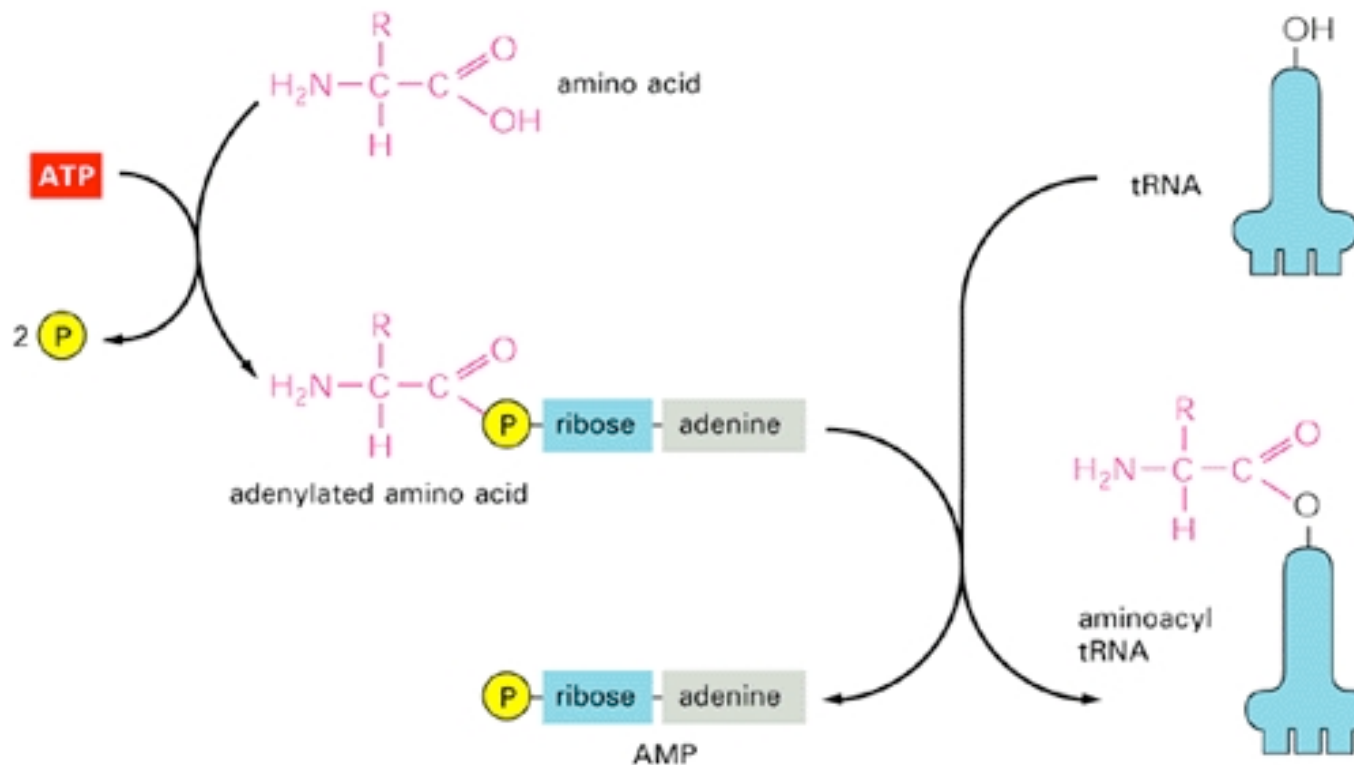


Image adapted from: National Human Genome Research Institute.

