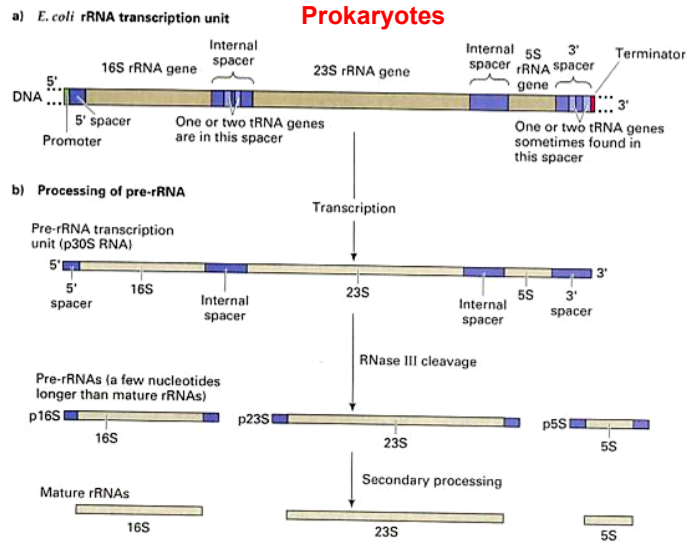
Translation: The last step in the transfer of information for gene expression.

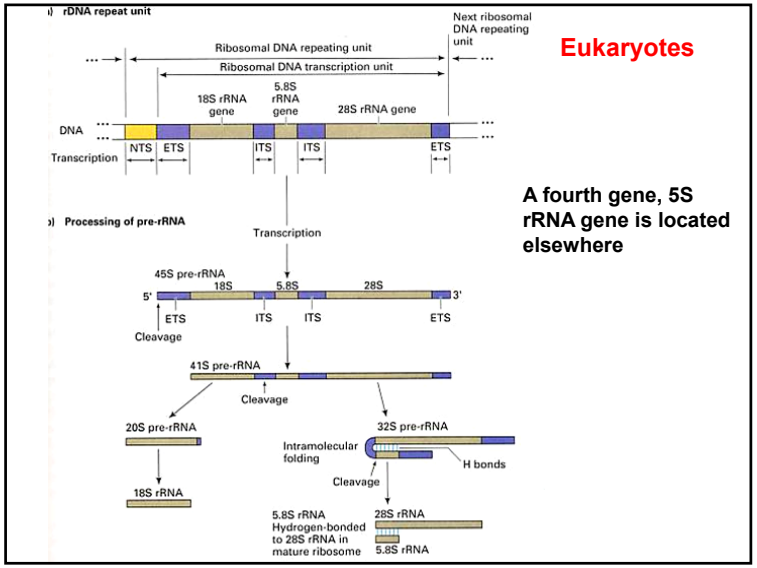
Who are the players?

mRNA (processed in the case of Eukaryotes)

Ribosomes (rRNAs and proteins)

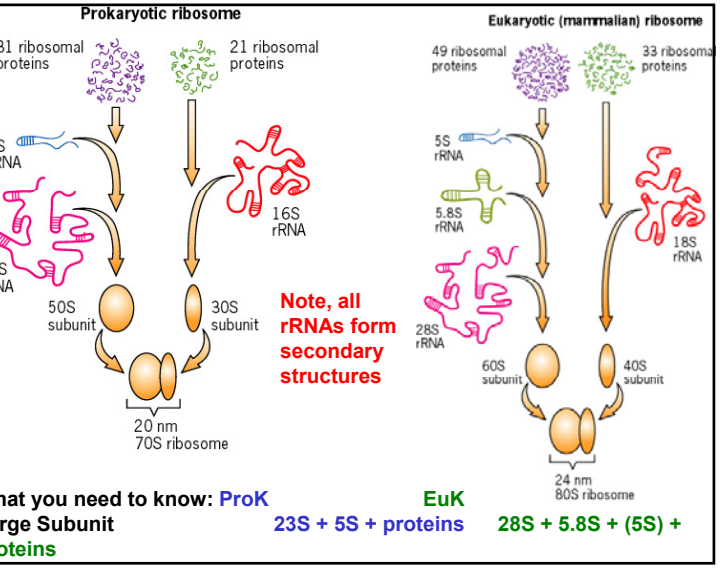
tRNAs (the predicted intermediate that is covalently bonded to amino acids)





Eukaryotes

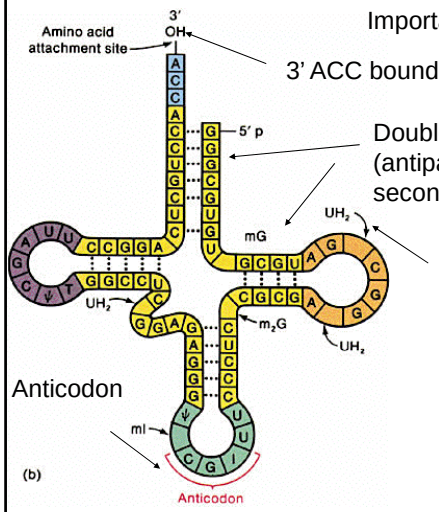
A fourth gene, 5S rRNA gene is located elsewhere



Note, all rRNAs form secondary structures

What you need to know: ProK EuK

Large Subunit 23S + 5S + proteins 28S + 5.8S + (5S) + proteins



Important Regions:

