Module I:

Introduction

20.109 Lecture 1

9 February, 2012

Module Overview

- **Introduction to**:
  - Fundamental concepts and techniques in molecular biology
  - Appreciating nucleic acids (***RNA*** in particular) as more than just information storage/transfer molecules
    - Structural
    - Catalysts
  - A powerful and accessible strategy (***SELEX***) for identifying nucleic acids (***Aptamers***) with desirable properties
    - Binding to a defined target
    - Catalysts

Module Objectives

- **Lectures**:
  - Conceptual and practical considerations for successfully selecting nucleic acid ***aptamers*** with desired properties [***SELEX***]
  - Become comfortable with nucleic acid [DNA and **RNA**] libraries
    - Design
    - Manipulation
    - Characterization
  - Broadly consider the practical applications of ***aptamers***
    - Cell biology [e.g. post-transcriptional regulation]
    - Technology [e.g. biosensors]
    - Therapeutics [e.g. anti-clotting, macular degeneration]

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 (*In vitro* 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

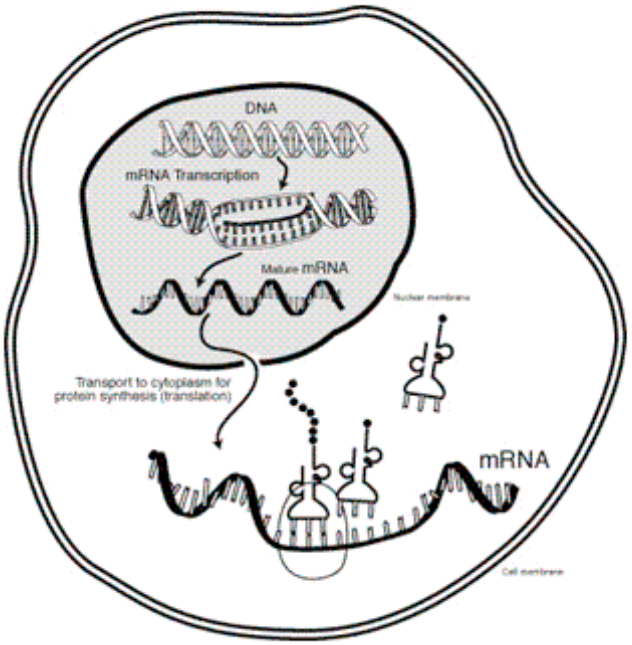
Today's Objectives

- Provide a context for appreciating RNA as a macromolecule capable of specific interactions
  - Small molecules
  - Proteins

- Appreciate that principles derived from our understanding of naturally occurring systems inspire aptamer development

- Understand that:
  - Atomic level interactions underlie these binding events
  - Binding reactions occur in 3-dimensional space

The Central Dogma



DNA

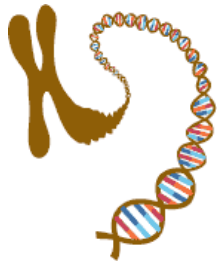
transcription

RNA

translation

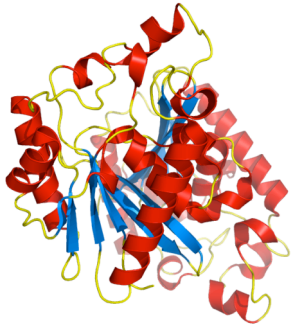
Protein

Information storage
- Double stranded
- Helical structure
- Encodes genes



What is RNA good for?

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



RNA has diverse functions, too!

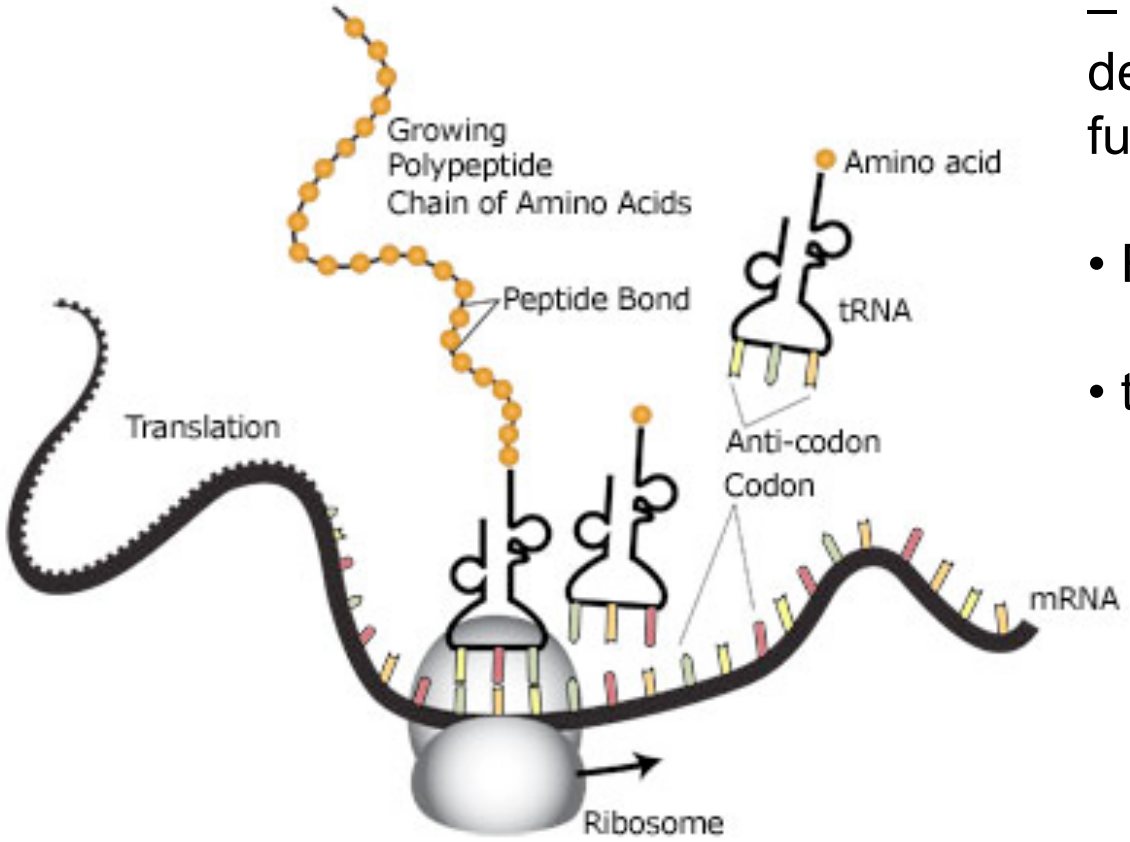


Image adapted from: National Human Genome Research Institute.

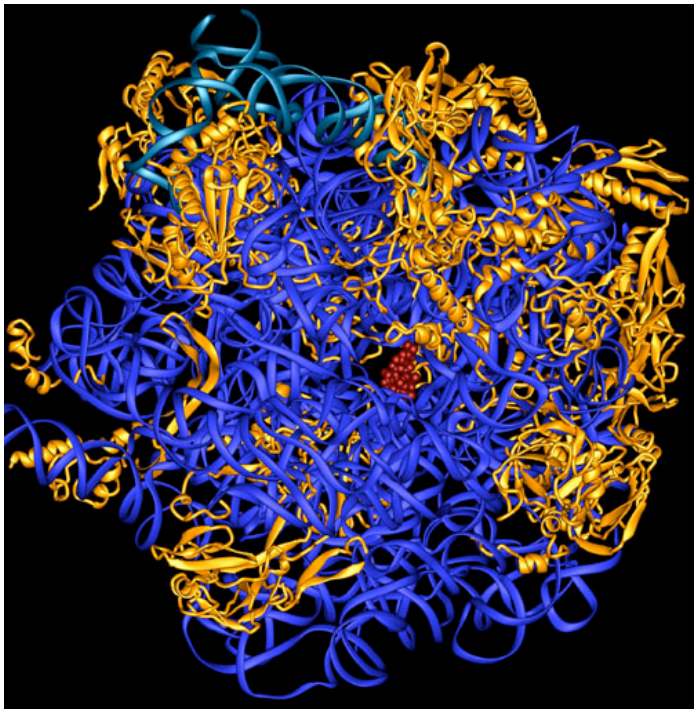
How does mRNA decoding take place during translation?

