Chap. 11--Regulation

• Substrate and Product
• Allosteric Enzymes (up/down)
  – ATCase
• Colavent Modification of Enzymes (on/off)
  – Phosphorylation (glycogen)
  – Proteolytic cleavage (eating and blood)
Substrate level control

Enzymes work rapidly at high [S]

Enzymes are inhibited by high [I]

Ex--hexokinase is stimulate by glucose and inhibited by G6P.
Why regulate enzyme activity?

**Need to respond to environmental signals (general energy/ ATP level)**

**Need to coordinate reaction pathways (use or store glucose?)

Need more than substrate and product control--Molecules not related to these may be important.
4 different enzymes catalyze this series of reactions.

Product E inhibits the first enzyme.

Feedback regulation
L30 represses both splicing translation of its transcript
Purine synthesis proceeds by a branched pathway.

Overall levels may be regulated before the branch (by pyrimidines).

A and G levels are reciprocally regulated where GMP inhibits the G branch and AMP inhibits the A branch.
Allosteric Enzymes

Multisubunit

T & R Conformations

Low [S] slow

High [S] fast
ATCase

Stimulated by ATP

Inhibited by CTP
(a) ATCase: T state

(b) ATCase: R state
ATCase has 6 catalytic and 6 regulatory subunits.

Subunit interfaces are very important for allostery

Activators bind Regulatory Subunit R state preferentially

Inhibitors bind Regulatory Subunit T state preferentially
Zn$^{2+}$ is important for structural (not catalytic) reasons.
Phosphorylation often serves as an on/off switch for enzymes.
Proteases are produced as inactive zymogens (red) and are (auto)activated by proteases. Trypsin cleaves many zymogens.
The active site of chymotrypsin is formed by proteolytic cleavage enabling Nter 16 to form a salt bridge with Asp 194.
Trypsin cleaves initially.

Residues 13-15 and 146-149 are removed autocatalytically so the final structure is held together by S--S linkages.
Thrombin is a serine protease. Hemophilia is caused by mutations in proteins in these pathways.
The gene for Factor VIII is on the X chromosome so males have only one copy and usually die young.
Red blood cells get caught in fibrin network. Thrombin is very precise.
Inappropriate clotting leads to heart attacks and strokes.

Clot removal requires plasmin whose production is catalyzed by plasminogen activator (TPA).

Recombinant TPA is being used therapeutically.
Experimental Approaches
Main Questions

• Biosynthetic Pathway?
• Reaction Mechanisms?
• Enzymes?
• Control Mechanisms?
  – (effectors, inhibitors, coordination)
Experimental Approaches

• Feeding Experiments
  – (normal and mutant)
  – Radiolabel experiments

• In vitro approach & enzyme purification
  – reconstitution experiments

• In vivo approach
  – NMR for $^{13}$C or $^{31}$P
Urine analysis--Basis of drug testing
E. coli feeding experiment--Make isotopically labelled Reactants or proposed intermediates, Allow metabolism to occur, recover isotopically labelled Molecules.
In-born errors of metabolism are often detected by accumulation of (poisonous) intermediates.
ATPase--

When isolated in the lab initially

$ATP = ADP + phosphate$

But in vivo

$ADP + Phosphate = ATP$

Enzymes catalyze forward and reverse reactions
Purified enzymes may not exactly mimic in vivo behavior.
Reconstitution Experiments

Isolate biochemical complex

Purify Separate Ingredients

Reassemble the Complexes and Test for Activity

Can any component be omitted? Mutated? Substituted by Homologous protein from another organism?
Ribosomal Reconstitution

Any one protein may be omitted---> 80% active in peptide bond formation.>>>>>Catalytic Site not on a protein??
Before exercise
Creatine phosphate
ATP $\gamma,\alpha,\beta$ phosphates

Pi

1 min. exercise

19 min. exercise

10 min. recovery
In Vivo Experiments

Glucose monitoring and pumps for diabetics

1. Test glucose in urine (initial diagnosis)

2. Test glucose in blood (OUCH!!)
   Dipstick biochemical analysis.

3. Insert glucose monitors
   (biochemical or spectroscopic analysis)
Newer in Vivo Experimental Approaches (Based on Genomics)

DNA Chip Technology

DNA → mRNA → protein

Western Blots and 2D Total Protein Gels

See page 254

SDS → Charge → Hybridize Antibody
Direct Methods of Measuring Protein Expression Levels

DNA chips--high density DNA arrays --
1. Attach DNA sequences of interest to chip (PCR products for each gene with Fluorescent label)
2. Hybridize with mRNA of interest (& controls)
3. Analyze with Fluorescent microscope
The effects of aging on gene expression in the hypothalamus and cortex of mice

Cecilia H. Jiang*, Joe Z. Tsien, Peter G. Schultz*, and Yinghe Hu*

* Genomics Institute of the Novartis Research Foundation, 3115 Merryfield Row, San Diego, CA 92121; and Department of Molecular Biology, Washington Road, Princeton University, Princeton, NJ 08544
Table 1. Gene expression changes in the hypothalamus from young (2 months) and old (22 months) BALB/c mice