3' ACC bound to aa

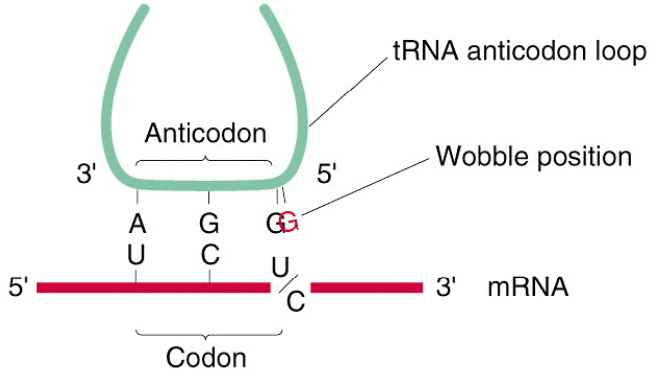
Double stranded RNA (antiparallel) forming secondary structure

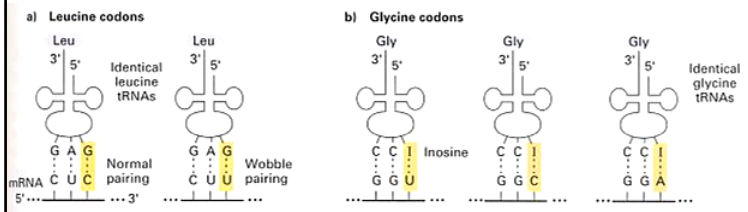
Several modified bases

How many tRNAs does a cell need?

61?

No, about 32-35





There are two unusual pairings that can occur between the 3rd base of the codon and the corresponding base of the anticodon.

1) Inosine is a modified adenosine in the tRNA. It has unique and varied pairing capabilities when in the 3rd position of the anticodon!!!

I = (U, C, A)

2) G = U

Which sequences or molecules are NOT involved in RNA processing in Eukaryotes?

A) 5'- GU…A…AG -3'

B) 5'- TATAAA -3'

C) 7mG

D) 5'- AAUAAA -3'

E) snRNA

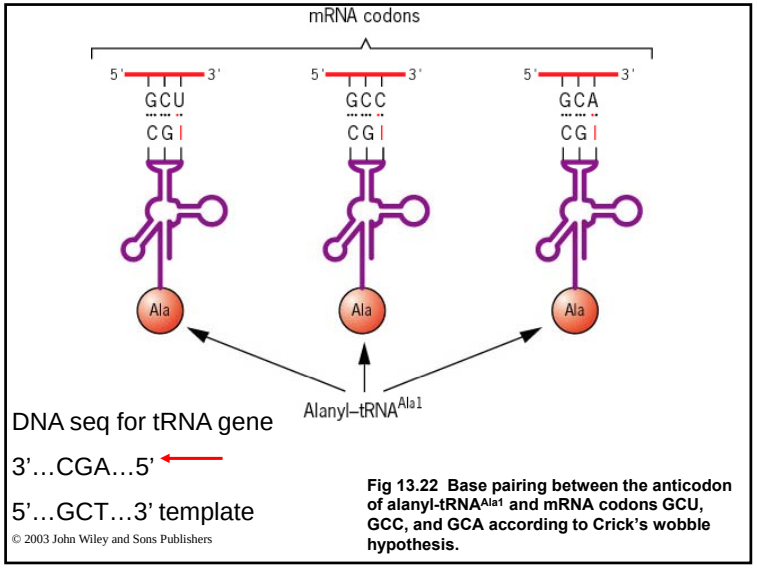


Fig 13.22 Base pairing between the anticodon of alanyl-tRNA^{Ala1} and mRNA codons GCU, GCC, and GCA according to Crick's wobble hypothesis.

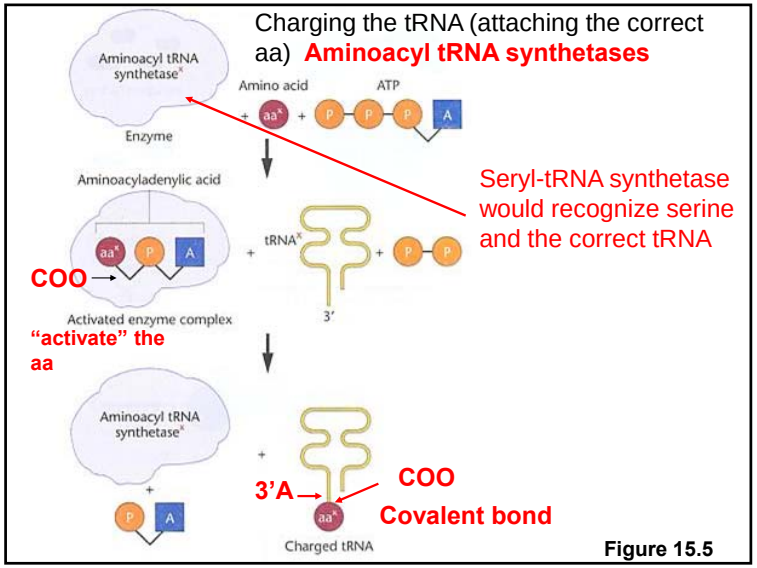


Figure 15.5

How do these enzymes recognize proper aa and tRNA?