– Two particularly critical players depend on RNA for proper function

- Ribosome
- tRNA

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)

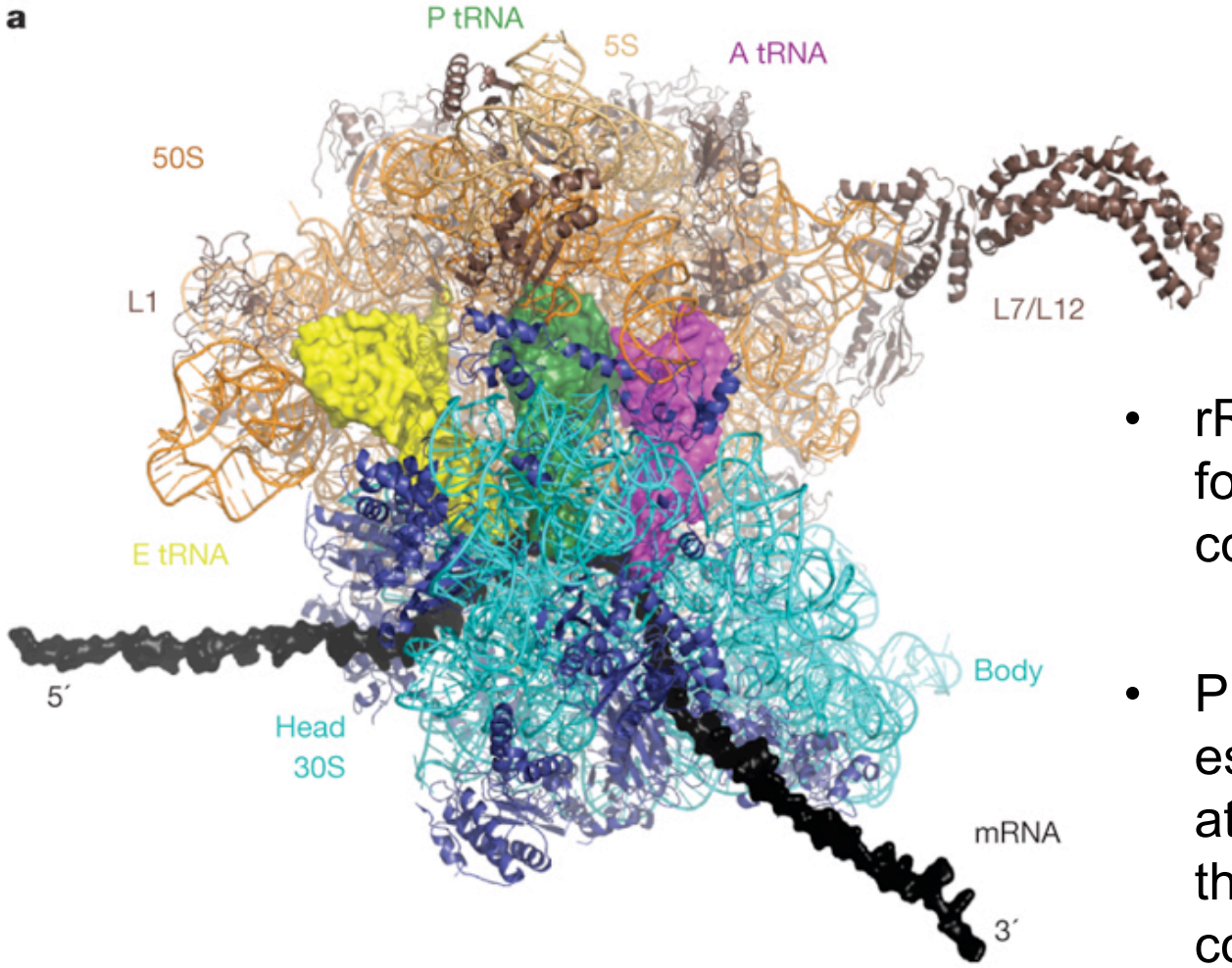


50S ribosomal subunit (*D. radiodurans*)
Blue = ribosomal RNA (rRNA)
Gold = protein

F. Schlunzen *et al*, ***Nature*** 413, 814-821 (2001)

Ribosomal RNA functions

- *Structural*
  - Organize the protein components in the ribosome

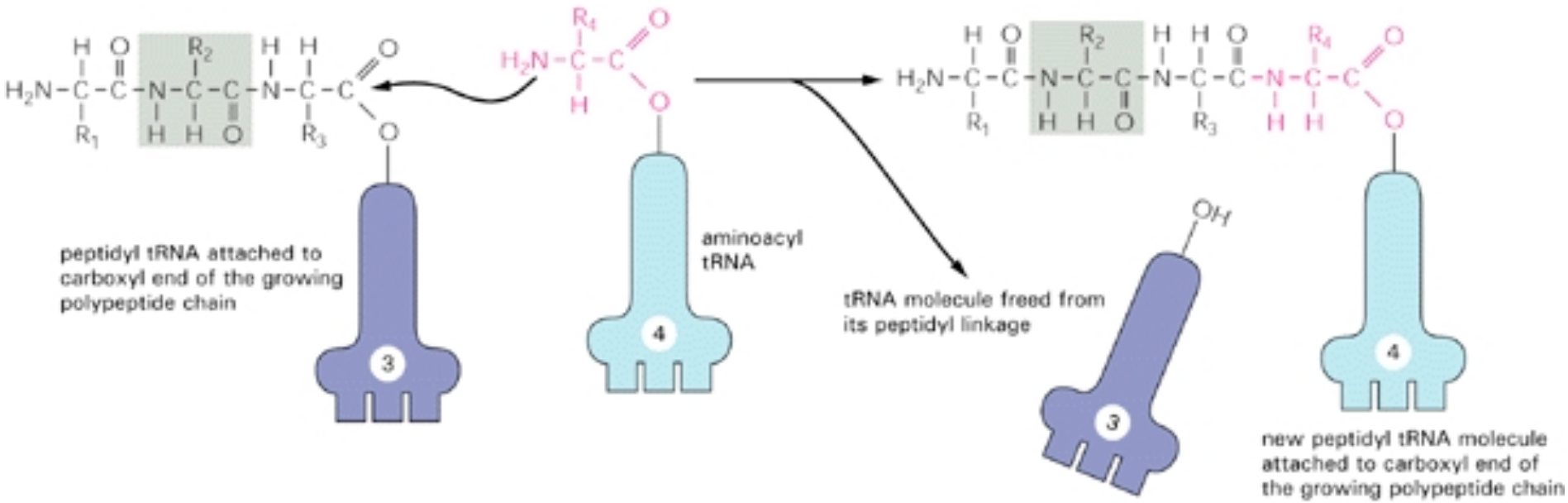


- rRNA serves as a scaffold for the associated protein components
- Proper assembly requires establishing very specific atomic contacts between the RNA and protein components