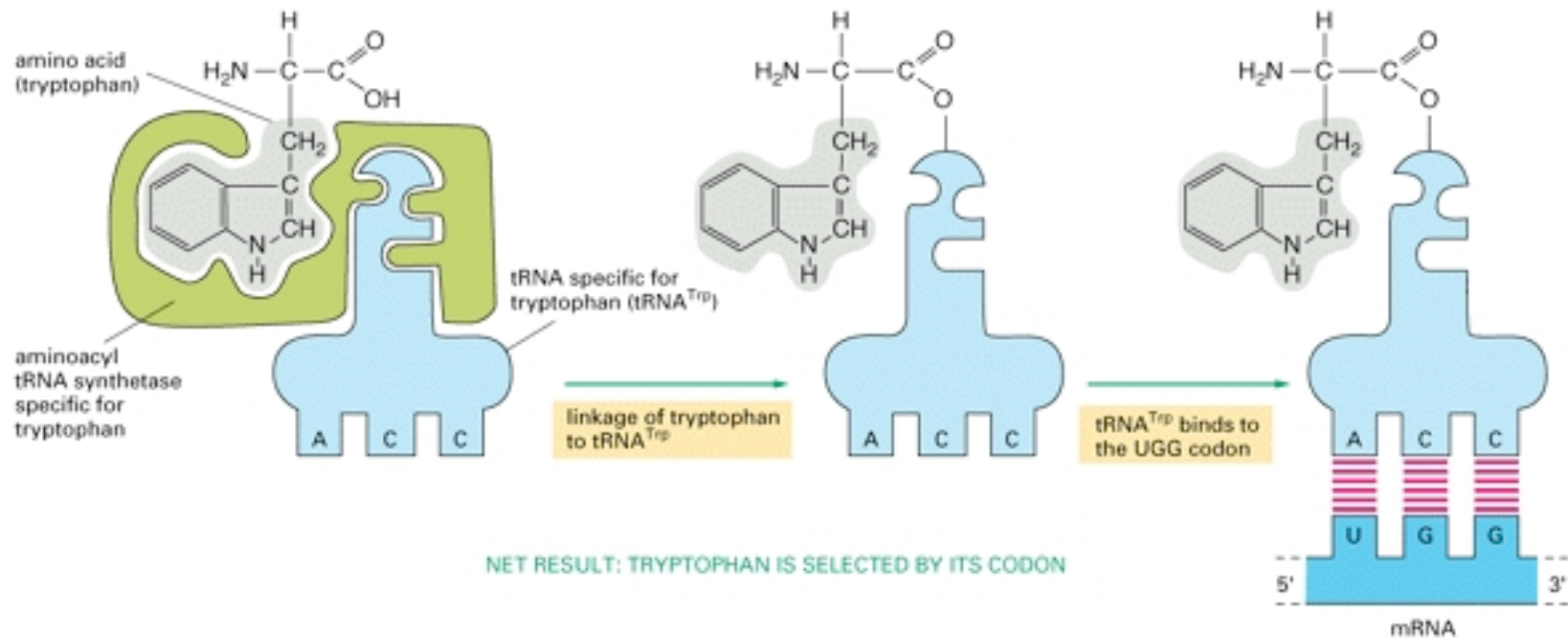
tRNA structure and function

- Each tRNA must be “charged” with its cognate amino acid
- Reaction carried out enzymatically
 - Aminoacyl tRNA synthetase (aaRS)