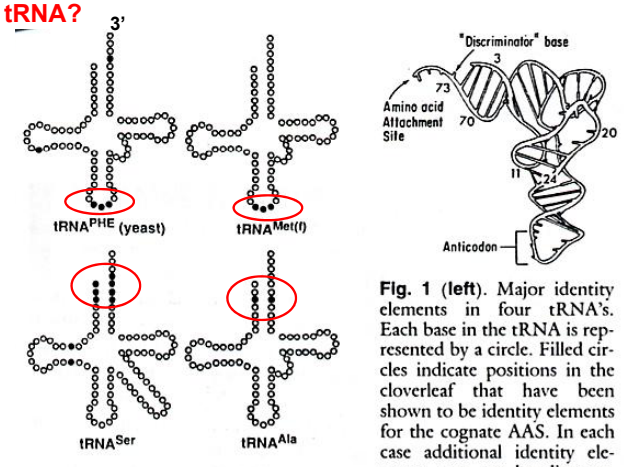
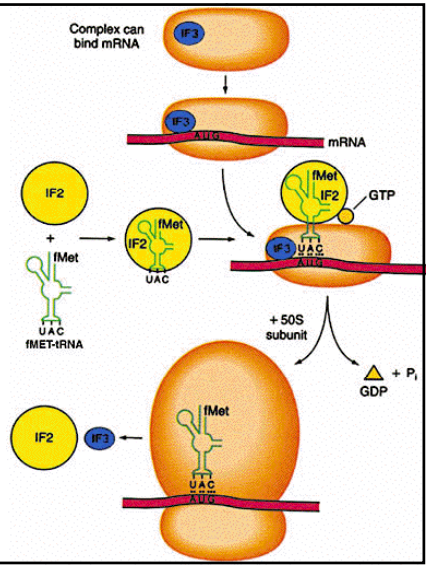


Fig. 1 (left). Major identity elements in four tRNA's. Each base in the tRNA is represented by a circle. Filled circles indicate positions in the cloverleaf that have been shown to be identity elements for the cognate AAS. In each case additional identity elements may yet be discovered. **Fig. 2 (right).** Location of elements in the tertiary structure of tRNA. A representation of the x-ray crystal structure of yeast tRNA^{Phe} is

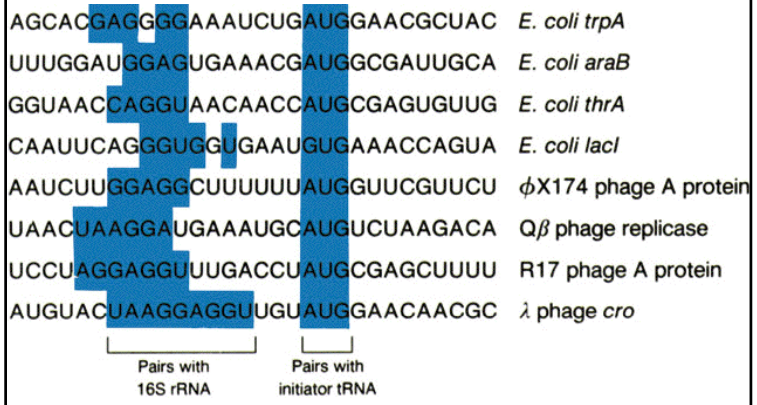
Translation in Prokaryotes

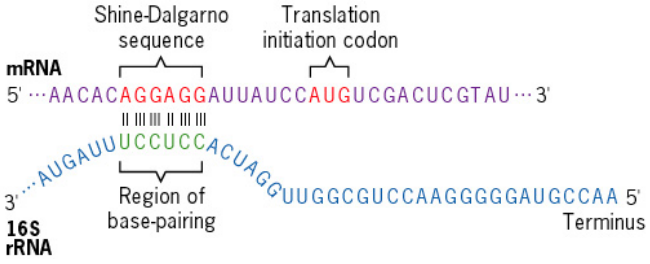
1) Small subunit of ribosome binds to mRNA (IF3 keeps subunits separate).

How does the small subunit of the ribosome recognize a mRNA?



Shine-Dalgarno or "GAGG" sequence.



