Lecture 35
5/12/04

Review Sunday 1-4pm room 2-147
TD review starts at 2:30

Continued from lecture 34 (see cartoon diagram)
Degradation through poklyubiquitination- (eucaryotes)

Specificity comes from E3 (remember ~100 E3's inside the cell)
You could imagine one type of E3 would interact with positively charged N-terminal amino acid, and another would recognize hydrophobic N-term.
Several ubiquitins are added on to a lysine on the protein by E3 and E2
Ubiquitination may have many roles inside the cell- we are only discussing its role in protein degradation in yeast.

Roles of E1,E2,E3 in ubiquitination (See handout 4d for cartoons of all of this)

1) ATP activated Ub through adenylation

2) E1 binds covalently to C-term of Ub through a thioester

Need fewer E1-> higher turnover rate than E2, E3

3) Ub transferr to E2, again as a thioester

4) Substrate needs N-end amino acid + lysine-> E3 interacts with substrate and E2 to catalyze attachment oof Ub to lysine through isopeptide linkage

5) Next Ub is added onto the lysine 48 of the previous Ub (E2, E3 repeat same process until 3-4 Ub are added)

6) PolyUb is reqcognized by the 19S cap to human proteosome and the substrate is degraded

7) Ub can be cleaved off intact and then reused

Example of proteins targeted for degradation (regulation through rapid degradation)
TF= transcription factor- heterodimer of alpha and beta
Acts as "oxygen sensor", when there is a lack of oxygen, TF activates transcription of a # of genes
Can stimulate angeogenesis- new blood vessel growth to find more O_2

To deactivate this transcription, degrade 1 of the 2 subunits

In the presence of normal O2, there is post-translational modification to hydroxylate a proline on the alpha subunit. This interacts with E3, targets alpha for degradation by the proteosome

This is how the system is capable of "oxygen sensing" without O2, the reaction can't occur, alpha is not degraded, transcription is activated

Quick 5.08 Summary

Biochemistry

-amazing complexity

-must relate in vitro studies to in vivo reality

-Macromolecular machines- conserved through 3 kingdoms (use simpler bacterial system as a starting point for understanding)

-FAS, PKS, NRPS

-Ribosome, translation

-"Foldosomes," "Proteosomes"

Methods

1) reconstitution – self-assembly
2) chemical probes (photoaffinity labeling, crosslinking, fluorescence)
3) site directed mutagenesis, unnatural amino acids
4) natural product inhibitors- freeze conformation
5) cryoEM, x-ray, NMY structure
6) kinetics (dynamics) pre-steady state and steady state
7) electrophoresis
8) use of radioactivity
9) mass spectrometry
10) FRET