Lecture #12
The Cell as a Machine

Protein Translation and Translocation

Alberts et al., Chapter 6

Now that we have the mRNA spliced in the nucleus, the methyl G cap put in place and the poly A tail, we are ready to move it to the cytoplasm. Within the nucleus, there are a number of RNA binding proteins that in essence package the RNA into much smaller dimensions than the 1-2 microns (3000-6000 bases) possible for some of the RNAs. The next problem is to exit the nuclear pore, which has a diameter of about 20 nm. This is accomplished by a facilitated diffusion of the mRNA through the pore with perhaps some substitution of proteins on the mRNA particle.

Movement of mRNA to cytoplasm (specific localization)

There are transport sequences on some mRNAs that cause them to be moved to one region or another of the cytoplasm before initiating protein translation. The transport of mRNA in cytoplasm has been studied most in oligodendrocytes (myelin-forming cells) and neuronal dendrites. In those systems relatively large complexes of mRNA and the motors (as large as 0.5-0.8 microns) are transported along microtubule tracks.

Basic complex for protein synthesis

The proteins that combine with ribosomes to form the translational complex with the mRNA have been described. In general, the eIF1-4 proteins are part of the complex between the 40s ribosome, mRNA and the methionine-tRNA (the initial amino acid in proteins). Basic complex procedure involves a ribozyme action of the ribosomal RNA, whose structure is known at the atomic level.

Initiation complex involves tRNA and several proteins that then recruits the large ribosomal subunit. Multiple initiation events occur on the same mRNA which gives rise to polyribosome complexes. Rate of synthesis is about 20 aa/s or 25 seconds to make 1 copy of a 500 aa protein (about 50 kDa). For a cell that is rapidly growing (24 hour division time), there is the need to produce 4×10^{-9} gm X 0.18 = 7.2×10^{-10} gm of protein or 1.5×10^{-14} moles of a 50 kDa protein (about 10^{10} molecules). If there are 2×10^{5} molecules synthesized in 24 hours at the rate of 1/25 s (1800/day), then you need 100 mRNAs active all of the time. Even if you take the picture in Alberts, Chap. 6 to be correct (5 ribosomes/100 nm of mRNA or 100 aa, assuming 1 aa/nm or 3 bases/nm), then there will be 25 ribosomes/message and you will only need 4 active mRNAs all of the time.

Quality control of protein being synthesized (chaperones)

Co-Translational assembly

Control of translation by protein concentration

Problems:

1. The transport of actin mRNA to the periphery has an unknown purpose. However, some people have suggested that actin is being synthesized to increase the concentration at the site of synthesis. If we assume that each actin mRNA (1050 bases in the coding region) is fully active as noted above (i.e. there is 1 active ribosome per every 60 bases of mRNA and the polymerization rate is 20/s), then we can calculate the concentration of actin in the periphery after 12 hours of synthesis from one message (1/2 the cell cycle). If the actin is polymerizing into filaments as it is synthesized in the periphery (in a space of 20 μm^2 by 0.4 μm thick), then the effective concentration will be? If the new actin is in the globular form that has a diffusion coefficient of 10^{-9} cm^2/s, then how long will it take the new proteins to diffuse to the other side of the cell, about 40 μm away? Will the concentration at the mRNA be significant higher at the end of the 12 hours or can we assume that all of the globular actin is uniformly distributed in cytoplasm?

2. If we have a multi-subunit protein such as myosin, which has 4 light chains and 2 heavy chains, then how would you coordinate the assembly of a functional molecular complex?