Insert Overview of Translation here 2 pages.

Between sections 5 and 6

Translation continued

```
3'  TAC CCC CAG GAC TGC ACC TAC TCA TTT ATG AAA ATG  5'
5'  ATG GGG GTC CTG ACG TGG ATG AGT AAA TAC TTT TAG  3'
```

Transcription of this DNA produces
the following mRNA.

```
5'  AUG GGG GUC CUG ACG UGG AUG AGU AAA UAC UUU UAG  3'
```

Translation of this mRNA produces
the following protein

NH_3 M G V L T W M S K Y F COOH

The ribosomes begin translating at the start codon, move down the mRNA in the 5' to 3' direction and stop translating when they encounter a stop codon. The protein is being synthesized in the amino to carboxy direction.

For simplicity's sake, we will discuss in detail only prokaryotic translation.

Before we proceed let's define two regions of the ribosome.
P-site stands for Peptidyl-site.

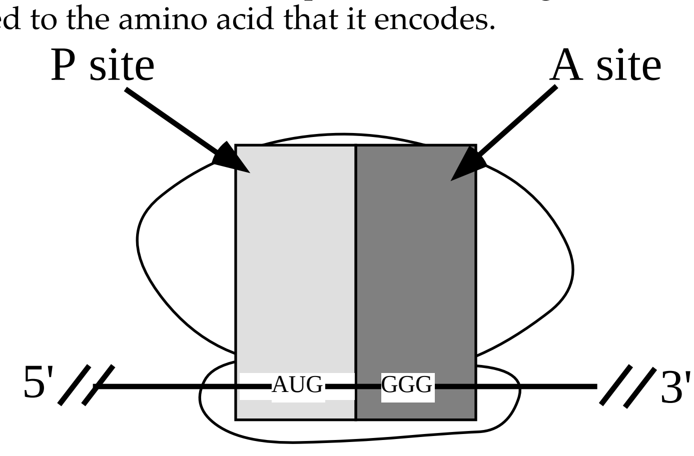
> The P-site is the area of the ribosome that holds a tRNA that is attached to the growing polypeptide chain.

A-site stands for Acceptor-site.

> The A-site is an area of the ribosome that accepts the next charged tRNA. A charged tRNA is attached to the amino acid that it encodes.

The complete P and A sites are present only in the 70 ribosome.

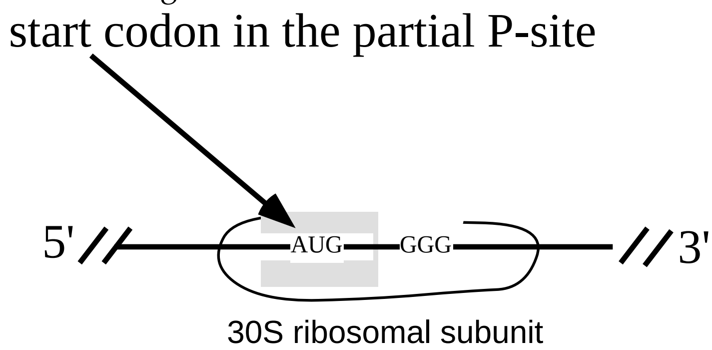
By itself, the 30S subunit has what we call a partial P-site and a partial A-site.



Initiation

Initiation is not a function of the complete ribosome but is undertaken by the separate subunits which assemble on the mRNA. Examine the figure called ***Overview of Initiation and Elongation*** during the following discussion.

One job of the 30S ribosomal subunit is to recognize the mRNA start codon and to attach to the mRNA so that the start codon is in the partial P-site.



How does the 30S subunit recognize the start codon?

In prokaryotes, this is fairly well understood. Prokaryotic mRNAs contain a ribosome binding site that is located 5' to (in front of) the start codon. This sequence is 5' AGGAGG 3'. It is called a Shine-Dalgarno sequence and it is found about 10 bases 5' to the start codon. The 16S rRNA, in turn, contains the sequence 5' CCUCCU 3' . These two sequences are antiparallel and complementary to one another.