F. Schlunzen *et al*, ***Nature*** 413, 814-821 (2001)

Ribosomal RNA functions

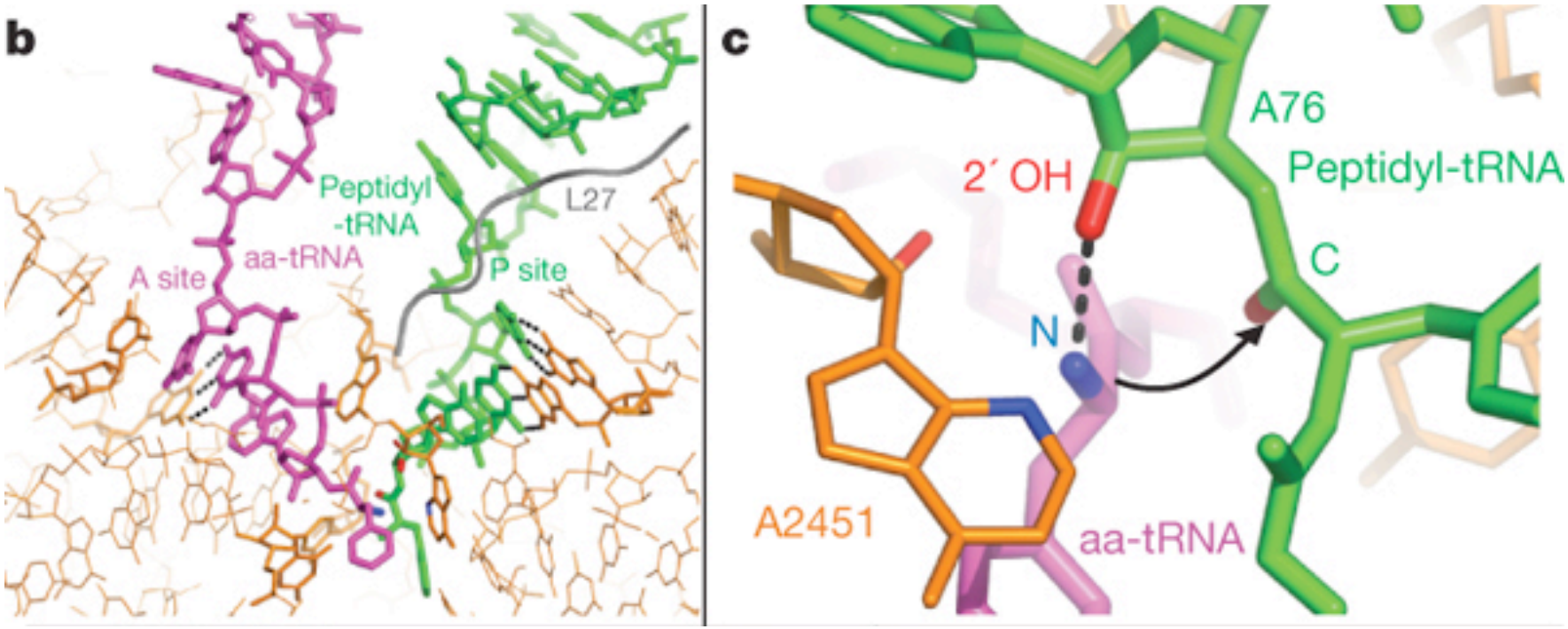
- *Enzymatic*
  - 23S rRNA involved in catalyzes the peptidyl transfer reaction step during translation
    - Binding site for the aminoacyl tRNA is located in the 23S rRNA
  - Rate enhancement over non-enzymatic reaction: ~ 10^5!



Bruce Alberts *et al*, Molecular Biology of the Cell (NCBI Bookshelf)

Ribosomal RNA functions

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



Schmeing & Ramakrishnan, **Nature**, 461, 1234-1242 (2009)

rRNA enzymatic activity as a drug target

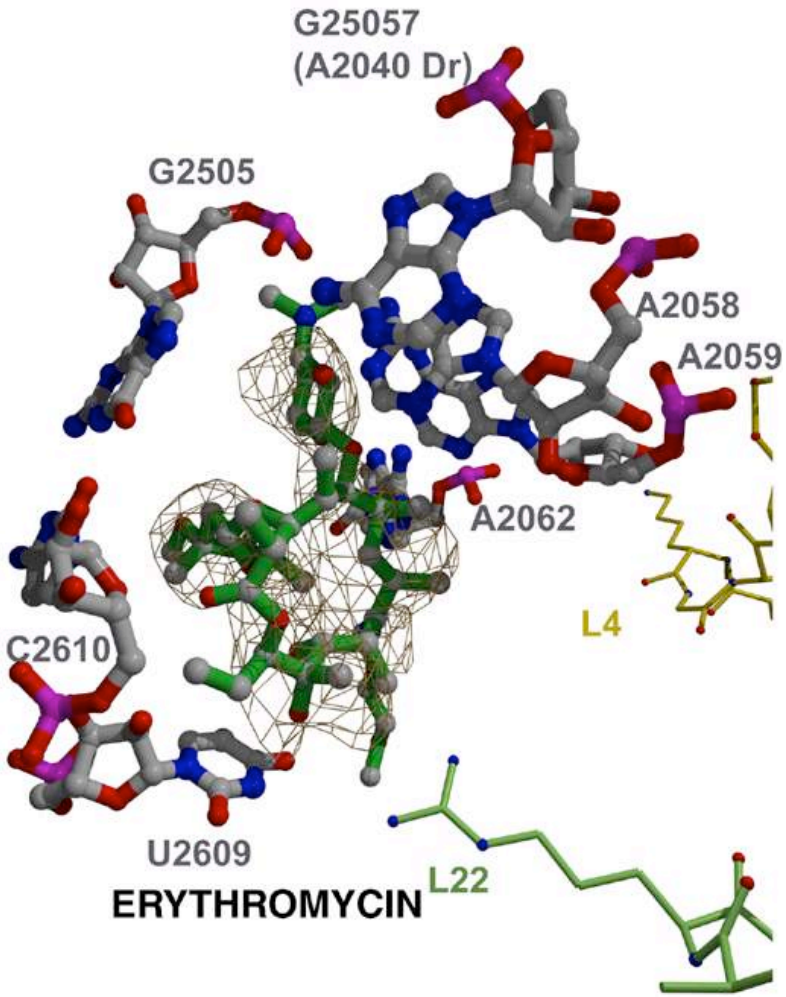
- *Antibiotics*
  - Used to treat microbial pathogens
  - Several different classes
  - Target different cellular processes
  - Selectively inhibit pathogen but not host pathways

  - 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 <u>exclusively</u> 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