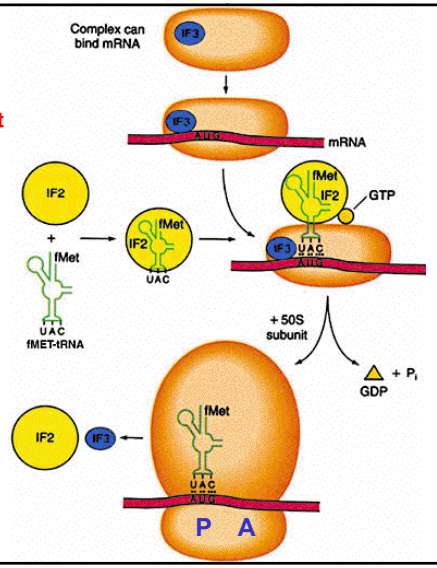


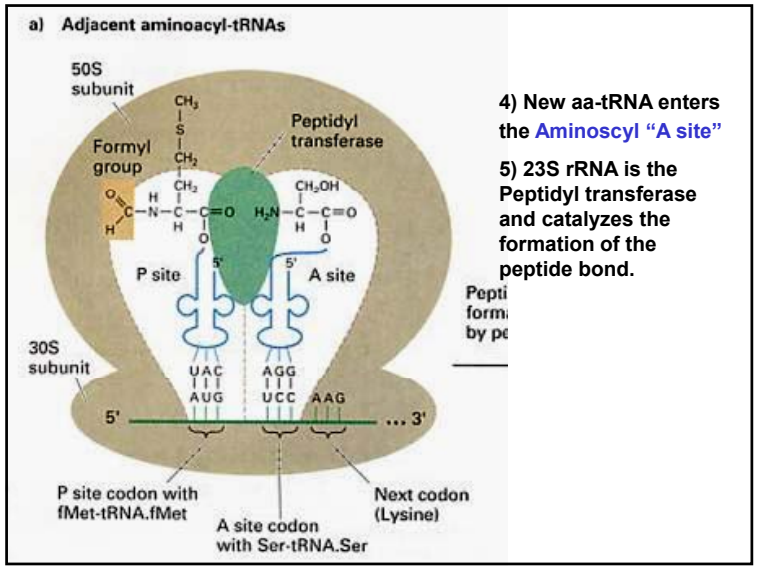
Base-pairing between the Shine-Dalgarno sequence in a prokaryotic mRNA and a complementary sequence near the 3' terminus of the 16S rRNA is involved in the formation of the mRNA/30S ribosomal subunit initiation complex.

1) Modified N-formylmethionine-tRNA is placed in the peptidyl "P-site" of the small subunit. Only tRNA that directly enters P-site!!

IF2 and IF1 involved.

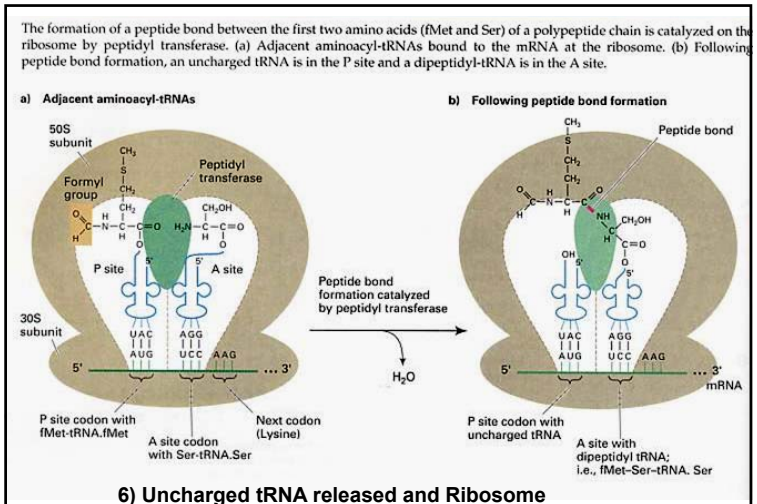
- Large subunit joins the party



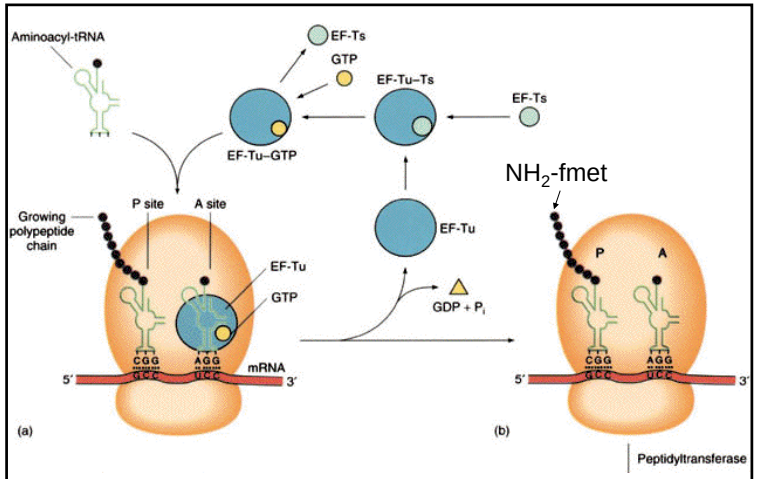


4) New aa-tRNA enters the Aminoscyl "A site"

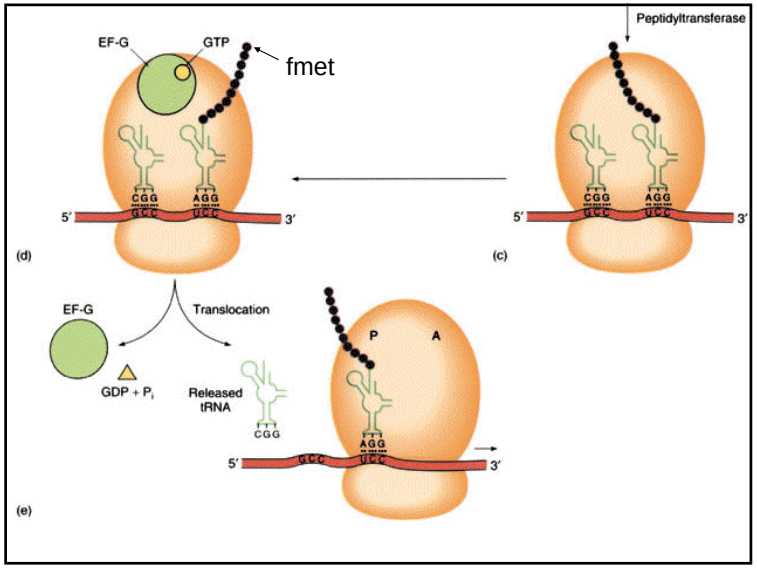
5) 23S rRNA is the Peptidyl transferase and catalyzes the formation of the peptide bond.

