Lecture 19

- Fig 8.12 A special initiator tRNA starts the polypeptide chain
  - Protein synthesis starts with a methionine amino acid usually coded by AUG
- When you make a peptide bond, there are two sites on ribosome that are important
  - A site - where newly amino acyl tRNAs enter
  - P site - has the growing polypeptide
- Both the A and P site are attached by their carboxyl group in a amino acyl bond to the OH on a terminal ribose of terminal A on the tRNA and are covalently attach
  - The tRNA is making contact, not only with the codon in the mRNA but part of the structure of tRNA makes contacts with the rRNA and ribosomal protein in both the large and small subunit.
- Initiation vs elongation
  - the first tRNA that enters has to go to the P site not the A site, otherwise there would be nothing to join together, you need two different tRNAs each with an amino acid to make the first peptide bond.
  - need initiation factors to deliver the initiating tRNA into the half P site. The way the ribosome works is that initiation involves just the RNA, the initiation factors, the initiating tRNA with thyamine goes into the half P site.
    - half because the ribosome is only the small subunit and doesn't have the large subunit yet. Once the complex assembled then the unique initiating factors due to GTP hydrolysis end up dissociated and the large subunit joins the small, now the ribosome is ready for elongation.
      - have the first codon from the start of the tRNA in the half P site and an empty A site for the second amino acyl tRNA to enter and the A site will sit over the second codon.
- Prokaryotic and eukaryotic ribosomes do initiation steps slightly different
  - one assembles on the mRNA first
  - Eukaryotes assemble the factors on the small subunit first then join the message later.
  - Both have a special initiation factor.
    - prokaryotes it is called IF2 and in eukaryotes its called eIF2; they uniquely recognize a unique tRNA that ends up initiating Methionine and only that tRNA goes into the empty P site.
    - In bacteria, part of the recognition of IF2 is having the reaction where to get the Met charged by synthase on this initiating tRNA a formyl group is added to the amino group.
      - Normally, an intermediate amino can have a blocked amino group that terminates protein synthesis. Always adding the second amino acid as a free amino group and adding a carboxyl group of the amino acid before. If you have a blocked amino group you can't make a peptide bond. The first amino acid can have a blocked amino group because the second amino acid is going to have the free amino group.

- Eukaryotic protiens don't have a formylated Met, they use the different features of the tRNA to distinguish it from the elongating tRNA of from Met.
- Both have special factors that only recognizes the intiating factors of tRNA.
- Once the first peptide bond is made the formyl group is cleaved off.
  - In bacteria the first or second amino acid is cleaved off as well.
- Fig 8.13 different methionine tRNAs are involved in initiation and elongation
- the initiator tRNA has unique structure features that distinguish it from all other tRNAs
- The NH_2 group of the methionine bound to bacterial initiator tRNA
- For bacterial tRNA the subtle features that make IF2 recognizable, if there is not all unpaired base in the acceptor stem and you change a base to a U the regular elongation factor eFTU will recognize the incorrect base and put it into the A site rather than the half P site.
- eIF2 looks for different features for eukaryotic initiating tRNAs.
- Even though proteins start with Met or have a terminal Met the initiating Met is recognized by IF2, which is the initiating factor that goes into the empty half P site. Any other tRNA for any other amino acid, including internal Met are recognized by eFTU (in eukaryotes it is eEF1).
- Fig 8.16 Initiation involves base pairing between mRNA and rRNA
- An initiation site on bacterial mRNA consist of:
  - The AUG initiation codon
  - Proceeds with a gap of ~10 bases by the shine-delgarno polypurine hexamer
- How does the ribosome know when you have a message that has multiple AUG, how does it know which AUG is the correct start?
  - Can have AUGs in the 5' UTR that are bypassed in favor of the correct start.
  - In eukaryotes and prokaryotes there is a system to position the ribosome so the P site sits over the right start codon.
  - In prokaryotes the small subunit has the smaller ribosomal RNA as part of it
    - the rRNA plays many roles in the ribosome
    - it anchors ribosomal proteins creating the frame work for what the ribosome should look like
    - It mediates by have alternative base pair schemes, it mediates many conformational changes the ribosome undergoes as parts of initiation, elongation, and termination. The ribosome undergoes many conformation changing, the rRNA is changing its confirmation. The rRNA makes contact with the initiation factors with the RNA and mRNA.
- The 16s rRNA is the smaller one in the small subunit. The 23s rRNA is the one in the large subunit.
  - Eukaryotic is 18S and 28S

- The last part of the 3' end of the 16s rRNA matches the sequence upstream of the AUG. The sequence is called a shine-delgarno sequence and is always 10 bases from the correct start codon. It forms complementary base pairs with the rRNA and small subunit.
  - If a shine-delgarno sequence is not present then translation is very inefficient and doesn't start with the right codon.
- common that a when you have to express a protein in eukaryotes or prokaryotes to have to include a ribosome binding site, it is the site that positions the ribosome so the P site sits over the correct AUG and not a internal AUG. The correct AUG is selected based on having a shine-delgarno sequence.
- Different between eukaryotes and prokaryotes
  - In prokaryotes, the ribosome positions directly over the AUG by the base pairing of the message that has to be shine delgarno and the rRNA.
  - In eukaryotes, the initiating factors assemble a small subunit over a CAP then helicases that are part of the initiation factors, have the ribosomes slide down the untranslated region and it rests over a different sequence.
    - don't have a shine-delgarno.
    - the sequence of the 16S rRNA is missing in 18S rRNA, instead they have a Kozak sequence and ribosomal proteins recognize it.
      - Kozak sequence is immediately adjacent to the AUG.
  - Eukaryotes start at the 5' end and slide down
  - Prokaryotes start directly over the AUG
  - Fig 8.18 small subunits scan for initiation sites on eukaryotic mRNA
    - eukaryotic 40S ribosomal subunits:
      - bind to the 5' end of mRNA
      - scan the mRNA until they reach an initiation site
    - a eukaryotic initiation site consists of a 10 nucleotide sequence that includes an AUG codon
      - 60S ribosomal subunit joins the complex at the initiation site.
- There is a methylated CAP and part of an initiation factor called IF4G, H, I. It recognizes both the poly A tail and the CAP and it assembles the small subunit directly then helicases unwind the secondary structure of the RNA. Most single stranded RNA have stems and loops that would be hard to get through and the helicases disrupt the base pairs so that the Met can slide and the kozak would be in the A site.
  - Stem = a helical region involved complementary base pairs
  - Loops = region connecting the stems.
- The important thing is having a purine three bases from the AUG, usually the base next to the AUG is the G. When a protein is being expressed you need a eukaryotic cell for bacteria, you have