Lecture 2

tRNA: Structure & Function

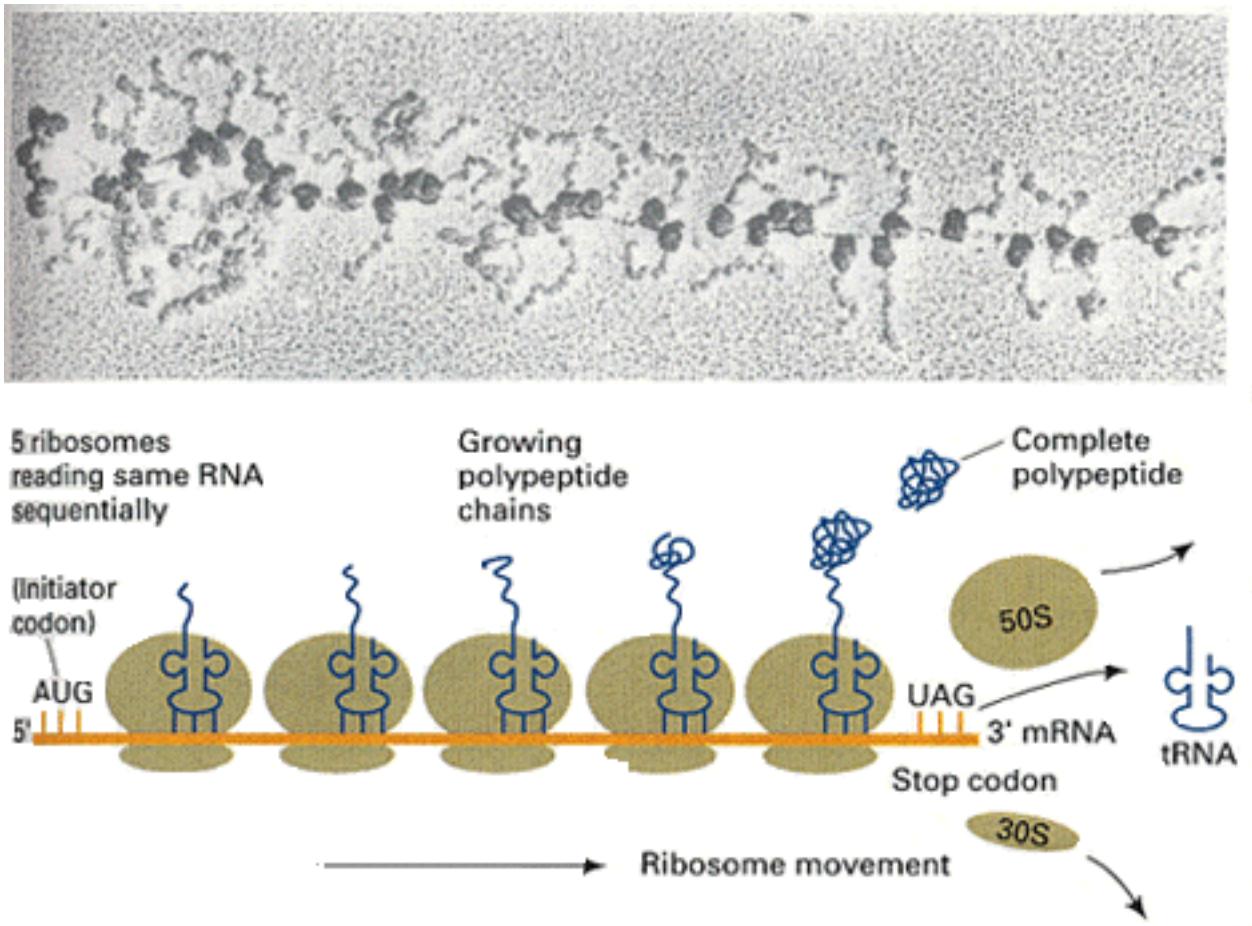
Major players in *protein synthesis*:
mRNA, tRNA and the ribosome

mRNA	**Messenger RNA**, a copy of DNA blueprint of the ***gene*** to be expressed.	**Information**
tRNA	Aminoacyl **transfer RNA**, also called ***anticodon*** or ***adaptor*** molecule. One or more tRNAs for each amino acid.	**Supply**
Ribosome	A very large complex of several rRNAs (ribosomal RNA) and many protein molecules. Total molecular weight over 2 million dalton.	**Factory**

Protein	Polypeptide chain with sequence dictated by the mRNA sequence. Also called the ***gene product***.	**Product**

Protein Synthesis

Electronmicrograph of a so-called *polysome*: one mRNA strand (faint horizontal line) with many individual ribosomes attached (dark blobs). The newly synthesized *polypeptide* chains (*proteins*) can be seen as irregularly shaped extensions from the ribosomes:



The bottom panel shows a schematic representation of the process in the upper panel.