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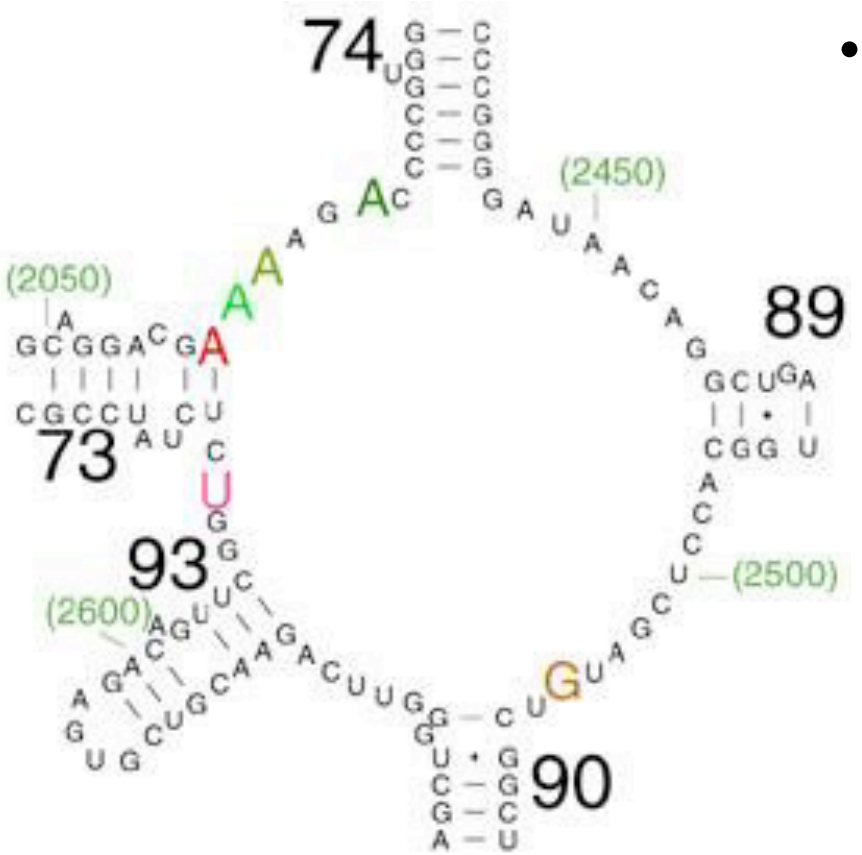
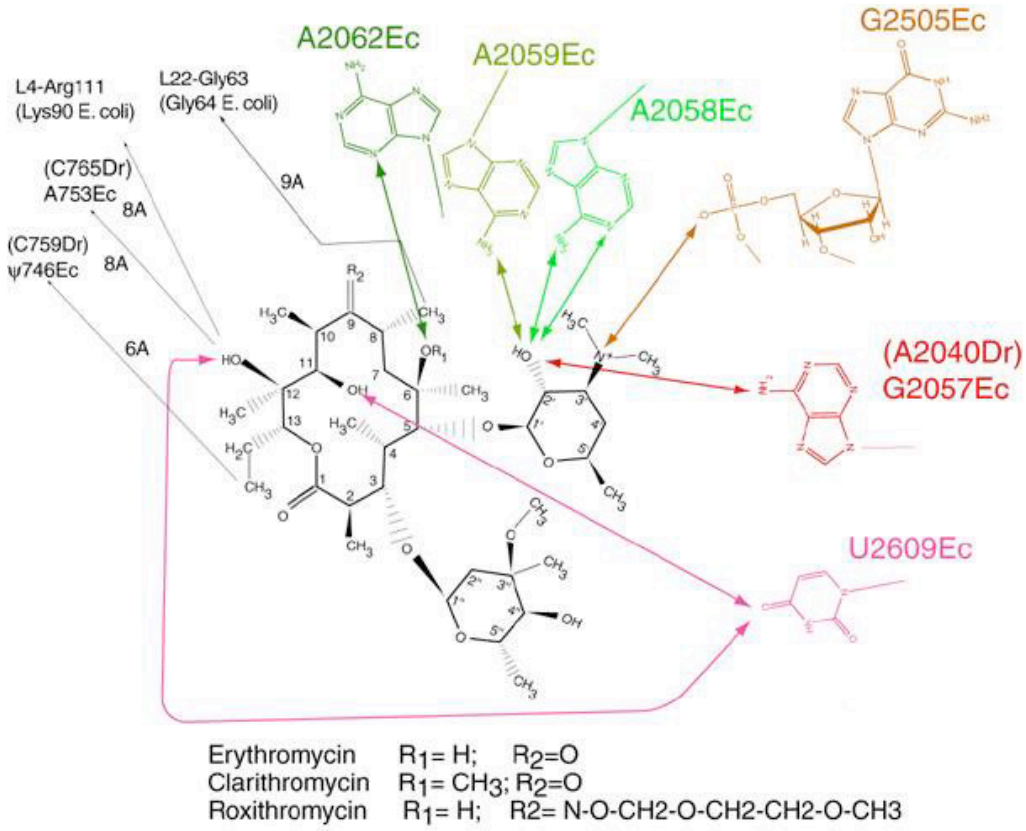
Exploiting rRNA enzymatic activity



- Specific atomic level interactions underlie erythromycin interaction with the rRNA



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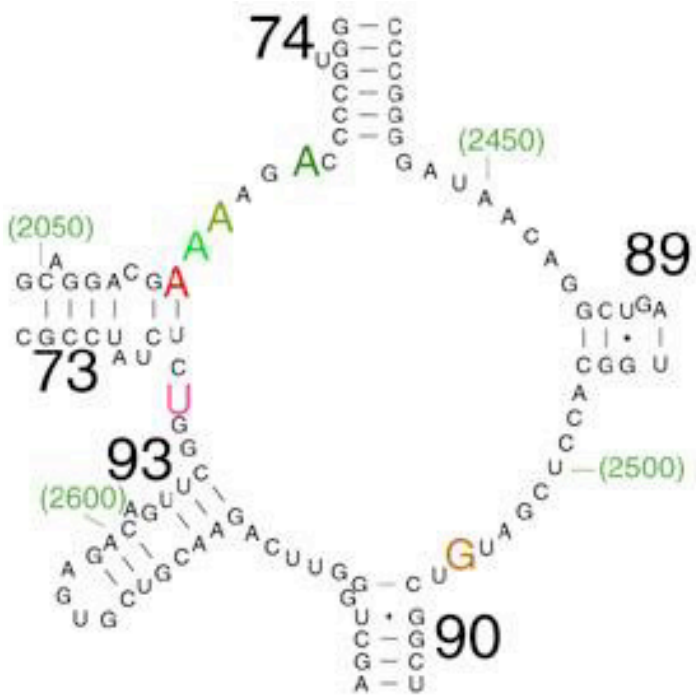
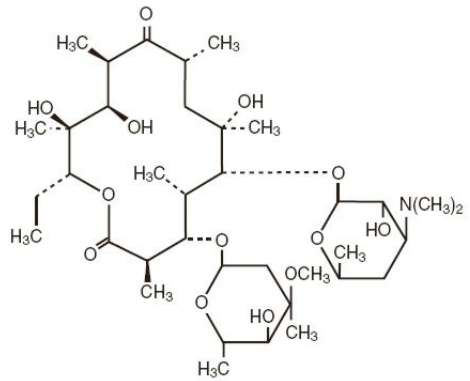
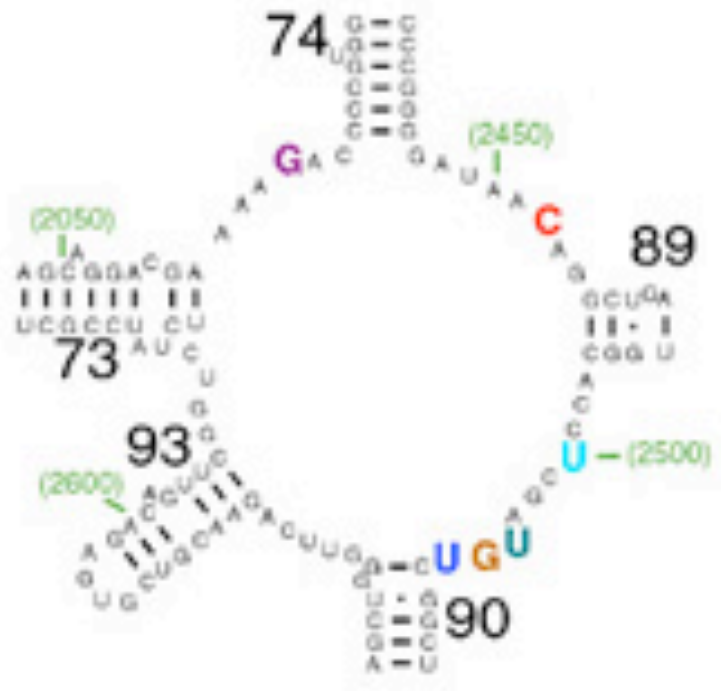
Structurally distinct antibiotics can bind to the same rRNA