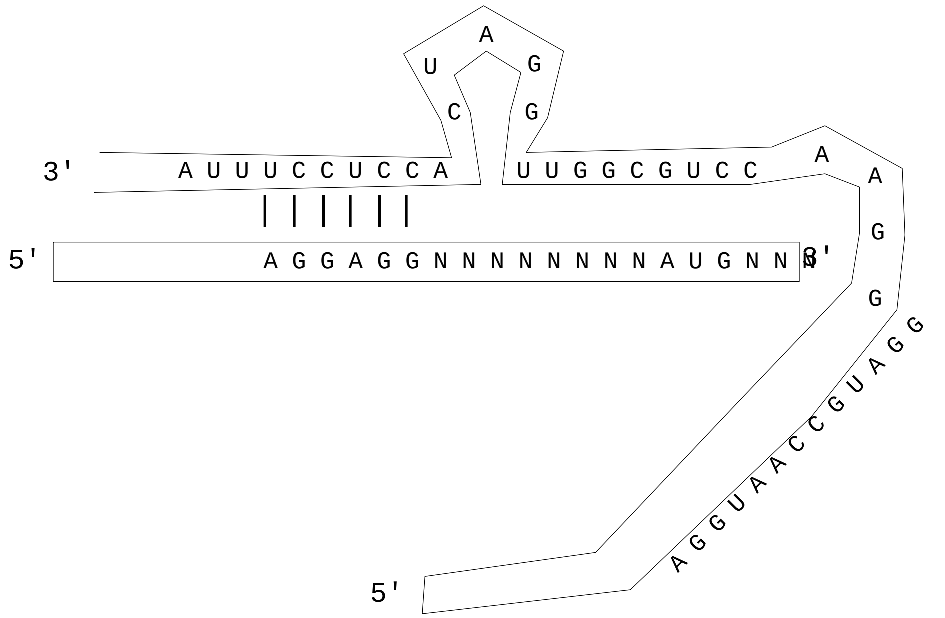
Example of a mRNA Shine-Dalgarno sequence and its relationship to a start codon.

5' NNNNN**AGGAGG**NNNNNNNNN**AUG**NNNNNNN 3'   N = any nucleotide.

The 16S rRNA binds the Shine-Dalgarno sequence which places the start codon in the partial P-site of the 30S subunit.

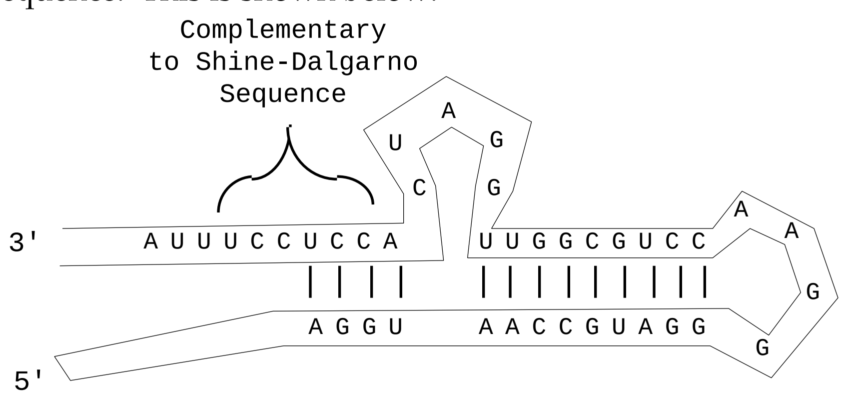
16S rRNA H-bonding with a Shine-Dalgarno sequence.

The bigger molecule is the 16S rRNA and the smaller molecule is a mRNA.



But wait! In order to translate the ribosome can't be bound to the mRNA in this fashion! It is believed that once the 70S ribosome has been assembled on the mRNA that *intramolecular* base pairing between different portions of the 16S rRNA displaces the mRNA's Shine-Dalgarno sequence. This is shown below.