Now let's magnify a ribosome to the size of a "Big Mac" (factor 10,000,000). At this magnification an *E. coli* bacterium would

be about 10 meters (or 30 feet) in diameter. You would be about 10,000 miles tall:

Ribosome	20 nm or 200 Å	**Big Mac** 20 cm	8 inches
tRNA	5 nm or 50 Å	5 cm	2 inches
mRNA (900 bases)	450 nm or 4,500 Å	450 cm	15 feet
Extended (*unfolded*) protein (300 amino acids)	90 nm or 900 Å	90 cm	3 feet
Globular (*folded*) protein (300 amino acids)	5 nm or 50 Å	5 cm	2 inches

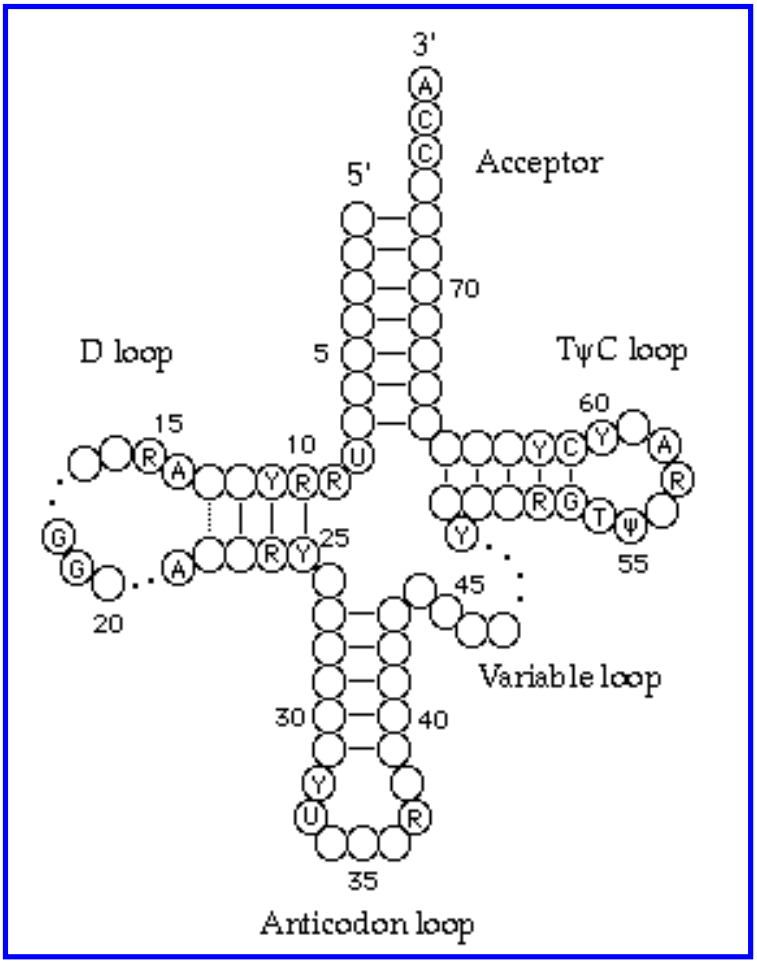
1 nm (nanometer) is 10^{-9} meters.
1 Å (angstrom) is 10^{-10} meters or 0.1 nm.

Next: tRNA Structure

Please report typos, errors etc. by EMAIL (mention the title of this page).