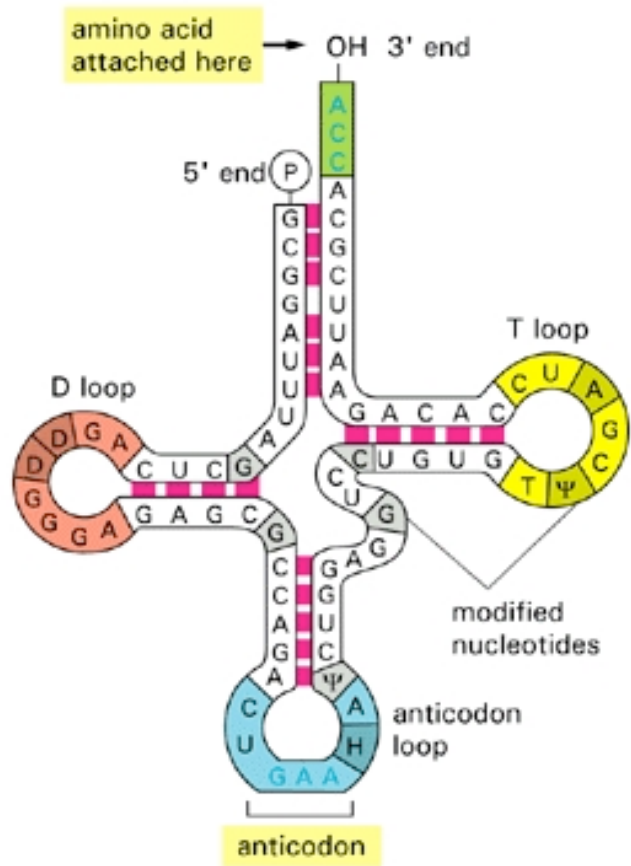
tRNA structure and function

- Specificity of aminoacylation reaction
 - Charging a tRNA with the right amino acid
 - *How might this be achieved?*
- ***Each tRNA is recognized by a specific aminoacyl tRNA synthetase***

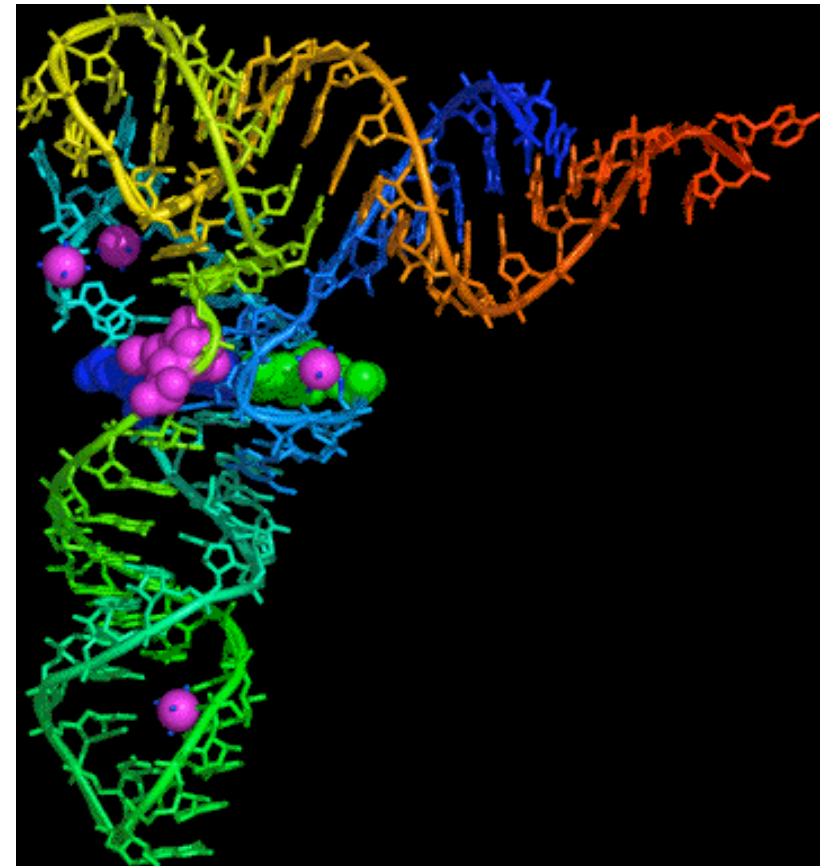


tRNA structure and function

- How is this possible?