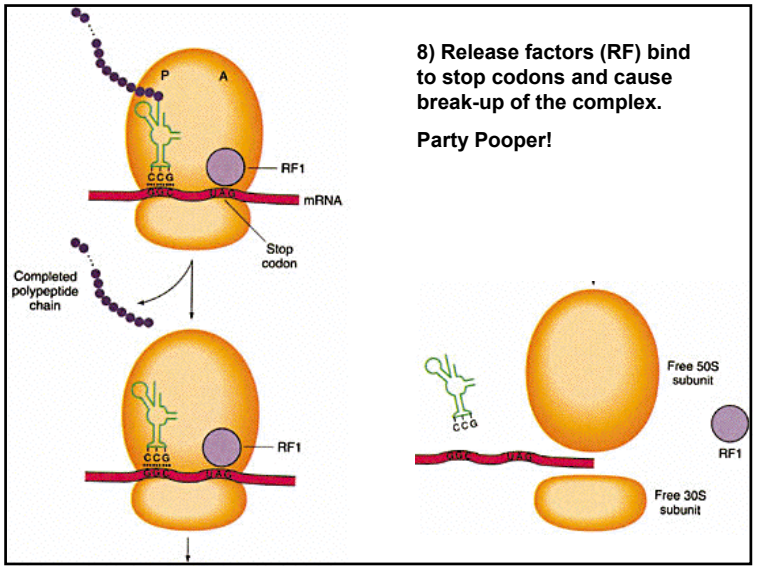


6) Uncharged tRNA released and Ribosome translocates forward with peptidyl-tRNA moving into the P site



7) Process continues, elongation factors (EF) bring new aa into A site.





8) Release factors (RF) bind to stop codons and cause break-up of the complex.

Party Pooper!

Eukaryotic Translation

Process is similar to Prokaryotic, but generally more complex (and less well understood). Some basic differences are:

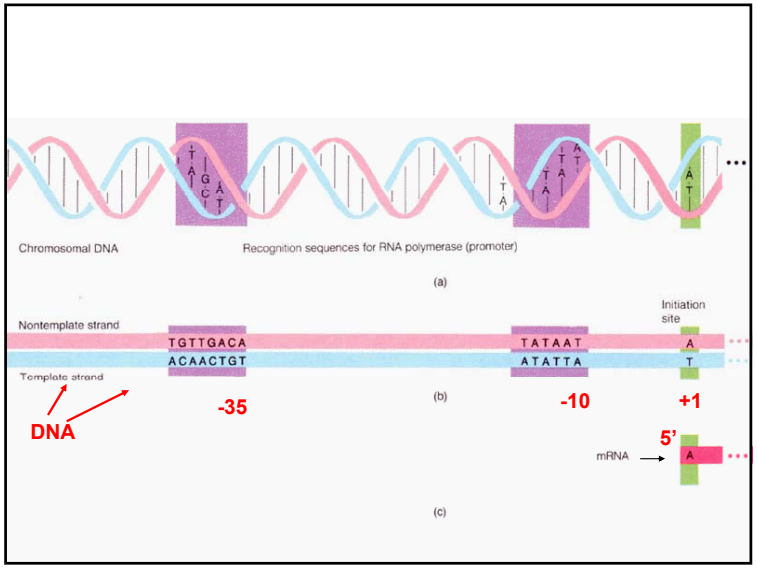
1) mRNAs are long-lived (generally hours vs. minutes, but this is variable). Cap and tail aid this longevity.

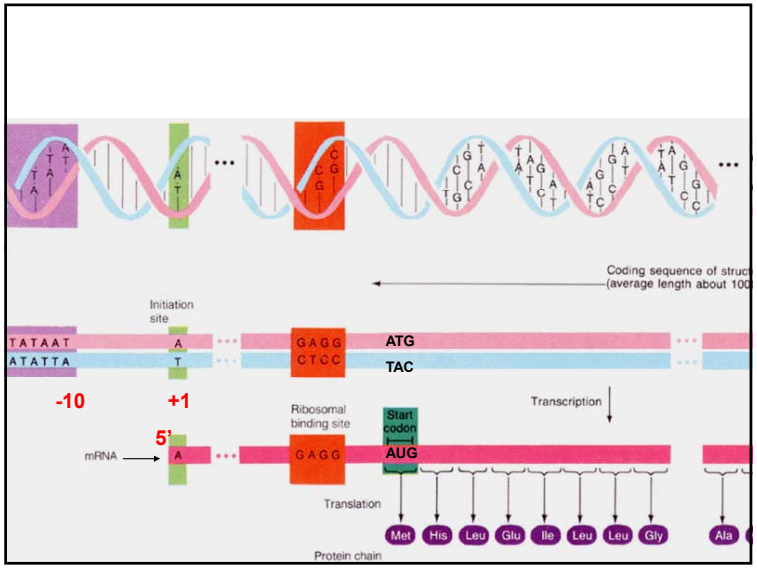
2) translation occurs in the cytoplasm away from transcription.