tRNA Structure

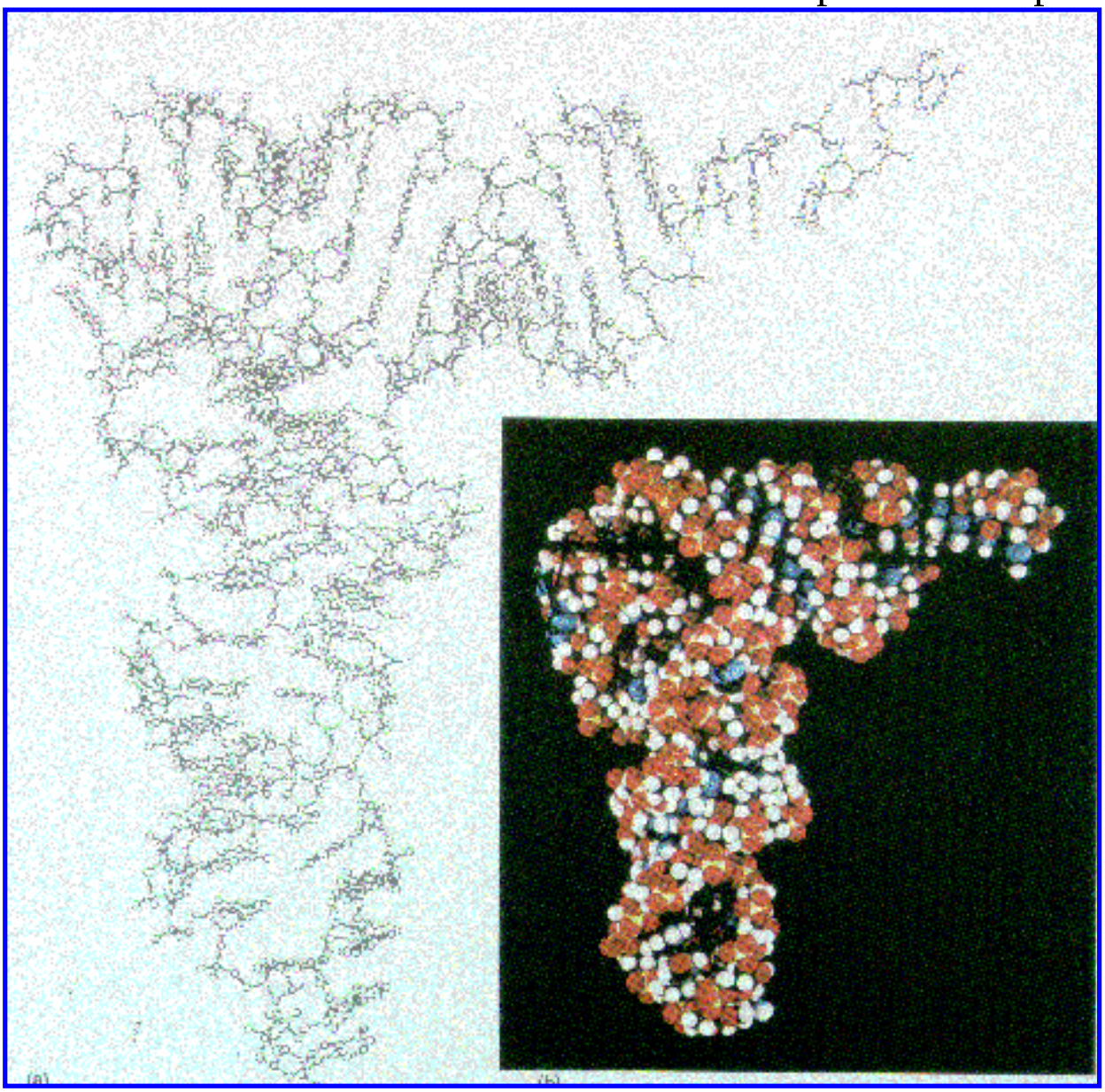
Primary & secondary structure



- All tRNAs have similar sequences of 73 to 93 nucleotides
- 3' end *always terminates* with the sequence **CCA**, with the 3' hydroxyl of the ribose of the terminal **A** being the point of covalent attachment of the amino acid
- Contain a number (7-15%) of unique/modified bases. These are post-transcriptionally modified after synthesis by RNA polymerase.
    - In particular, adenosine (**A**) in first or 5' position of the anticodon (corresponding to the third or 3' position of the codon) is always modified to inosine (**I**) which lacks the amino group on the purine ring. Inosine can base-pair with **A**, **U** or **C** and thus accounts for much of the ***degeneracy*** of the Genetic Code ("***Wobble Theory***").
- tRNAs have ***cloverleaf*** secondary structure due to four base-paired ***stems***
- The cloverleaf contains three non-base-paired ***loops***: D, anticodon, and TpsiC loop

Tertiary structure

- The tertiary structure of tRNA is best described as a compact "*L*" shape.
- The ***anticodon*** is a single-stranded loop at the bottom of the Figure which later base-pairs with the triplet codon
- The amino acid is attached to the terminal **A** on the upper right.