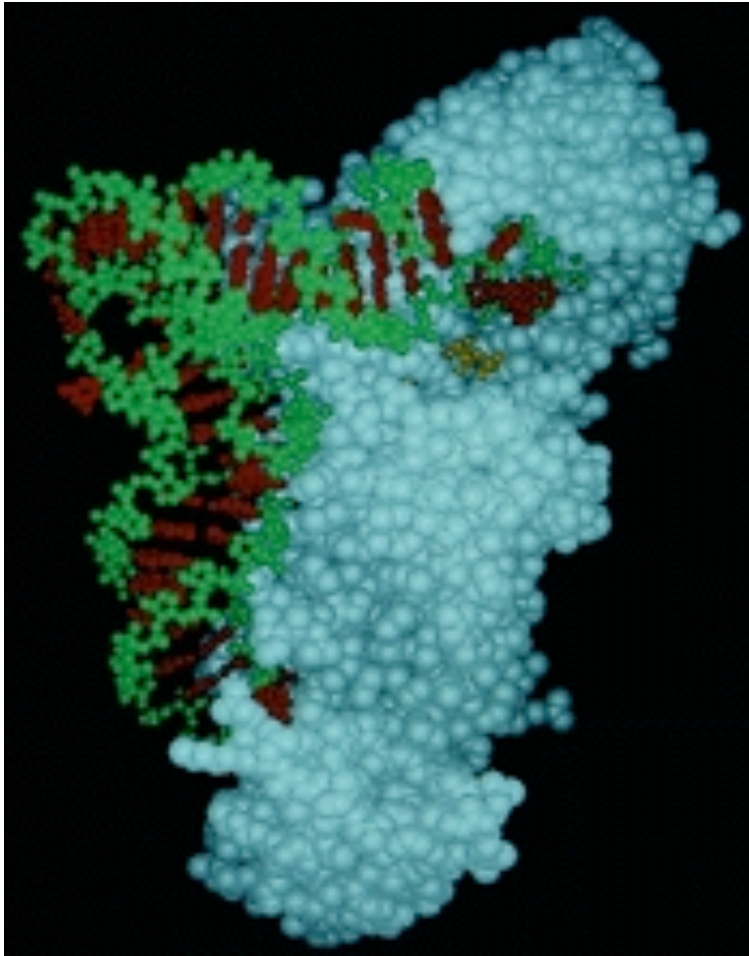


2D tRNA view



3D tRNA view

tRNA structure and function

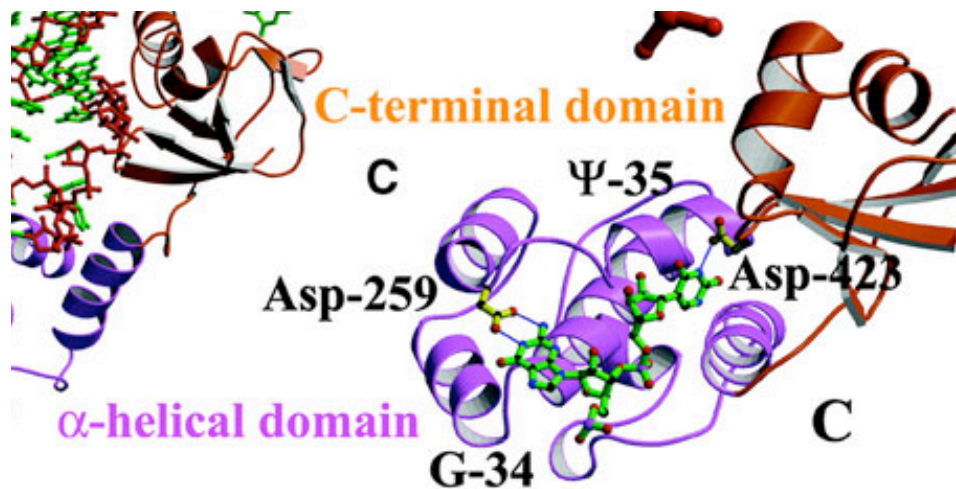
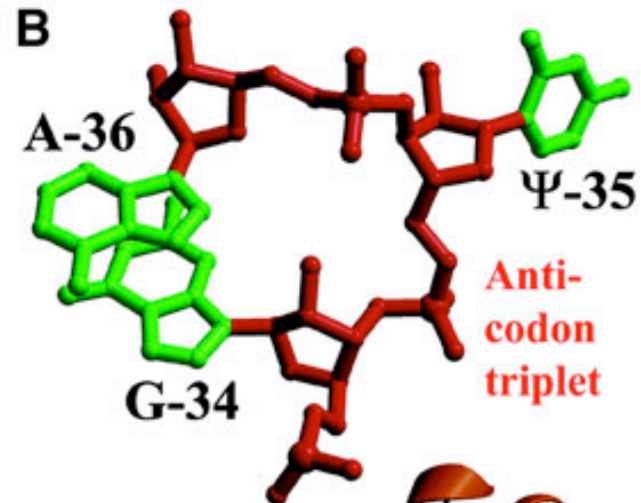
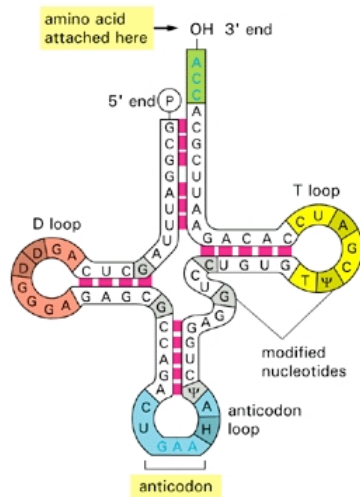