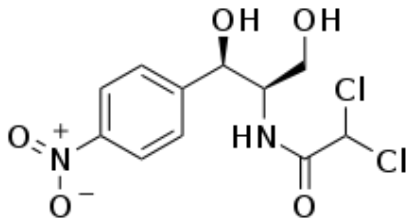
Erythromycin *contacts*





Chloramphenicol *contacts*





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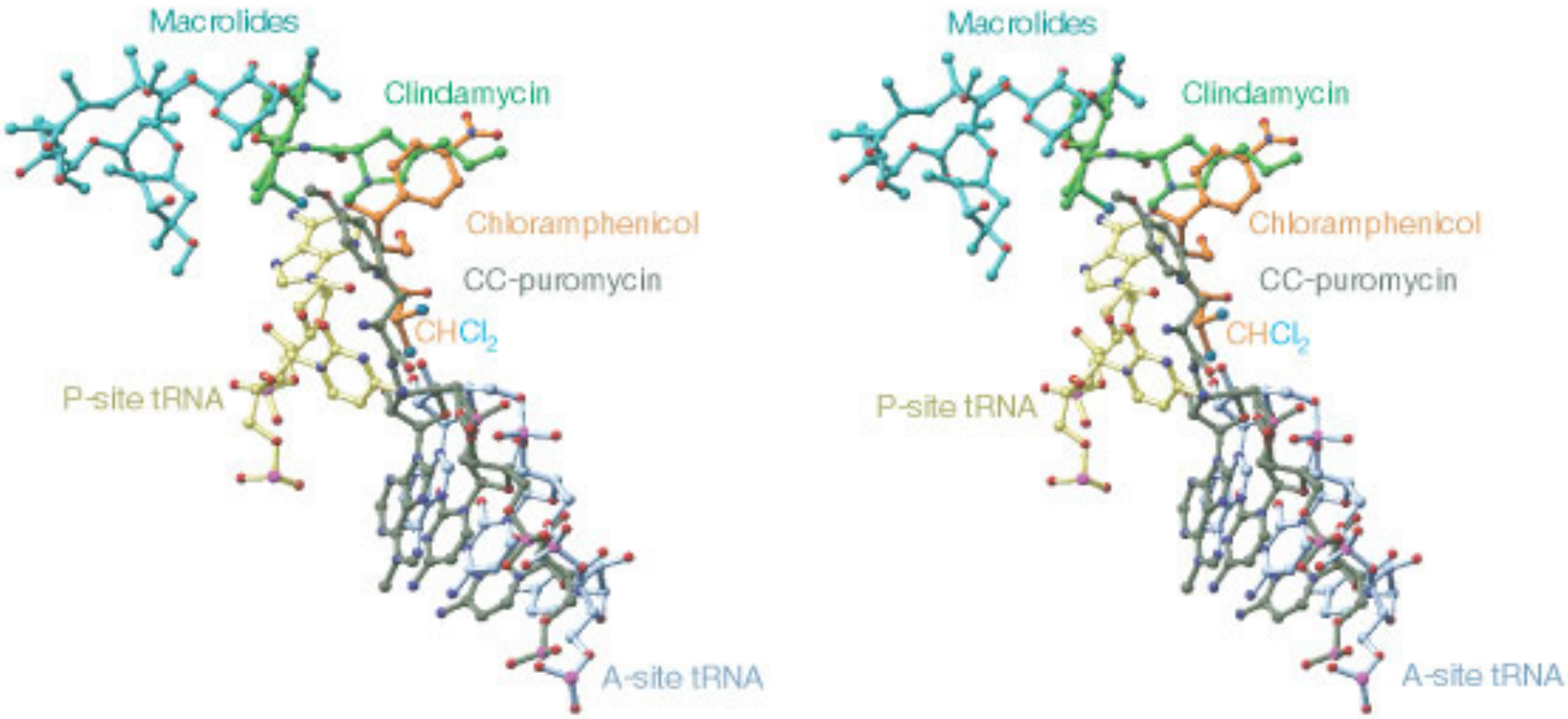
Structurally distinct antibiotics can bind to the same rRNA



- 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?

F. Schlunzen *et al*, ***Nature*** 413, 814-821 (2001)

Now what about tRNA?

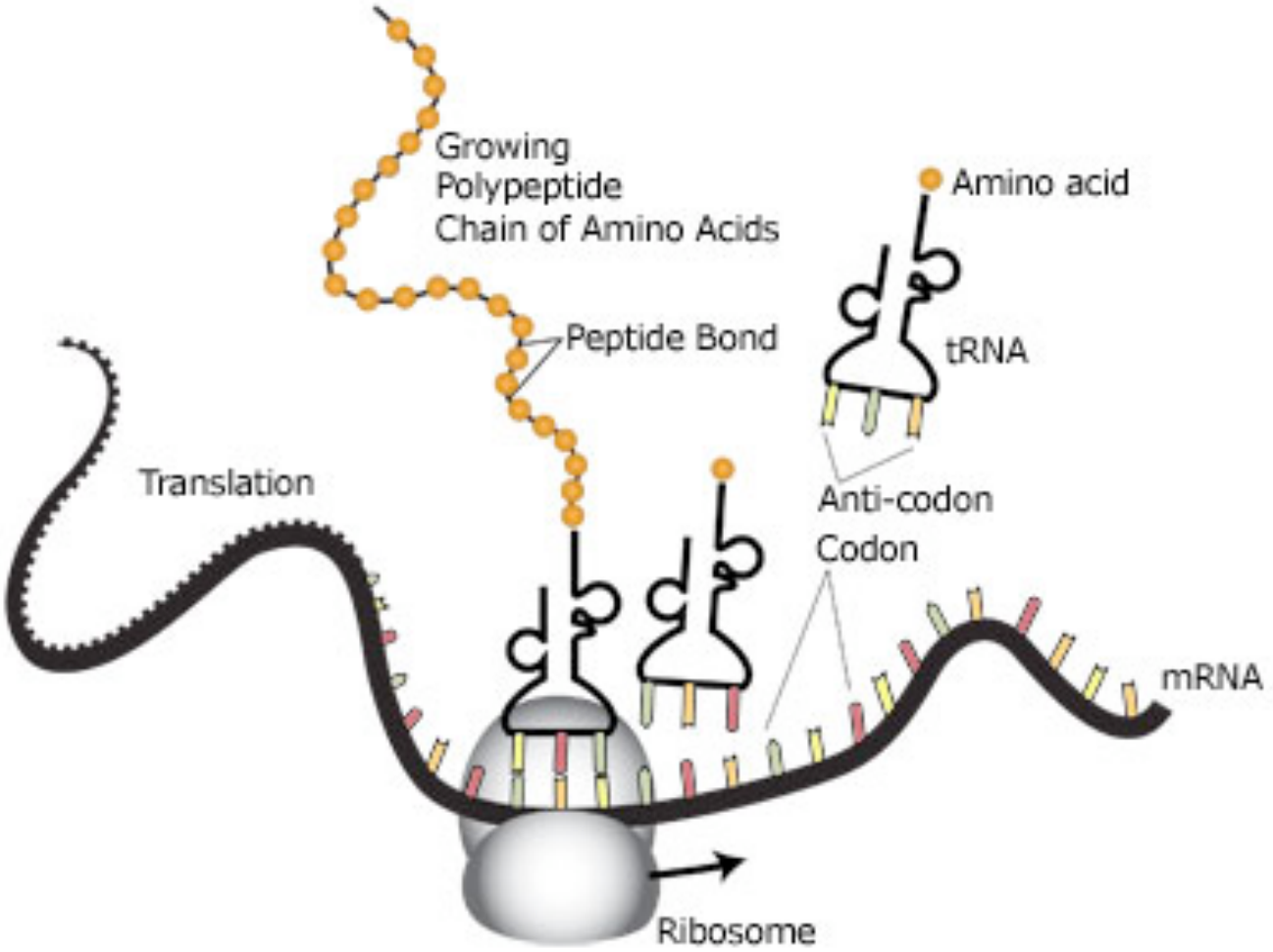
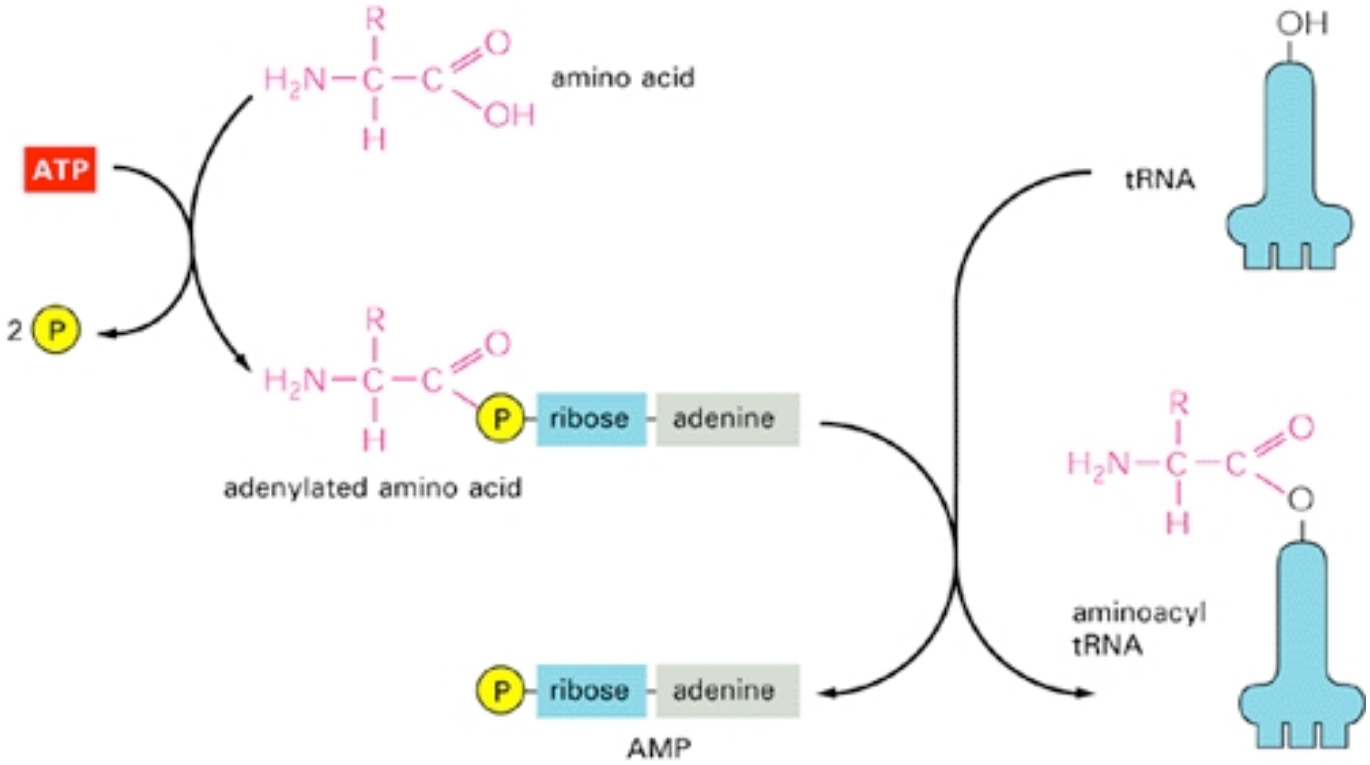