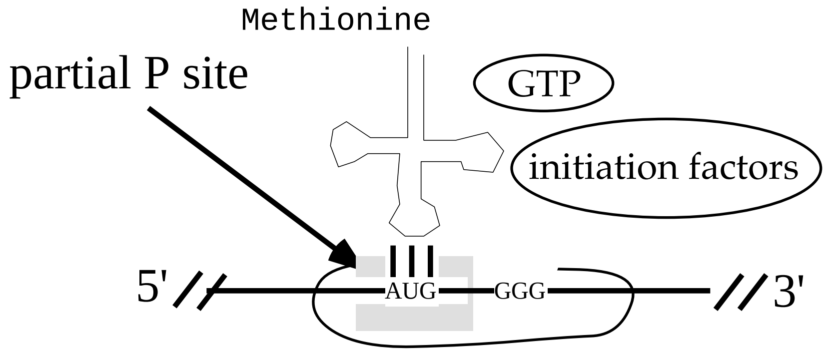
Intramolecular base pairing between two regions of the 16S rRNA masks the Shine-Dalgarno complementary sequence.



30S pre-initiation complex

Once the 30S subunit has bound the mRNA it can accept a tRNA. Only one type of tRNA can enter the half P-site. It is called the initiator methionyl tRNA and is abbreviated as $tRNA_i^{met}$. This particular charged tRNA is the only one that can enter the partial P-site. It cannot enter a complete P-site. It is reserved for use in the pre-initiation complex and is not used during elongation..

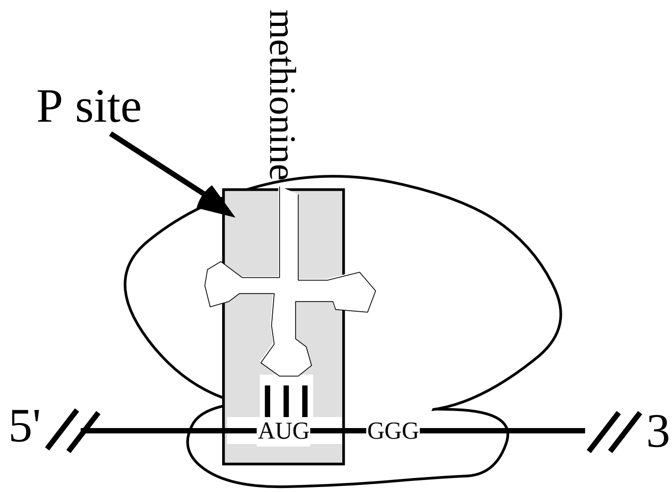
The 30 S pre-initiation complex consists of a $tRNA_i^{met}$ charged with methionine, GTP, initiation factors, the mRNA and the 30S ribosomal subunit.



30S pre-initiation complex

To complete the initiation process the 50S subunit binds, the initiation factors are released and energy in consumed in the form of one high-energy phosphate bond derived from GTP.

Completed 70S initiation complex which has a complete P and A site.



70S initiation complex

What purpose do the initiation factors serve?

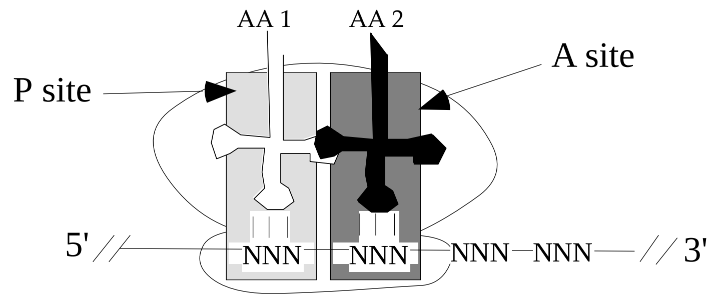
The initiation factors help to make translation proceed in one direction. The assembly of the 70S initiation complex is very much like an enzymatic reaction. It even consumes energy in the form of GTP. Like all reactions it is reversible. The reversal of initiation, however, requires the interaction of all of the molecules described above. Once the 50S subunit binds, energy is consumed and the initiation factors dissociate from the complex. The loss of the initiation factors and the consumption of energy make it very unlikely that all of the players will be in the correct position to get the reaction to flow backwards. Therefore, once begun, initiation tends to flow only in the forward direction.