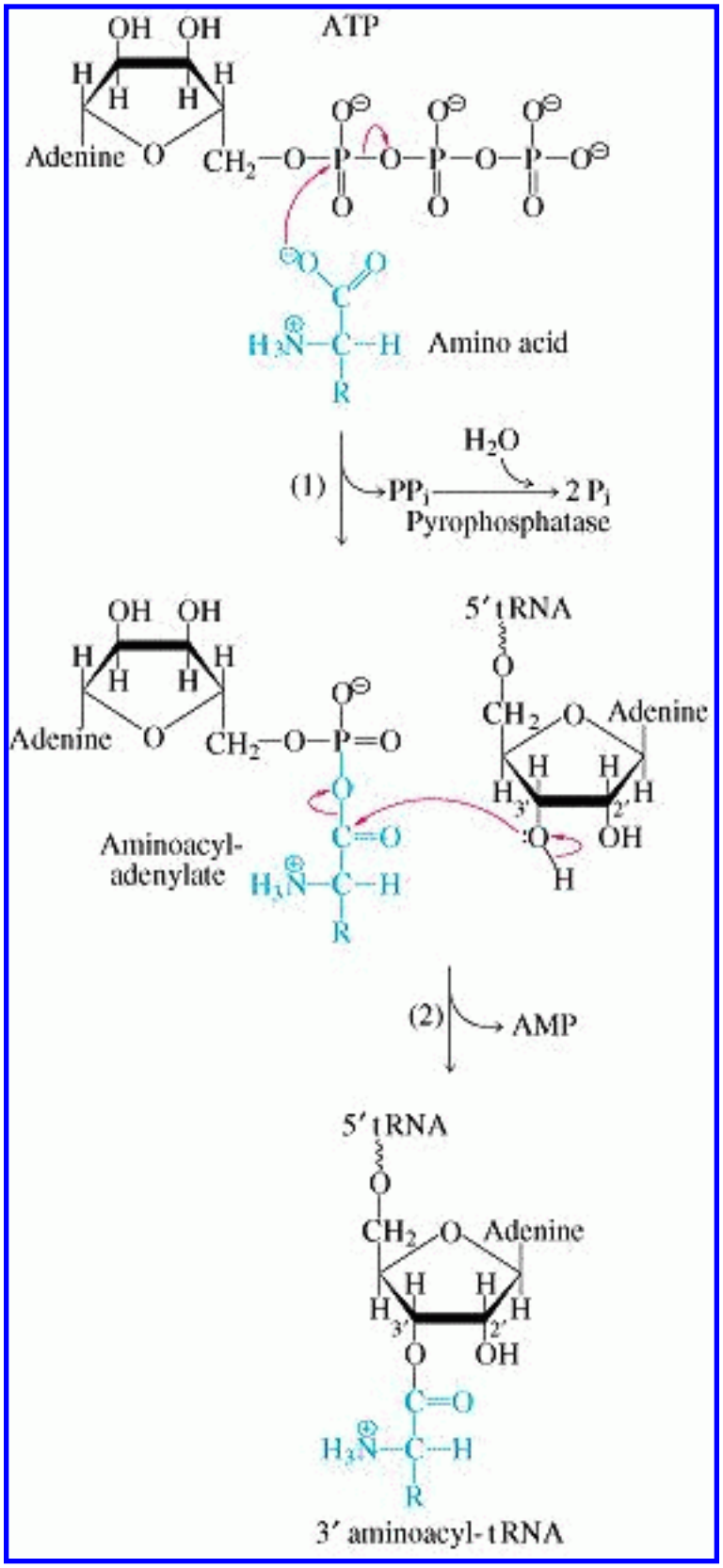
- The active sites (*anticodon* and *amino acid*) are maximally separated.
- As in proteins, the tertiary structure is dictated by the primary sequence.
- The tertiary structure is stabilized by base pairing and base stacking.
- Two areas (anticodon stem and acceptor stem) form double helix.

Next: tRNA Function

Please report typos, errors etc. by EMAIL (mention the title of this page).