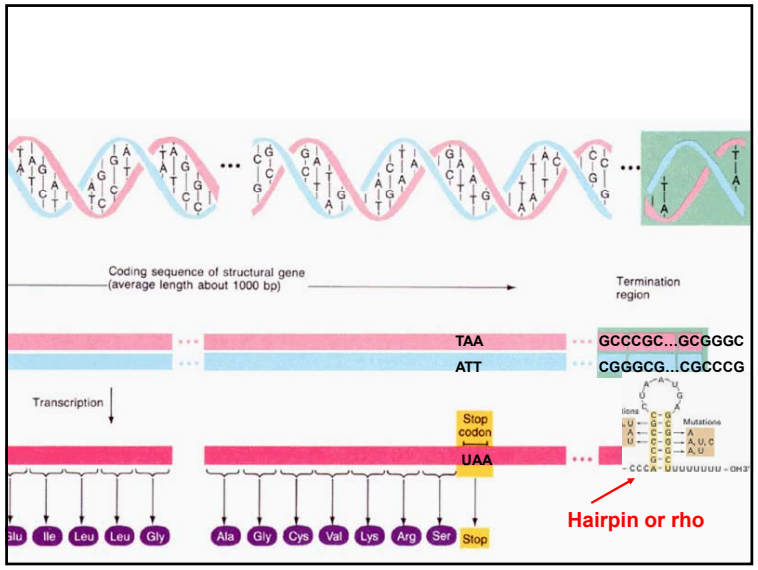
3) there are recognition sequences for translation initiation analogous to Shine-Dalgarno (GAGG); Kozak sequence (5'-ACCAUGG-3'). This plus the 7mG Cap are essential.

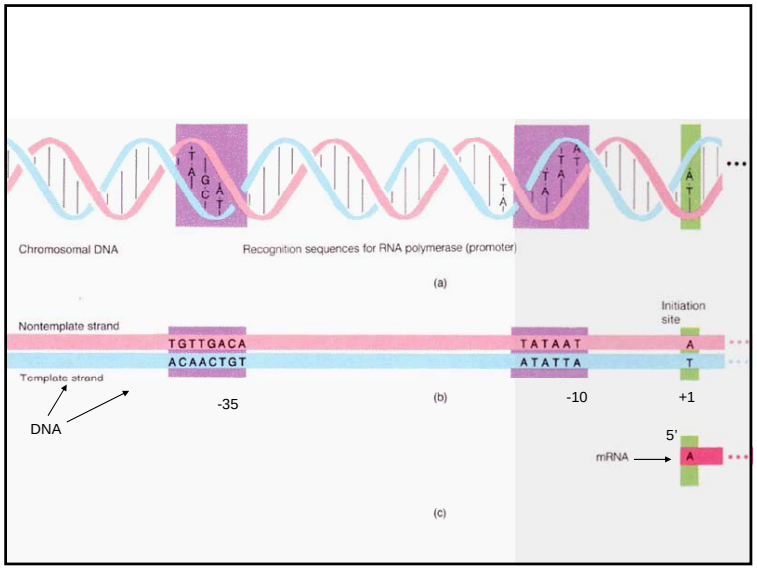
4) met not fmet is the first amino acid, a special tRNA is needed; $tRNA_i^{met}$

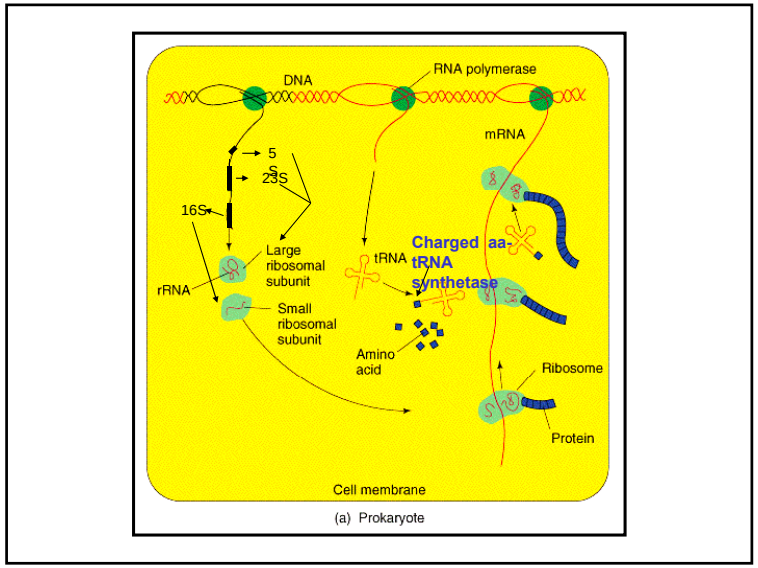
5) In general more complicated with more IF and EF proteins involved.