Furthermore, it is the initiation factors that recognize the $tRNA_i^{met}$ and enable it to enter the partial P-site.

Elongation

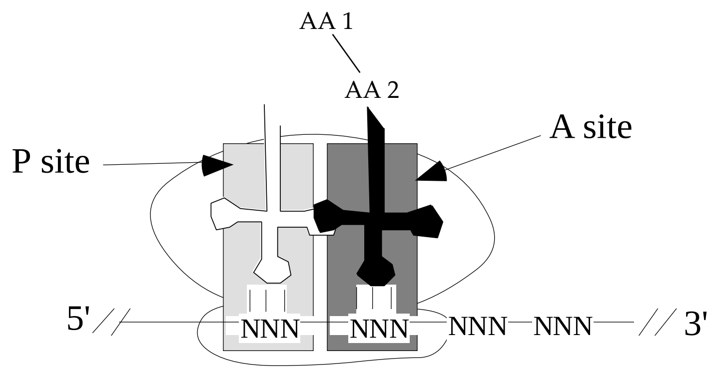
NNN stands for a codon in the mRNA. AA 1 stands for amino acid 1. AA 2 stands for amino acid 2 etc.

A charged tRNA enters the A site, H-bonding with the codon.



Peptide Bond Formation

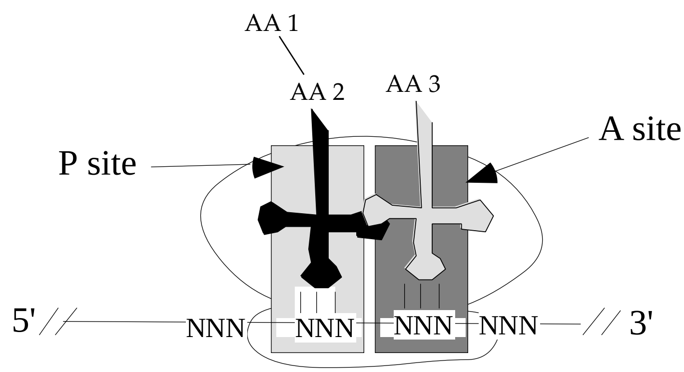
Catalysis of a peptide bond between AA1 and AA2. Peptide bond formation is catalyzed between AA 1 and AA 2.



Translocation

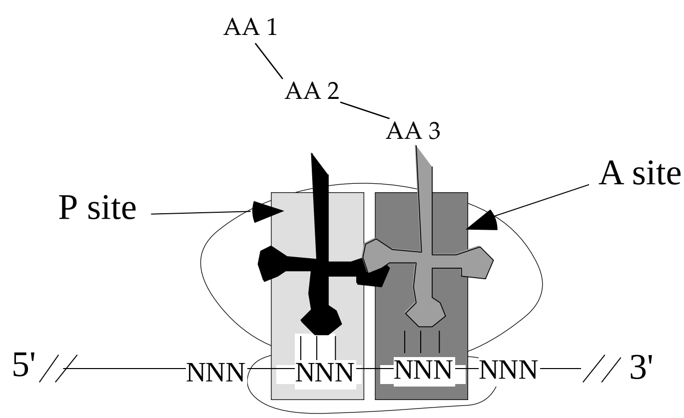
The ribosome translocates to the next codon, the uncharged tRNA leaves and a new charged tRNA enters the A site.

This step requires EF-G and consumes energy in the form of GTP.