Image adapted from: National Human Genome Research Institute.

tRNA structure and function

- Each tRNA must be “charged” with its cognate amino acid

- Reaction carried out enzymatically
  - Aminoacyl tRNA synthetase (aaRS)

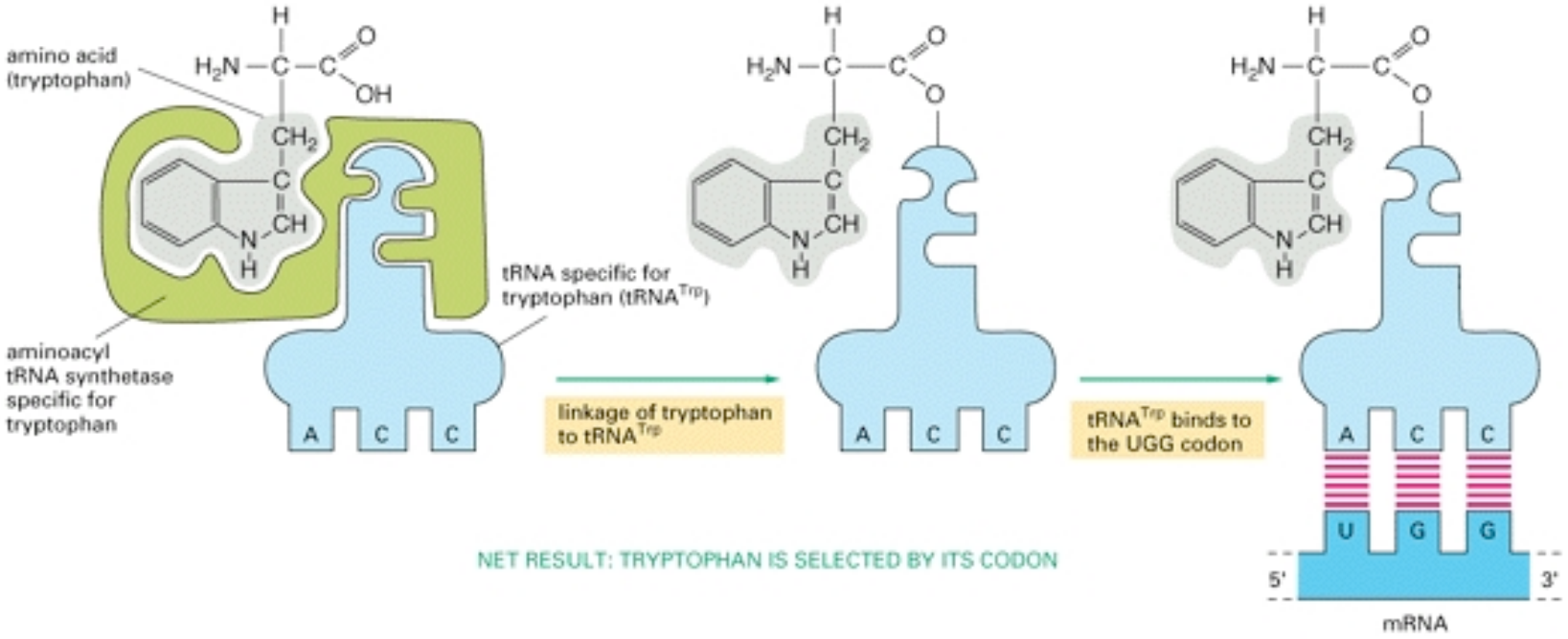


Bruce Alberts *et al*, Molecular Biology of the Cell (NCBI Bookshelf)

tRNA structure and function

- Specificity of aminoacylation reaction
  - Charging a tRNA with the right amino acid
  - *How might this be achieved?*

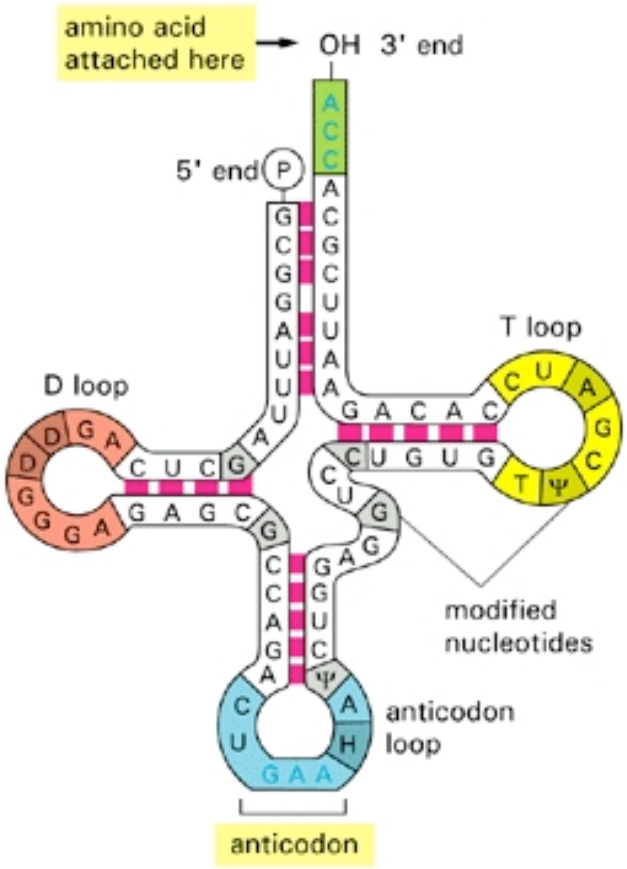
    - ***Each tRNA is recognized by a specific aminoacyl tRNA synthetase***