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic enzymes</td>
<td></td>
</tr>
<tr>
<td>NADH-ubiquinone oxidoreductase KFYI subunit</td>
<td>2.8</td>
</tr>
<tr>
<td>NADH oxidoreductase subunit MWFE</td>
<td>3</td>
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<tr>
<td>NADH-ubiquinone oxidoreductase chain 4L</td>
<td>2.1</td>
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<tr>
<td>NADH-ubiquinone oxidoreductase SGDH subunit</td>
<td>2.5</td>
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<tr>
<td>Cytochrome c oxidase subunit VIIb</td>
<td>2.2</td>
</tr>
<tr>
<td>ATP synthase O subunit</td>
<td>2.2</td>
</tr>
<tr>
<td>ATP synthase -subunit</td>
<td>2.7</td>
</tr>
<tr>
<td>Sarco/ endoplasmic reticulum Ca2+-ATPase</td>
<td>2.9</td>
</tr>
<tr>
<td>Na/K-ATPase</td>
<td>11</td>
</tr>
<tr>
<td>H+ ATPase subunit E</td>
<td>6.1</td>
</tr>
<tr>
<td>H(+)-ATPase (mvp)</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Genomic Expression Programs in the Response of Yeast Cells to Environmental Changes

Audrey P. Gasch, Paul T. Spellman, Camilla M. Kao, Orna Carmel-Harel, Michael B. Eisen, Gisela Storz, David Botstein, and Patrick O. Brown

Departments of Biochemistry and Genetics, Stanford University School of Medicine, Stanford, CA 94305-5428; Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-5430; Lawrence Berkeley National Labs and Department of Molecular and Cellular Biology, University of California Berkeley, Berkeley, CA 94720; and Howard Hughes Medical Institute, Stanford, CA
Glycolysis is Anaerobic
(no net oxidation)
In general, futile cycles are avoided.
(except if heat is needed).
Complete oxydation to CO$_2$

And ultimately O$_2$ is reduced to H$_2$O
Amino acids are directly recycled and used to make other molecules (neurotransmitters) that produce nitrogenous waste.
Lipids and fatty acids are more “energy dense” than carbohydrates.

You animals can’t make sugar from fat (but plants can)!
In Michael Faraday’s *Chemical History of a Candle*, respiration is compared to a candle burning. But energy is released gradually in a series of reactions.

Fuel efficiency is higher for less oxygenated fuels

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} \quad \Delta G^\circ = -2870 \text{ kJ/mol}
\]

\[
\text{C}_{16}\text{H}_{32}\text{O}_2 + 23\text{O}_2 \rightarrow 16\text{CO}_2 + 16\text{H}_2\text{O} \quad \Delta G^\circ = -36.74 \text{ kcal/g}
\]

\[
\text{C}_{16}\text{H}_{32}\text{O}_2 + 23\text{O}_2 \rightarrow 16\text{CO}_2 + 16\text{H}_2\text{O} \quad \Delta G^\circ = -93.0 \text{ kcal/g}
\]
Photosynthesis

*Uses light energy
To make proton Gradients and ATP.

*Uses CO₂ to make sugars
Regulation of metabolism enzymes

1. Enzyme production (DNA or RNA level--steroid hormones)
2. Compartmentalization and enzyme clustering
3. Covalent modification (phosphorylation)
4. Inhibitors and effectors (BPTI/trypsin)
Enzyme regulation--

Committed Step vs. Distributed model
Glycolysis and Phosphofructokinase (PFK)

Fructose-6-phosphate + ATP → Fructosebisphosphate + ADP
<table>
<thead>
<tr>
<th>Hormone Class</th>
<th>Target Organ</th>
<th>Protein(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoids</td>
<td>Liver</td>
<td>Tyrosine aminotransferase, Tryptophan oxygenase, α-Fetoprotein (↓), Metallothionein</td>
</tr>
<tr>
<td>Liver, retina</td>
<td>Glutamine synthetase</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Phosphoenolpyruvate carboxykinase</td>
<td></td>
</tr>
<tr>
<td>Oviduct</td>
<td>Ovalbumin</td>
<td></td>
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<tr>
<td>Pituitary</td>
<td>Pro-opiomelanocortin</td>
<td></td>
</tr>
<tr>
<td>Estrogens</td>
<td>Oviduct</td>
<td>Ovalbumin, Lysozyme</td>
</tr>
<tr>
<td>Liver</td>
<td>Vitellogenin, apo-VLDL</td>
<td></td>
</tr>
<tr>
<td>Progesterone</td>
<td>Oviduct</td>
<td>Ovalbumin, Avidin</td>
</tr>
<tr>
<td>Uterus</td>
<td>Uteroglobin</td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>Prostate</td>
<td>Aldolase</td>
</tr>
<tr>
<td>Kidney</td>
<td>β-Glucuronidase</td>
<td></td>
</tr>
<tr>
<td>Oviduct</td>
<td>Albumin</td>
<td></td>
</tr>
</tbody>
</table>

Hormones that regulate protein synthesis
Glucocorticoid acts on DNA transcription
Steroid receptors have a conserved “zinc finger” DNA binding domain.
Estrogen Receptor

Activities

DNA binding
Transcriptional activation
Protein dimerization
Hormone binding
Co-factor differences in catabolism vs anabolism

NAD\(^+\) vs NADPH
NADPH = N-ribose-5’-Phos-Phos-ribose-(2’ Phos)-adenine
Can a catabolic enzyme which uses NAD+ be “re-engineered” to use NADPH?

Biochemistry, 32, March 1993, pp 2739

Compare sequences of

Dihydrolipoamide dehydrogenase (NAD+)

Glutathione reductase (NADPH)