Peptide Bond Formation

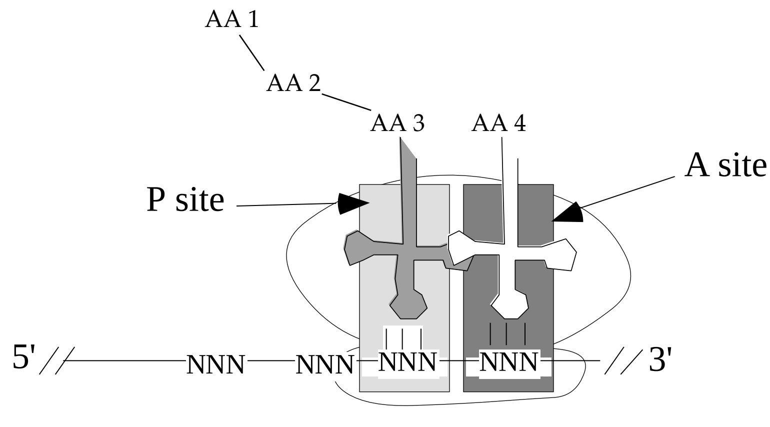
Catalysis of peptide bond formation between AA 2 and AA3.



Translocation

Uncharged tRNA leaves, the ribosome translocates to the next codon and a new charged tRNA enters the A site.

This step requires EF-G and consumes energy in the form of GTP.



This process of elongation occurs cyclically until a stop codon is reached.

Termination

The last step in translation. Termination occurs when the a stop codon enter the A-site of a ribosome. At this time the ribosome binds a releasing factor and GTP. The GTP is hydrolyzed (energy is consumed) and the last tRNA, the mRNA, the two ribosomal subunits and the protein dissociate.
Examine page 2 of the ***Overview of Initiation and Elongation*** .

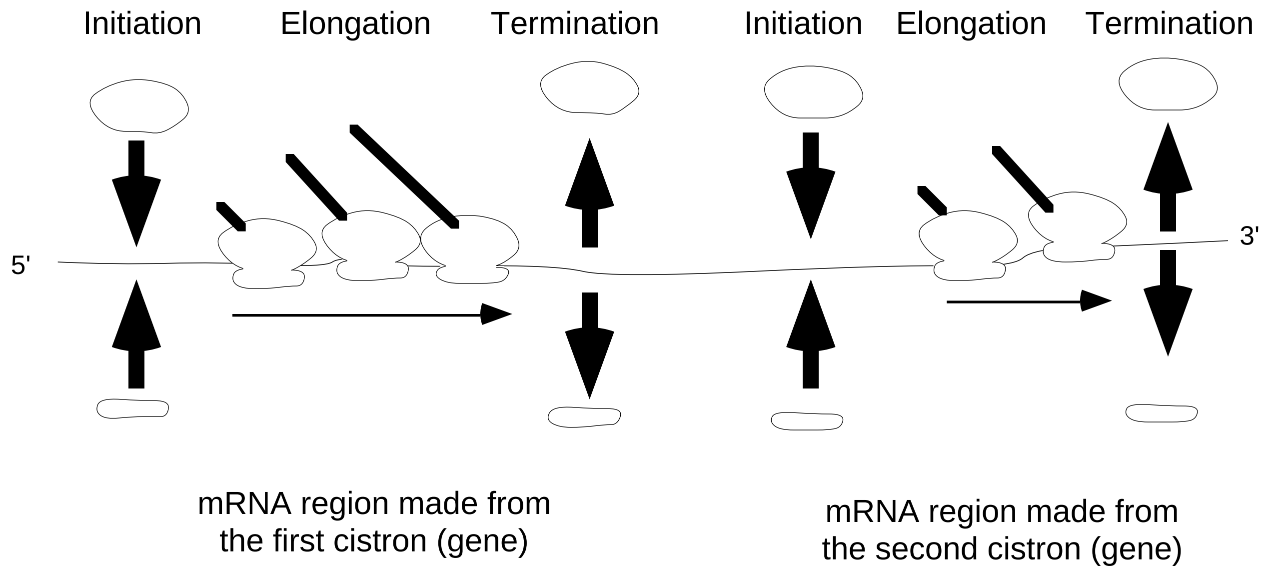
Do all proteins really begin with methionine?