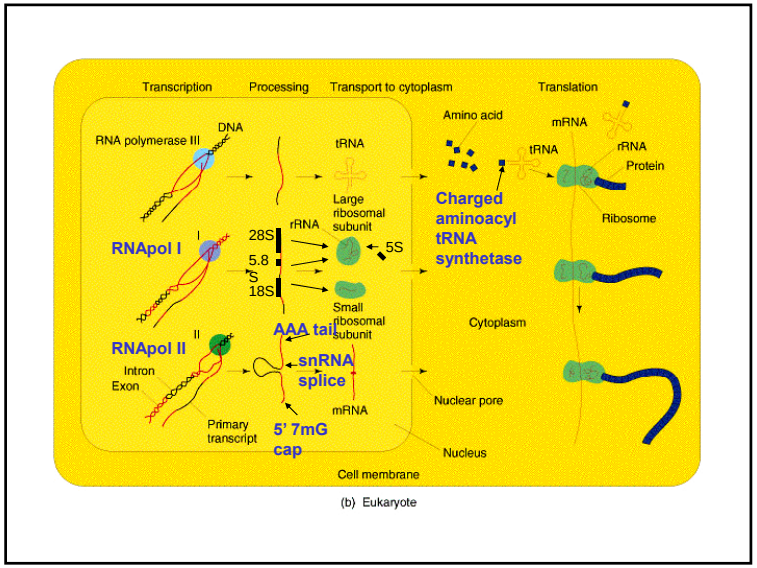


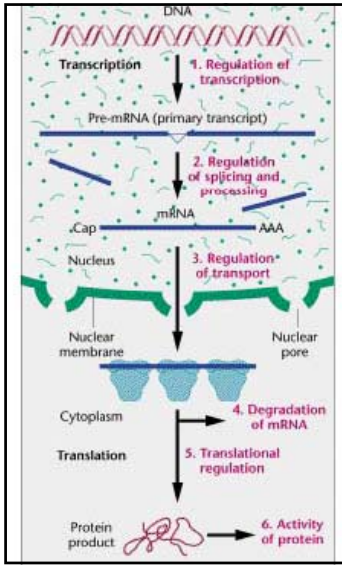



Coding sequence of structural gene
(average length about 1000 bp)
Termination region
TAA
ATT
GCCCGC...GCGGGC
CGGGCG...CGCCCG
Transcription
Stop codon
UAA
Mutations
Glu
Ile
Leu
Leu
Gly
Ala
Gly
Cys
Val
Lys
Arg
Ser
Stop
Hairpin or rho


Chromosomal DNA
Recognition sequences for RNA polymerase (promoter)
(a)
Nontemplate strand
TGTTGACA
ACAACTGT
TATAAT
ATATTA
Initiation site
A
T
Template strand
DNA
-35
(b)
-10
+1
5'
mRNA
A
(c)


DNA
RNA polymerase
mRNA
5S
23S
16S
Large ribosomal subunit
rRNA
Small ribosomal subunit
tRNA
Charged aa-tRNA synthetase
Amino acid
Ribosome
Protein
Cell membrane
(a) Prokaryote


Transcription
Processing
Transport to cytoplasm
Translation
RNA polymerase III
DNA
tRNA
Amino acid
mRNA
tRNA
rRNA
Protein
Large ribosomal subunit
Charged aminoacyl tRNA synthetase
Ribosome
RNApol I
rRNA
28S
5.8S
18S
5S
Small ribosomal subunit
Cytoplasm
AAA tail
RNApol II
Intron
Exon
snRNA splice
mRNA
Nuclear pore
Primary transcript
5' 7mG cap
Nucleus
Cell membrane
(b) Eukaryote



Control of Gene Expression

Transcription

Processing (Eukaryotes only)

Nuclear export (Euk. Only)

Translation

Recognition by Ribosomes

5'UTR such as

Shine-Dalgarno (Prok) or

Kozak, 7mG (Euk)

Secondary structure in 5'

3' UTR poly A tail, interactions 5'end (Euk only)

Degradation of mRNA

miRNAs (antisense sequences) siRNAs … (Euk only?)

Mendelian ⇨ Genes on DNA ⇨ RNA ⇨ Proteins ⇨ Mendelian Traits **Mendelian Traits**

Let's now begin to make the connection between genes and traits.

What is the relationship between the genotype and the phenotype?

We will do this by looking at the genetic basis of some well known traits.

A. Garrod 1902, 1908 Inborn Errors of Metabolism

For Alkaptonuria he recognized that afflicted had a build up of homogentistic acid (alkapton). He speculated that either:

1) alkapton was an abnormal product formed by "perverted" metabolism of tyrosine (**Gain of Function Mutation**),

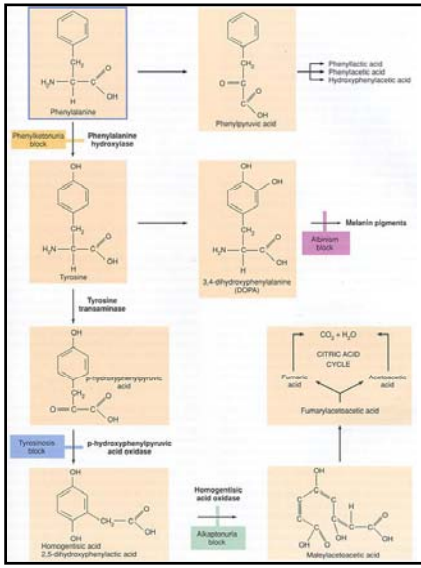
2) intermediate by-product of normal metabolism caused by a block in a pathway (**Loss of Function Mutation**).

Experiments:

Fed tyrosine and phenylalanine to Alkaptonuria patients and to normal people (himself).

