Lecture #14

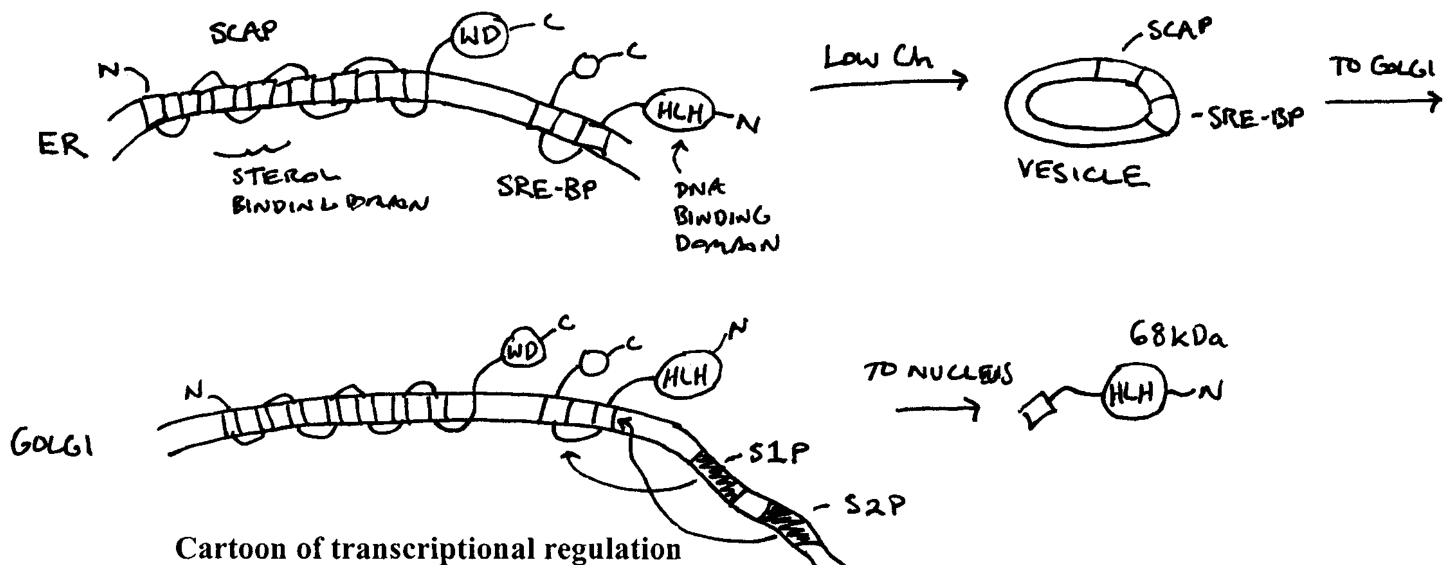
Lecture 14
3/5/03

Read: Translation chapter in Voet&Voet p.959-1004,
Review in Biochimie (2002) 84, 745-754 (elongation kinetics)
TIBS (2003) 28, 411-418 (50S structure)

Regulation of the transcription level by insoluble metabolites like sterols- hydroxylated cholesterol (Ch-OH) (See last lecture for descriptions of the key players-SCAP, etc)

Cartoon Level
-The SCAP and SRE-BP proteins are both in the ER membrane
-Low levels of Ch-OH cause the membrane to pinch off into a vesicle with both SCAP and SRE-BP now in the vesicle membrane
-The vesicle fuses with the Golgi membrane
-The membrane associated proteases S-1-P and S-2-P and located in the Golgi membrane
-These proteases cleave of the DNA binding domain of SRE-BP, which becomes soluble and moves to the nucleus
-S-1-P cleaves in the lumen of the golgi, while S-2-P actually cleaves within the membrane. The C-terminus of SRE-BP is liberated into the cytosol and finds its way to the nucleus
-In the nucleus, the liberated DNA binding domain of SRE-BP interacts with SRE (DNA sequence) forms a complex of transcription factors, and activates transcription for LDL-R and HMG-CoA reductase

With High Ch levels (and therefore high Ch-OH levels)
SCAP binds sterol, interacts with SRE_BP and InSIG in the ER membrane
-NO movement to the golgi
-No activation of transcription of LDL-R or HMG-CoA reductase



Cartoon of transcriptional regulation

examples of SRE-BP regulated promoters (contain the SRE DNA sequence)
-LDL-R, HMG-CoA reductase, HMG-CoA synthase, Farnesyl PP synthase, Squalene synthase, AcetylCoA carboxylase, Fatty Acid synthase

There are binding sites at the 5'-ends of the genes for many different transcription factors shown in cartoon

There is a complex regulation of the partitioning of AcetylCoA to make Ch and TAG and phospholipids at the transcriptional level

MODULE 3
Translation

Outline for Module 3

- I. Overview
  - A. Players
  - B. Initiation, elongation, termination, fidelity (main focus= elongation and fidelity)
  - C. Cartoon overview
- II. New Methods
  - A. Reductionist Approach- Isolate components and put them back together- try to get biochemical activity- allows manipulation of individual components.
  - B. Use antibiotics- freeze machine in defined states to assist in cryoEM (electron microscopy) ~10-30 angstrom resolution and x-ray methods ~2.4 angstrom resolution EM data can be fitted using atomic resolution data from crystallography
  - C. Chemical methods
    1. crosslinking (3-D information)
    2. photoaffinity labeling (3-D info)
    3. hydroxide radical footprinting (Noller, showed no proteins involved in peptide bond formation
    4. fluorescence methods
       - a) monitor protein conformational changes
       - b) protein-protein or protein-nucleic acid interactions (both in vitro and in vivo
    5. Pre-steady state kinetics-> role of GTP
- III. tRNA synthases- Fidelity
- IV. G-proteins (use GTP)-elongation process
    1. EF-Tu (EF = elongation factor)
    2. EF-G

    Switch?? Motor?? How do you distinguish between the two?
- V. Peptide bond formation- RNA catalysis (RNA world?)
- VI. New Technology- incorporation of unnatural amino acids into any protein inside the cell

I. Overview

PLAYERS:

1. tRNA- carries amino acids that will be incorporated into the peptide chain

SEE page 1 handout 3a for pictures of the flattenend and 3-D structures

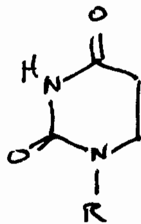
Locate the TpsiC loop, psi = pseudouridine, the variable loop, the anticodon loop and the D loop, D = dihydrouracil

Always has the sequence ACC at 3' end
The "A" carries the amino acid
Anticodon region interacts with mRNA

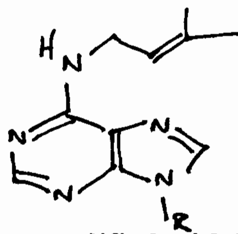
MEMORIZE the structures of nucleic acids and base pairing. This is part of the basic biochemistry vocabulary

tRNAs often have many modified nucleic acids

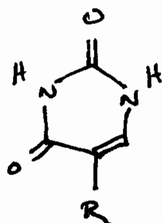
examples:



dihydrouracil (saturated uracil)



Adenine modified with IPP



Pseudo uracil (attached to ribose through a carbon)