Yes and No. Translation of all protein does begin with methionine. But for some proteins an enzyme comes along and clips off the 15 to 30 amino acids from the N-terminus. Therefore, mature proteins do not all begin with methionine.

Polycistronic mRNA

Translation differs in two fundamental ways in prokaryotes and eukaryotes. We have already discussed the fact that only prokaryotes have concomitant transcription and translation. The second way is that prokaryotes produce *polycistronic* mRNAs while eukaryotes produce only *monocistronic* mRNAs. The word cistron is synonymous with gene.

Translation of a polycistronic mRNA.

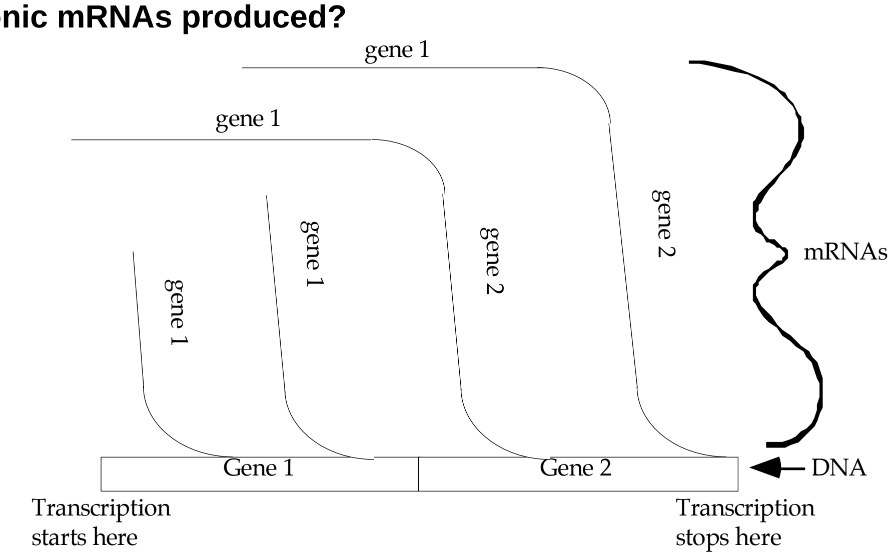


As a rule, each cistron is treated as a 'separate' mRNA by the ribosomes. That is, the processes of initiation, elongation and termination are completely independent. Each cistron has its own Shine-Dalgarno sequence, its own start codon and its own stop codon. An occasional exception to this rule exists. Ask me about it if you like.

How are polycistronic mRNAs produced?

Polycistronic mRNAs are transcribed as a single transcription unit, that is, a single mRNA that contains the information from more than one gene.

Don't forget that translation of these mRNAs is also occurring simultaneously with transcription.



What types of genes are expressed in the same polycistronic mRNA?

Polycistronic mRNAs are usually produced from genes that are involved in the same biochemical pathway. For instance, the five enzymes needed by *E. coli* to synthesize tryptophan are produced from a single polycistronic mRNA. All of these genes are organized 'head to tail' in the bacterial genome and are transcribed as one long mRNA. This is a very efficient way to control gene expression. Can you figure out why?

Definitions

elongation factors	Generic abbreviation is EF for prokaryotic factors. These are proteins that associate with ribosomes cyclically, during the addition of each amino acid to the polypeptide chain.
initiation factors	These are proteins that associate with the small subunit of the ribosome specifically at the stage of translation initiation.
monocistronic mRNA	A mRNA that contains the coding region of only a single gene. The opposite of monocistronic is polycistronic. Eukaryotes produce only monocistronic mRNAs. Prokaryotes can produce either monocistronic or polycistronic mRNAs.
polycistronic mRNA	A single mRNA that carries the information from more than one gene. Usually several genes from same metabolic pathway. The information from each gene can be independently translated. The opposite of polycistronic is monocistronic. Prokaryotes produce both polycistronic and monocistronic mRNAs. Eukaryotes produce only monocistronic mRNAs.