Alkaptonuria people had more alkapton, normal had constant levels.

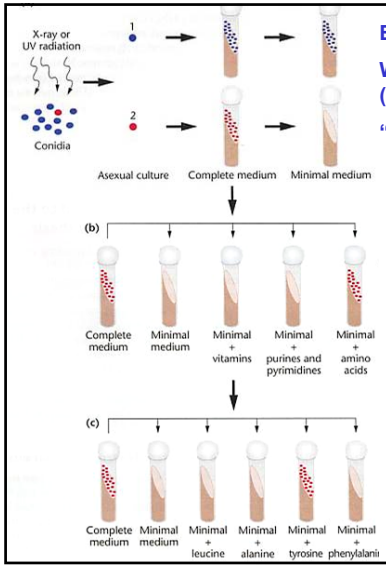
Concluded alkapton is normally metabolized.



After examining many cases (e.g. albinism), Garrod concluded:

Hereditary information controls chemical reactions and metabolism in humans.

Inherited disorders are the result of genetic errors



Beadle & Tatum 1941

Working with bread mold (Neurospora).

"One gene, one enzyme"

In which pathway is the mutation?

AA pathway!

In which pathway is the mutation?

Tyrosine pathway

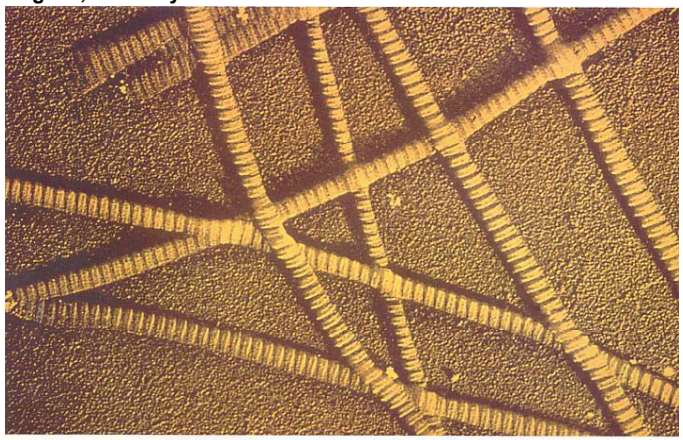
Further analysis of multiple tyrosine mutants by???

Complementation tests and pathway analysis (adding known aa precursors to tease apart steps in pathway).

A → B → C → tyr

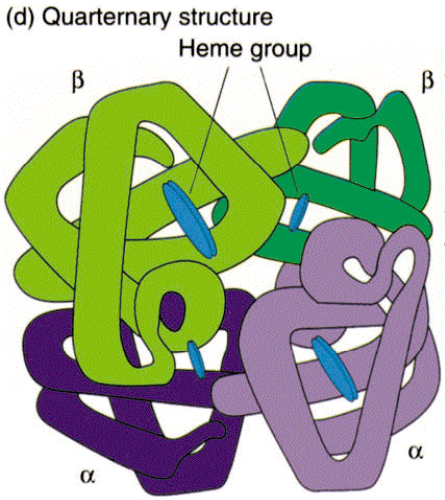
Fig. 15.11

"One gene, one enzyme" idea has been modified several times.



An electron micrograph of collagen fibers, the most abundant protein found in vertebrates.

Collagen is the most abundant protein in vertebrates, yet it is not an enzyme.



Even some proteins are made up of polypeptides from multiple genes!

More commonly the statement is:

"One gene, one polypeptide".

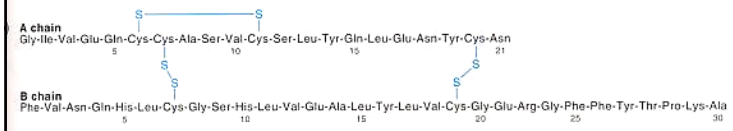
Is this correct?

Proteins encoded by gene have diverse functions:

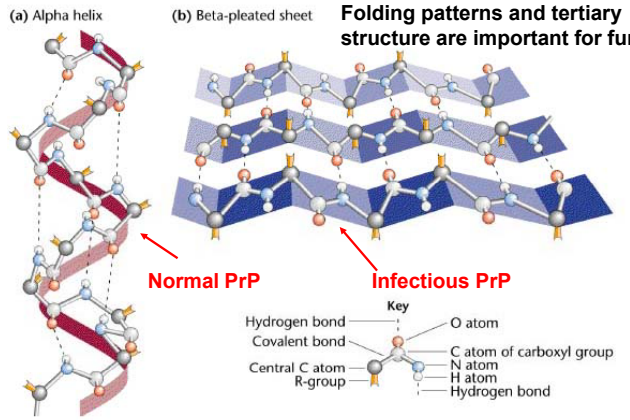
- Enzymes
- Structural Proteins
- Contractile Proteins
- Transport Proteins
- Immunoglobulins
- Histones

Proteins have complex structures allowing them to carry out the diverse functions.

Bovine Insulin -- secondary structure



Disulfide bonds between Cysteine amino acids



Folding patterns and tertiary structure are important for function

Prion Diseases S. Prusiner 1997 Nobel Prize working with "Kuru"

Mad Cow and Scrapie Disease: Bovine Spongiform Encephalopathy (BSE), Creutzfeldt-Jakob Disease (CJD)