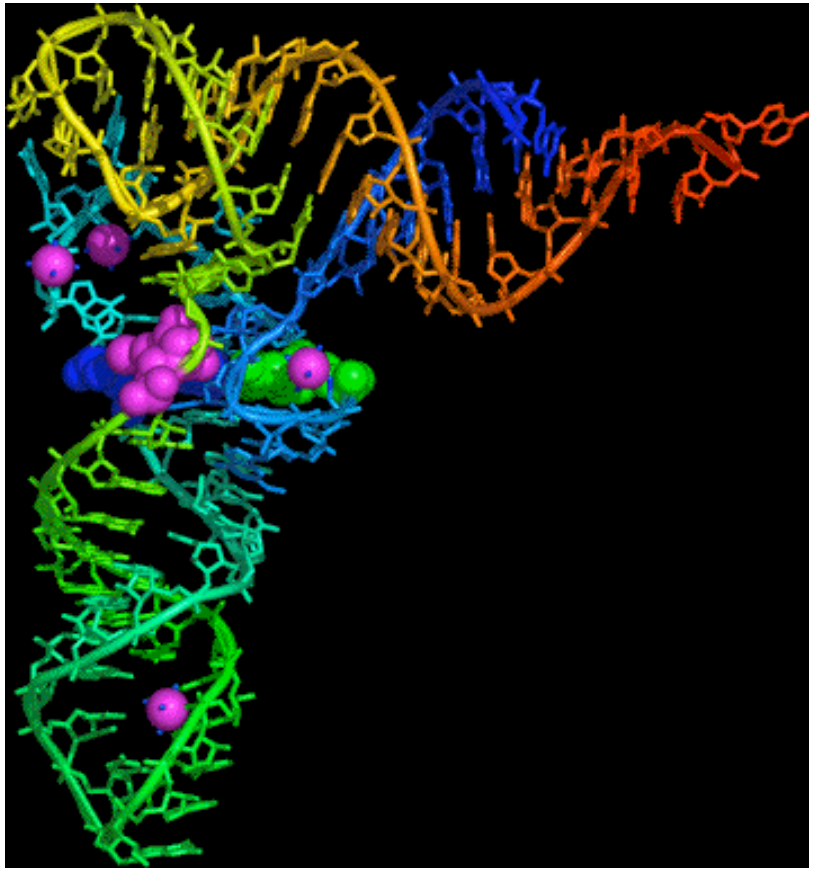
Bruce Alberts *et al*, Molecular Biology of the Cell (NCBI Bookshelf)

tRNA structure and function

- How is this possible?



2D tRNA view



3D tRNA view

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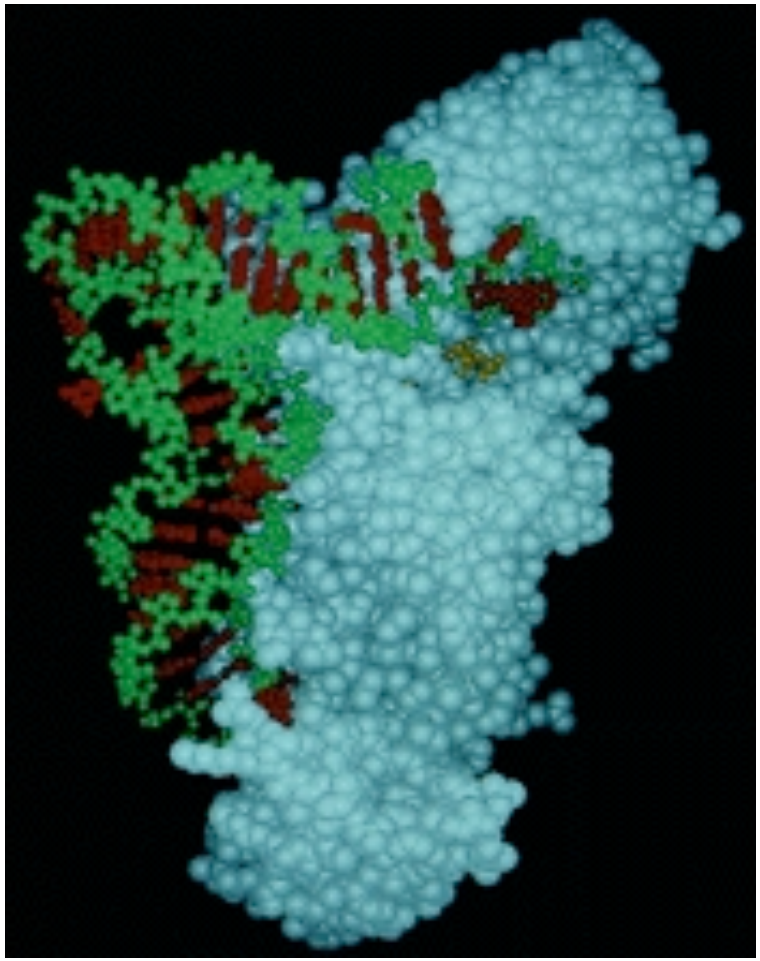
tRNA structure and function



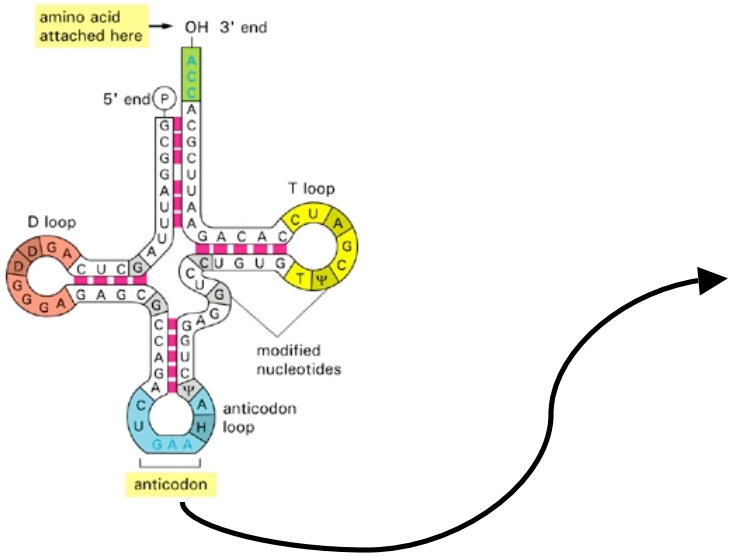
Aminoacyl tRNA synthetase in complex with tRNA

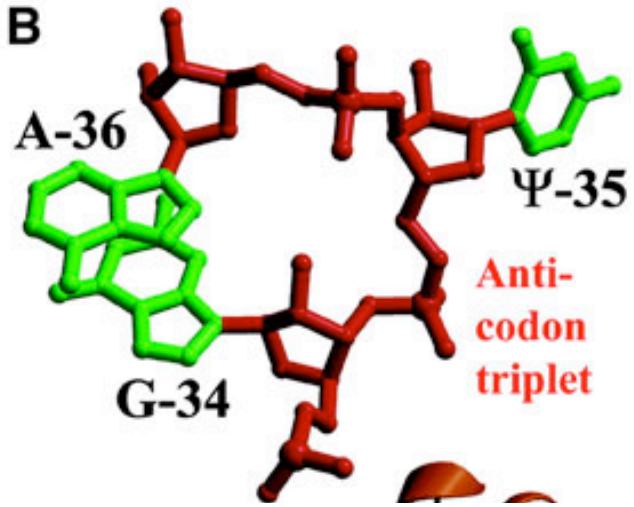
- Aminoacyl tRNA synthetases very specifically recognize the 3D structure adopted by their cognate tRNA

