Lecture #13

Lecture 13
3/3/04

For next time, read for Module 3: Voet&Voet 959-1004, or an equivalent biochemistry chapter on the translation process

Today & tomorrow: Leadley lecture at 4pm

So far with Cholesterol (Ch), we have discussed:
biosynthetic pathway (parallels with FAS, new ways of forming C-C bonds)
Now we are focusing on regulation: How is Ch sensed? (It is an insoluble molecule)
What is controls the balance between dietary intake of Ch and synthesis?

Last time we started talking about the Brown and Goldstein experiments that led to the discovery of the LDL-receptor.
Remember the working hypothesis:

Liver cells with LDL-receptors
Apo protein B on LDL particle has a patch recognized by the LDL-receptor
Receptors MUST BE CLUSTERED!

-Each receptor has a "zip code" or amino acid tag –NPVY, that attracts the protein clathrin
-Membrane pinches off to form clathrin coated vesicle
-Uncoating, clathrin is removed
-Vesicle fuses with endosome (compartment with pH~6), the receptor stays in the membrane while LDL is left in the interior of the endosome
-Receptor can bud off and return to outer membrane (recycled)
-endosome fuses with lysosome (compartment with low pH ~5)
-lysosome is a bag of proteases and hydrolytic enzyme that break down the apo protein into amino acids and de-esterify Ch esters
-release free Ch

The B&G experiments compared patients with FH (familial hypercholesterolemia) with normal patients. Ch regulation actually occurs in hepatocytes (liver cells), but is faithfully replicated in some fashion in skin fibroblast cells (easier to study)

Expt. 1&2
p8. handout 2d "Regulation of HMG-CoA reductase"

response of normal and FH patient cells to removal (1) and addition (2) of Ch containing lipoproteins to the media.

Results: Normal cells respond to changes in the concentration of lipoproteins in the media, by regulating the activity of HMG-CoA reductase (changes amount of Ch synthesis)

FH cells can't sense Ch in media, the levels of HMG-CoA reductase activity remain high and constant.

Conclusion: Feedback regulation via Ch is altered in FH patients relative to normal patients.

This suggested the hypothesis that Ch was a feedback inhibitor of HMG-CoA reductase.

Expt 3
Can we get Ch inside the cell easily?
Yes, mix with ethanol, goes into cell through passive diffusion.
Then, examine HMG-CoA reductase activity

Both the FH patient and control responded to the added Ch by decreasing HMG-CoA activity!

Implies that this is not classic feedback inhibition.
The FH defect must be in the mechanism of bringing Ch into the cell.

Expt. 4
p. 8 handout 2d, "Binding, Internalization, and Degradation of LDL by Normal Fibroblasts"

Monitor whether Ch in LDL particles can get into normal cells.
Methods: labeling with radioisotopes and scintillation counting.

-Labeled apoprotein B with radioisotope ^{125}I on tyrosines
(^{125}I is "hot," so a very sensitive assay)
-Label Ch with ^{3}H and fatty acids (FA) with ^{14}C
-Monitor isotopes by scintillation counting

-Take LDL particle with ^{125}I labeled protein and incubate cells at 4degreesC
At this temperature, the cell membrane is not fluid, and LDL can bind to receptors but won't be brought into the cell
-Wash away unbound LDL
-Raise temperature to 37degC
-monitor LDL that stays on surface versus LDL internalized (need a method to distinguish these)
Over time, the amount of surface bound LDL decreases, and the amount internalized increases (see figure p. 8 handout 2d)

-Once internalized, apoB is degraded in the lysosome, leaving iodinated tyrosine
Protein is insoluble in acid (precipitates), amino acids are acid soluble
-add trichloroacetic acid and compare insoluble to soluble labeled tyrosine to determine how much protein is intact vs. degraded
after a 10min lag phase, the amount of degraded apoB increases.

THEN, use these same experiments to compare FH to control cells
p.8 handout 2d, "LDL processing in normal vs. FH fibroblasts"

results for FH cells (see figures)
-LDL does not bind
-almost no internalization of LDL
-almost no hydrolysis of apoB (not much gets into cell in the first place)
-no change in rate of Ch synthesis
-almost no esterification of labeled Ch

The structure of the LDL receptor – ref. for structure Science, **298**, 2353 (2002)
p.7 handout 2d, "catch and release"
-7 repeats (sequences of the same ~40 amino acids) in LDL binding region
-only 1 transmembrane-spanning region
hypothesis
inside endosome-> pH drops-> change protonation state -> more affinity of LDL binding region for "Beta-propeller" region of LDL-receptor than for LDL particle

Mutations in the LDL receptor
1)no receptors
2)receptors can't go from golgi to plasma membrane
3) receptors reach surface of membrane but can't cluster
4) receptors can reach surface and cluster, but can't bind LDL
Defects and genetics can help us understand the processes occurring the cell

NEW regulatory method: How and why can the LDL-receptor and HMGCoA reductase respond to Ch?

Regulation at the TRANSCRIPTIONAL LEVEL (conversion of DNA->RNA)
A STEROL is the signal (a sterol is a form of hydroxylated Ch)

Hypothesis: Low levels of Ch and thus of sterol, result in a need for more LDL-receptors (to bring Ch into cell) and HMGCoA reductase (to synthesize more Ch)
Low Ch ->low sterol-> generate increased amounts of a transcription factor (TF) that activates a wide range of genes (including those for LDL-receptor and HMGCoA reductase)

PLAYERS:
1) Sterol responsive element-binding protein (SRE-BP) -> TF

-located in ER membrane (endoplasmic reticulum)
-Two transmembrane regions
-Domain near N-terminus with a helix-loop-helix motif (typical DNA binding motif)

2) SRE (sterol recognition element

The sequence of DNA recognized by the TF (SRE-BP)

3) SCAP (Sre-bp Cleavage Activating Protein)

-located in ER membrane
-7 transmembrane regions
-within membrane spanning region is a "sterol sensing domain"
(a sterol sensing domain is also found in the HMGCoA reductase)

4) InSIG (Insulin Sensitive Gene)

-located in ER membrane

5) S-1-P

-Serine protease

6) S-2-P

-metallo-protease with Zn^{2+}

Working Hypothesis: The transmembrane portion of the TF (SRE-BP) is cleaved within the membrane to liberate the TF that travels to the nucleus.