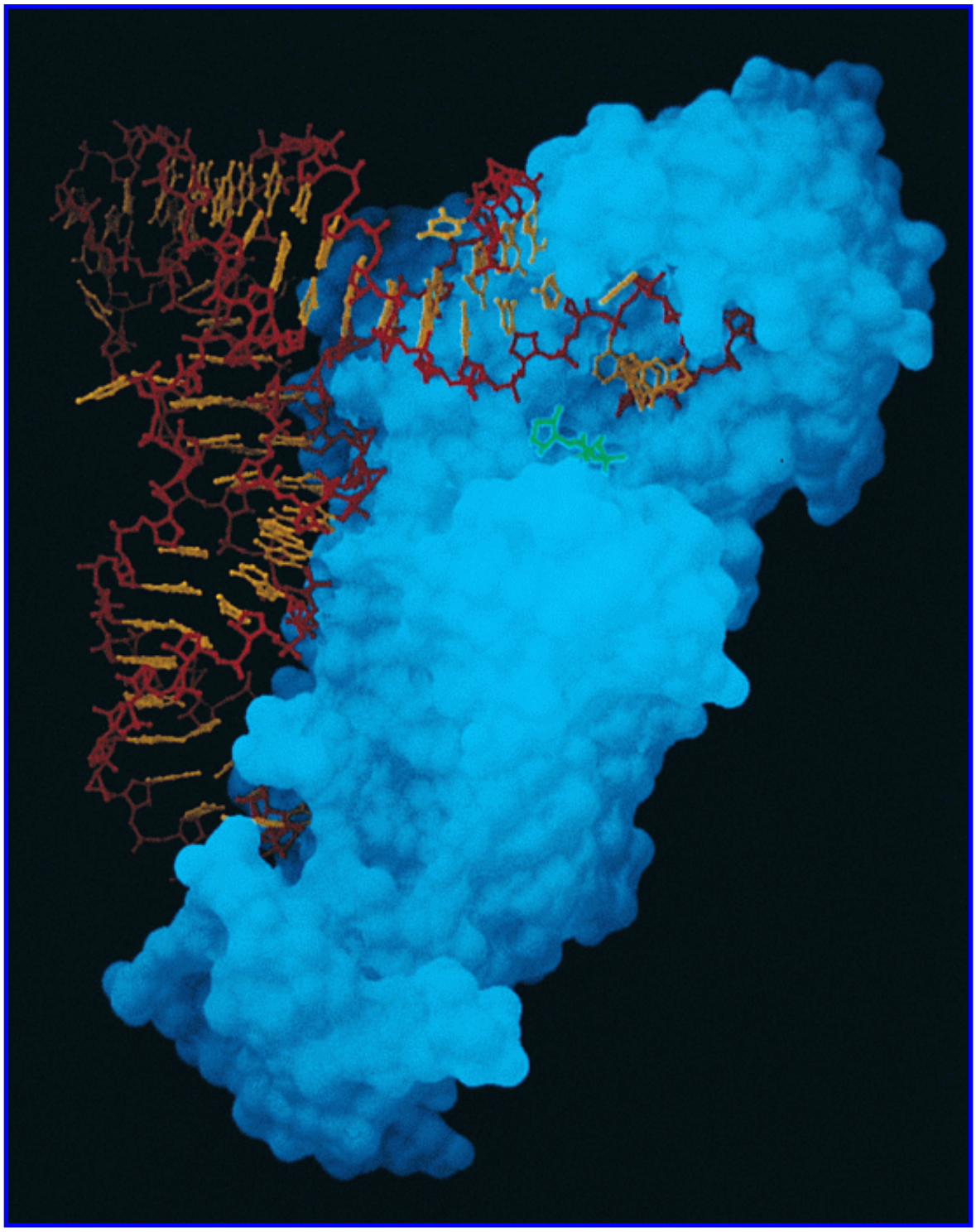
tRNA Function: Synthetases

- Each tRNA is *charged* with the proper amino acid via a covalent ester bond at their 3' end by a family of enzymes called ***aminoacyl-tRNA synthetases***. Each enzyme must recognize ***both*** the tRNA specific for an amino acid and the corresponding amino acid. This energy-consuming process is ATP-dependent and results in the cleavage of **two** high-energy phosphate bonds (one in going from ATP to AMP + PP, and one for the cleavage of pyrophosphate into two inorganic phosphates:

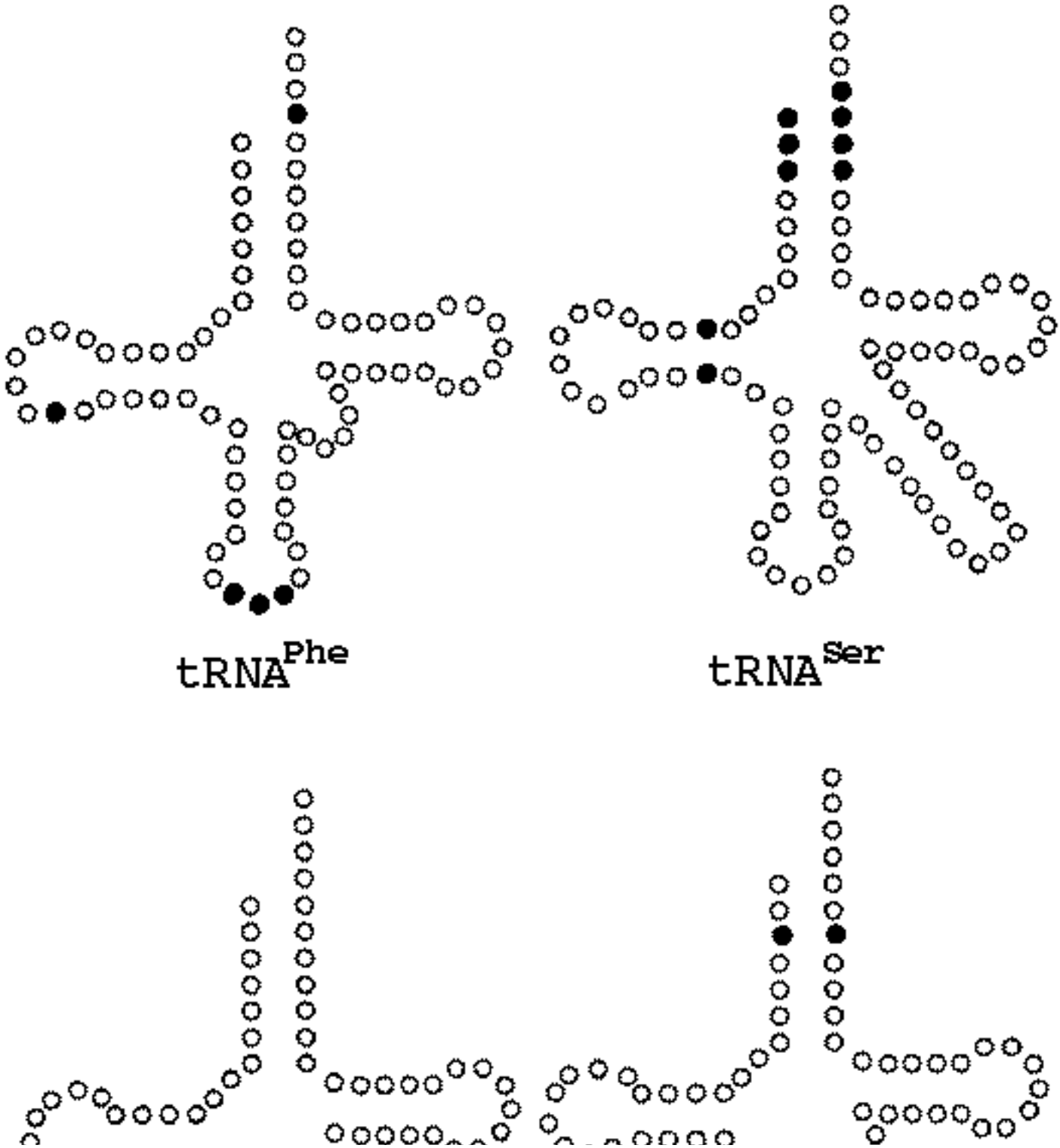


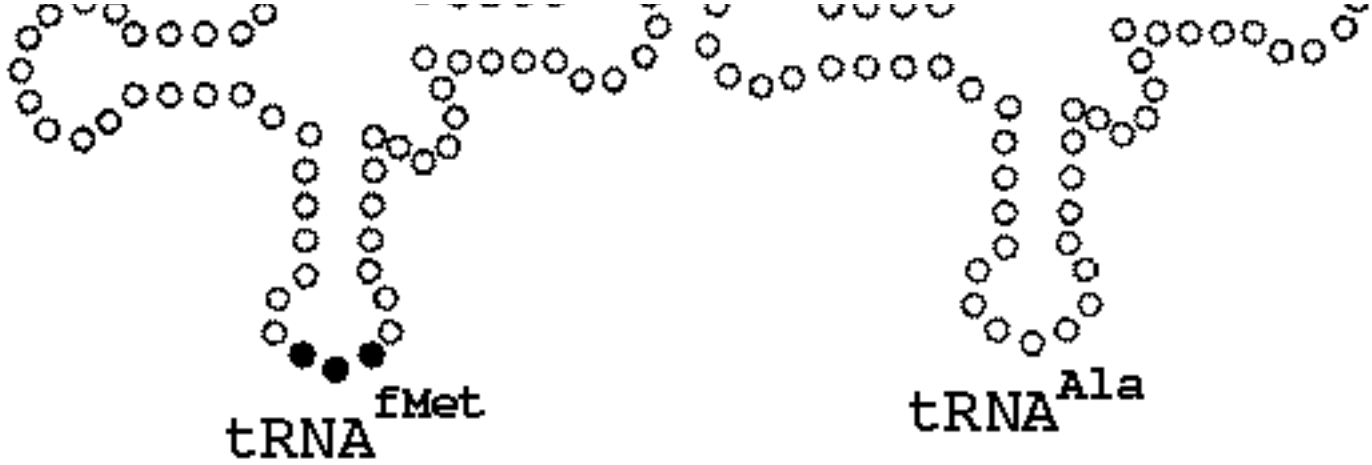
- There are 20 different aminoacyl-tRNA synthetases, one for each amino acid. Despite the fact that they all carry out very similar tasks, they vary greatly in size (40-100 kDalton).

- Since there are 61 amino acid codons, most tRNA synthetases must be able to recognize more than one type of tRNA (i.e. 6 codons for Arg). These tRNAs are called ***cognate tRNAs*** for that particular synthetase. This mapping is achieved through so-called ***recognition domains*** on the tRNA. tRNA shown with red backbone and yellow bases. tRNA synthetase shown as space-filling model in blue:



- The *recognition domain* includes unique of sections of the acceptor stem *and/or* the anticodon (black dots):



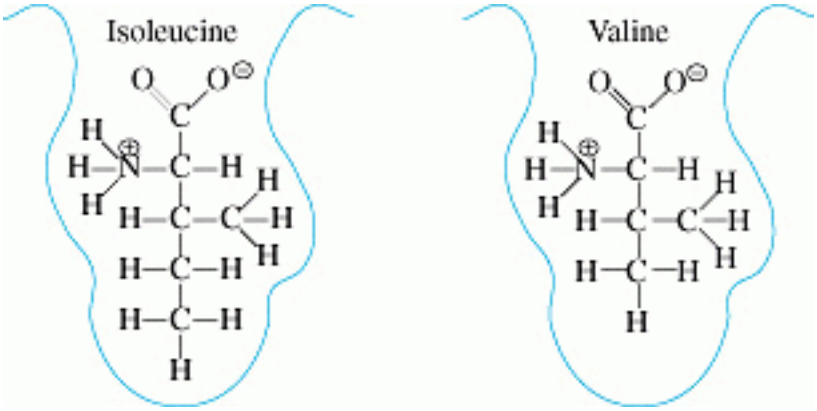


Accuracy & Proofreading

- The *accuracy* of charging tRNA with the proper amino acid is crucial because once charged, only the tRNA ***anticodon*** determines incorporation, ***not*** the attached amino acid.