**Aminoacyl tRNA synthetase
in complex with tRNA**

- Aminoacyl tRNA synthetases very specifically recognize the 3D structure adopted by their cognate tRNA

– *How is this achieved?*

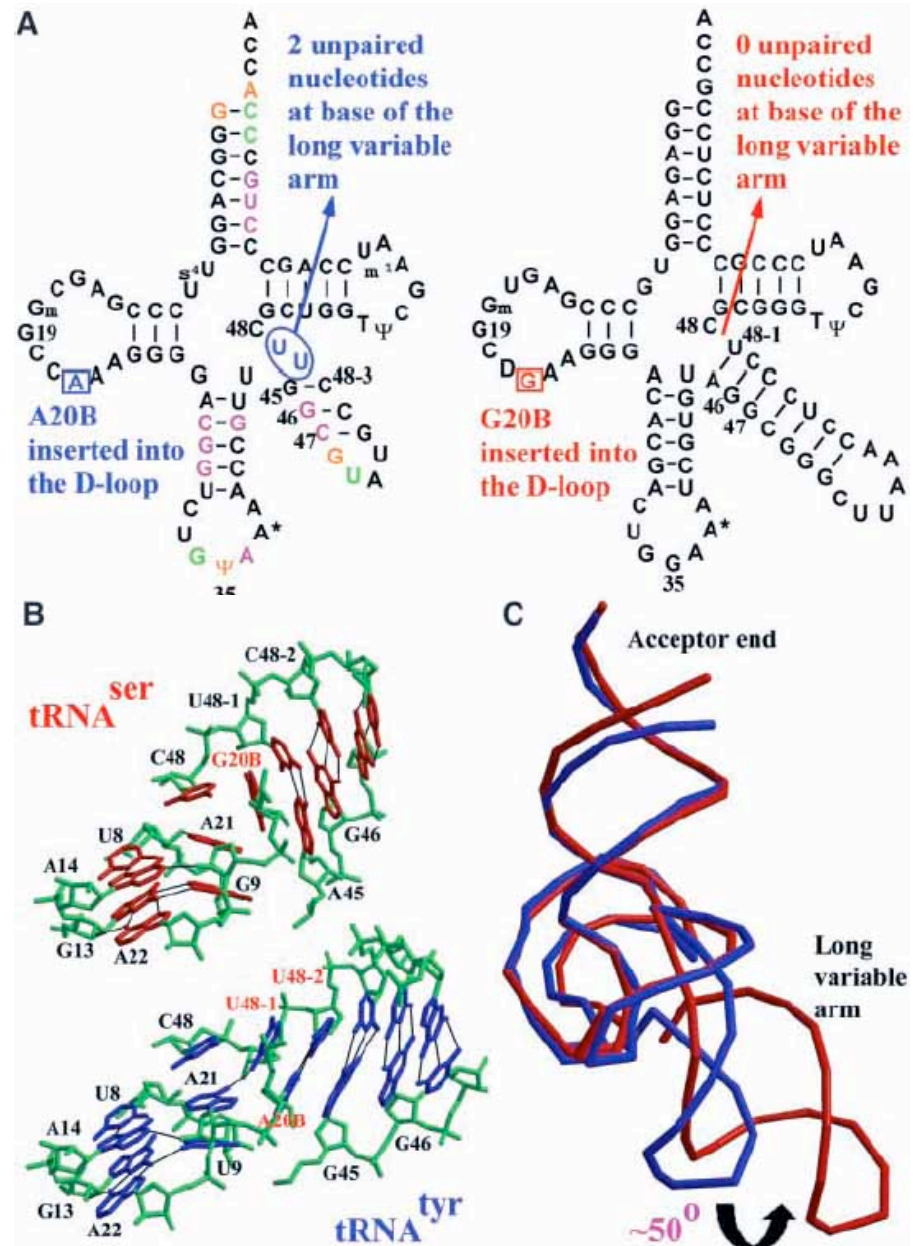
tRNA structure and function



- Specific tRNA recognition occurs due to atomic level interactions between the tRNA and enzyme
 - H-bonding
 - Electrostatic
 - Van der Waals

tRNA structure and function

- tRNAs differ in their sequence
- This translates into differences in structure
 - 2D structure
 - 3D structure
- Atomic level contacts only possible if the tRNA structure fits exactly with the aminoacyl tRNA synthetase structure*



Summary

- RNA plays very dynamic roles in biology:
 - Intricately involved in protein synthesis
 - Structural function
 - Enzymatic/catalytic functions
- These roles are facilitated by RNA's ability to:
 - Adopt very defined structures
 - Form molecular interactions with small molecules and proteins
 - Highly specific
 - High affinity
 - Facilitated by complementarity of the interacting partners
 - Atomic level interactions dictate these properties