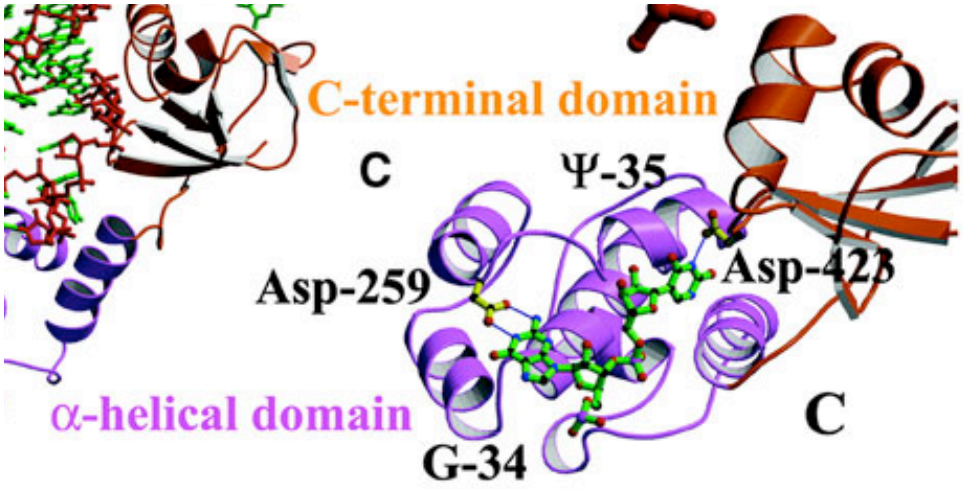
  – *How is this achieved?*

Bruce Alberts *et al*, Molecular Biology of the Cell (NCBI Bookshelf)

tRNA structure and function





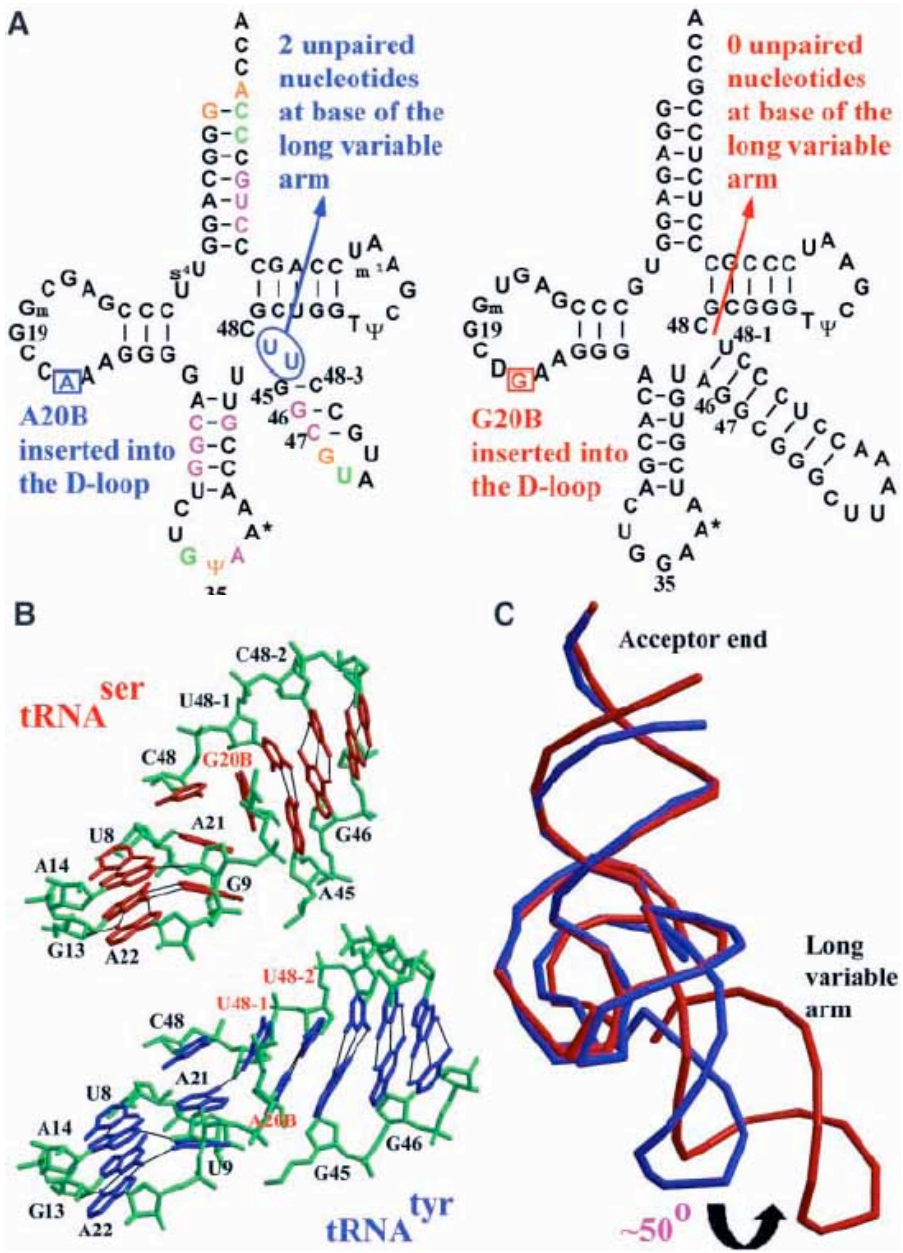


- Specific tRNA recognition occurs due to atomic level interactions between the tRNA and enzyme
  - H-bonding
  - Electrostatic
  - Van der Waals

Yaremshuk *et al*, **EMBO J**, (2002) 21:3829-3840

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*



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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

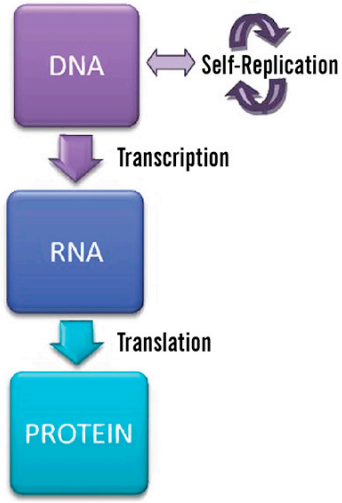
- **Skills**

  1. PCR to generate DNA library template

  2. RNA synthesis (*in vitro transcription*)
     – RNA handling precautions

  3. Nucleic acid purification methods

Central Dogma

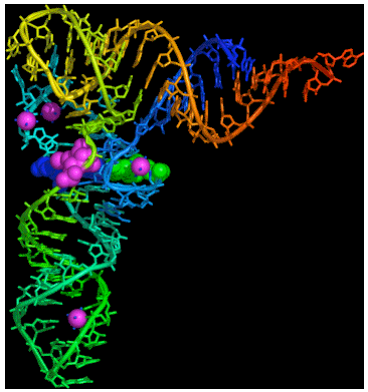


BioRad Thermal Cycler

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

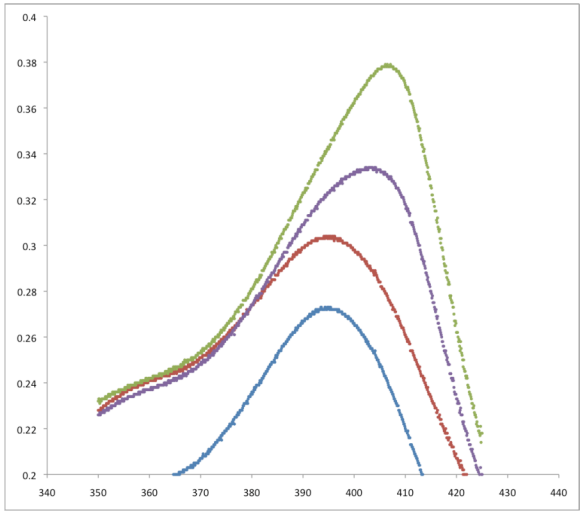
  3. RT-PCR: converting RNA into DNA
  – Expanding your post-selection library
  – Enriched for target aptamers (hopefully)

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