- The error rate of charging is very low: 1 in 10,000. This is achieved by two means:

  - the amino acid specificity pocket in a specific synthetase will only bind amino acids similar in size and charge.
  - the synthetase also has



proofreading capability which, once a wrong aminoacyl-adenylate complex is formed (1st step), will hydrolyze the complex ***before*** it can be covalently attached to the tRNA (2nd step).

Next: The Wobble Theory

Please report typos, errors etc. by EMAIL (mention the title of this page).

The Wobble Theory

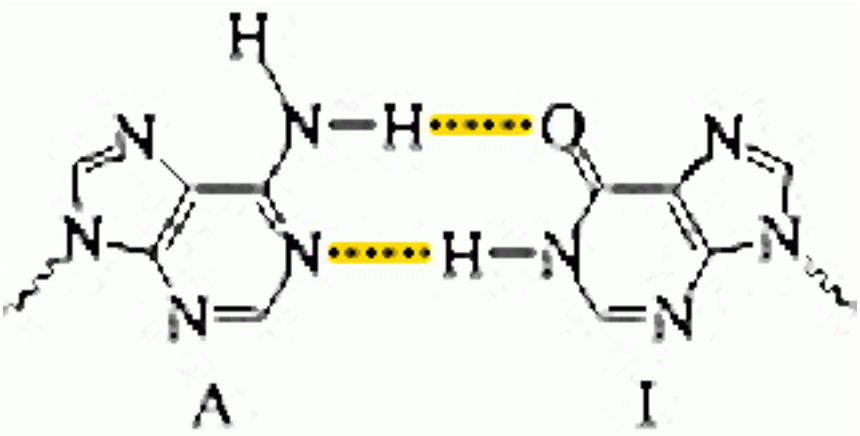
Assumption:

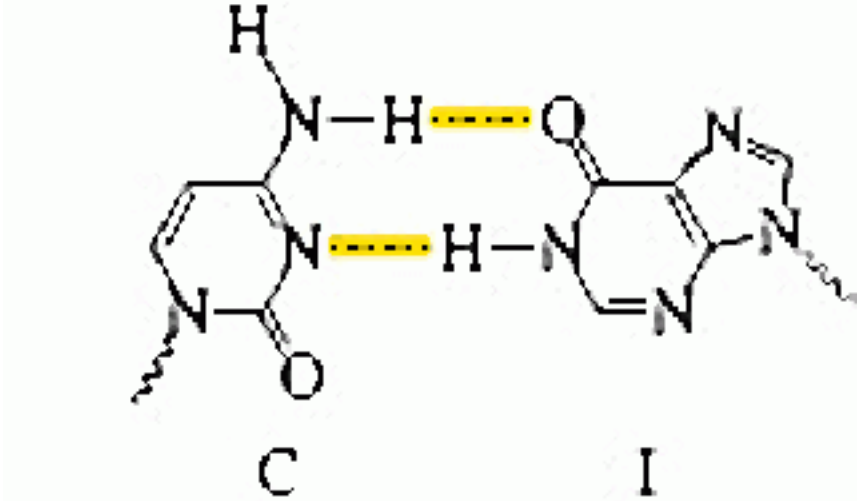
Each tRNA, defined by its 3-base anticodon, pairs only with its complementary codon on the mRNA.

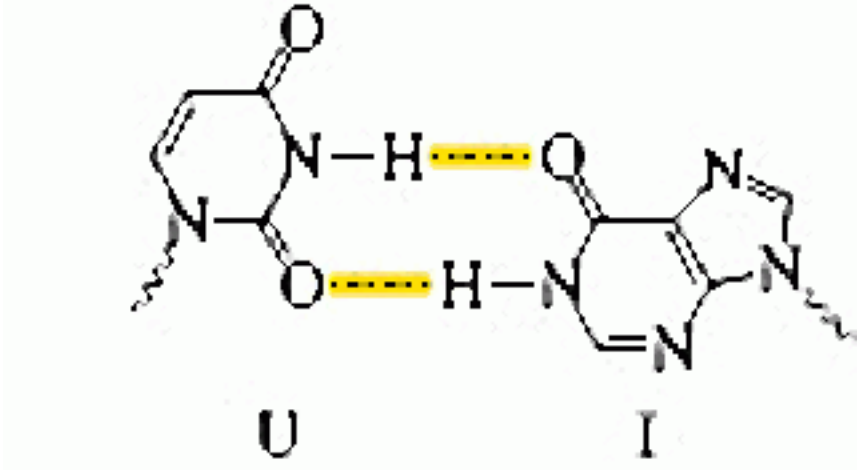
Discrepancy:

$tRNA^{Ala}$ was found to bind to codons GCA, GCC and GCU.