- ***As achieved by Nature, can we take advantage of RNA's plasticity to derive new functions?***

Module Workflow

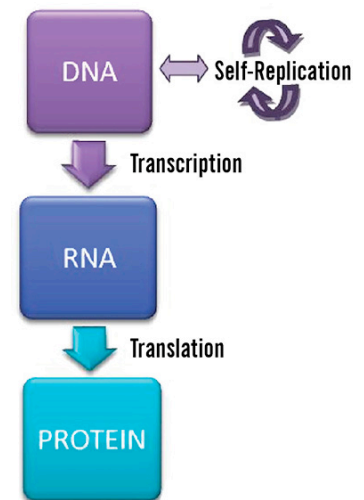
Phase I Objective

Reconstitute an RNA SELEX library

- **Skills**

1. PCR to generate DNA library template
2. RNA synthesis (*in vitro* transcription)
 - RNA handling precautions
3. Nucleic acid purification methods

Central Dogma



BioRad Thermal Cycler

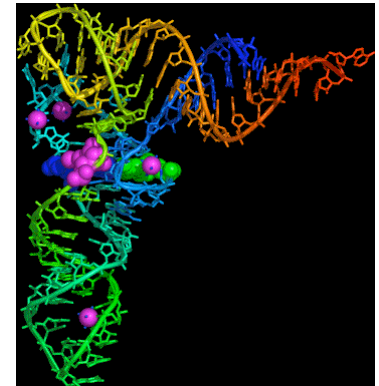
Module Workflow

Phase II Objective

Subject RNA library to selection pressure

- **Skills**

1. RNA refolding method
 - Restoring proper 3D RNA structure for appropriate function
2. Affinity based library enrichment
 - Controlling stringency during selection
3. RT-PCR: converting RNA into DNA
 - Expanding your post-selection library
 - Enriched for target aptamers (hopefully)



Module Workflow

Phase III Objective

Analyzing the success of your aptamer selection process

- **Skills**

1. UV-visible spectroscopy
 - Take advantage of unique heme spectral characteristics

2. Data analysis
 - Post-acquisition spectral analysis
 - Pooled lab data (hopefully) to understand how selection stringency impacts enrichment efficiency