Codon (5'->3') Anticodon (3'->5')	GCA CGU	GCC CGG	GCU CGA


A
I


C
I


U
I

The answer:

The $tRNA^{Ala}$ anticodon is actually CGI which pairs with all three codons.

Experimentally determined codon-anticodon pairing rules:

- The first two positions of the mRNA codon observe Watson-Crick base pairing rules (**A-U, C-G**)
- The third position exhibits ***wobble.***
- Wobble occurs because the conformation of the tRNA anticodon loop permits flexibility at the first base of the anticodon.

5' anticodon base (tRNA)	3' codon base (mRNA)
A (not observed)	**U** (Watson-Crick)
C	**G** (Watson-Crick)
G	**C** or **U**
U	**A** or **G**

I	**A** or **C** or **U**

***Caveat*: Since a single tRNA can respond to more than one codon, one tRNA could respond to codons for *two different* amino acids! This *would* lead to an ambiguous code. But since the Genetic Code is *unambiguous*, certain anticodons are disallowed.**

Next: The Dangers of Wobble

Please report typos, errors etc. by EMAIL (mention the title of this page).

The Dangers of Wobble

Since a single tRNA can respond to more than one codon, one tRNA could *in theory* respond to codons for *two different* amino acids:

Example: **AGC** and **AGU** both code for **Ser**. Their anticodon is **UCG** using wobble rules. But anticodon **UCA** would also pair with **AGU** (**Ser**). This anticodon (**UCA**) would be converted post-transcriptionally to **UCI** (**Hey, that's us!**) which would recognize **AGC** and **AGU** as intended, but also **AGA** which codes for **Arg** -- **not good, would result in an ambiguous Genetic Code**!

One anticodon for serine is UCG:

Codon (5'->3') Anticodon (3'->5')	AGC UCG	AGU UCG (wobble)
Amino acid	Ser	Ser

Now, in the following *hypothetical* example, anticodon UCA (*if it existed)* would also base-pair with codon AGU using wobble rules:

AGU UCA =>	AGU UCI (wobble)	AGC UCI	AGU UCI	AGA UCI (wobble)
Ser	Ser	Ser	Ser	Arg

But anticodon UCA would be converted post-transcriptionally to UCI which is able to bind three codons (wobble rules), including one for Arg! For this reason the UCA/UCI anticodon does not exist! And the codon AGA for Arg is actually covered by the anticodon UCU (wobble)!

Next: Supplemental Material

Please report typos, errors etc. by EMAIL (mention the title of this page).

Supplemental Material

This page won't be on the final!

Molecular Graphics:

This exercise will let you get a better 3-dimensional picture of the interaction of a tRNA with its tRNA synthetase. You will be able to rotate, scale and otherwise manipulate the structure on your screen.

First, you'll need to obtain & install a ***FREE*** molecular graphics program called RASMOL on your PC:

Click here to obtain the MS Windows/NT version

or

Click here to obtain the Apple Macintosh version

Unpack the distribution, you should then be able to run the program RASWIN (PC) or RASMAC (MAC). Click here for detailed help. If you get stuck or need help, please contact me (Hudel).

Second, you'll need to get a copy of the atomic coordinates from the Protein Data Bank:

PDB entry 1GTR

Save this rather large file (0.5 MBytes) with the "Save full entry to disk" option. Save the file as "**1GTR.pdb**". This file contains the complete atomic coordinates of GLUTAMINYL-tRNA SYNTHETASE COMPLEXED WITH tRNA AND ATP.

Now, run the program RASWIN/RASMAC and go to the "**Open...**" option under the "**File**" Menu. Select the file you just saved (**1GTR.pdb**). Then select the "**Backbone**" option under the "**Display**" menu. Your display should show something like this. For a description of the program, check the "**User Manual**" under the "**Help**" Menu.

Have fun!

Next: Summary

Please report typos, errors etc. by EMAIL (mention the title of this page).

Summary

Features of tRNAs

- tRNAs are the ***adaptor molecules*** that translate from nucleic acid triplet to amino acid.
- tRNAs are ***charged*** with amino acids by enzymes called aminoacyl-tRNA synthetases in an energy-dependent 2-step process.
- There are 20 specific synthetases, one for each amino acid.
- Since most amino acids have more than one possible codon, there are more than 20 different tRNAs (40 in *E. coli*).
- Not all 61 codons have a specific tRNA due to ***wobble*** in the 3rd codon position.
- tRNA synthetases highly specific and have ***proofreading*** capabilities, achieving a better than 1:10,000 error rate.
- The accuracy of charging tRNA with the proper amino acid is ***crucial*** because once charged, only the tRNA ***anticodon*** determines incorporation into the growing polypeptide chain, ***not*** the attached amino acid.

Next Lecture: The Ribosome, rRNA and mRNA

Please report typos, errors etc. by EMAIL (mention the title of this page).