Minor Covalent Modification of Enzyme Activity Adenylation

An alternative mechanism of covalent modification by ATP involves adenylation transfer to an AMP adenylyl group from ATP to the enzyme with the accompanying formation of inorganic pyro PO_4. While this is not known to occur in mammalian systems, adenylation is responsible for regulation of the glutamine synthelase and RNA polymerase in *E. coli*, e.g. glutamine synthetase.

$$\text{ATP} + \text{glutamate} + NH_3 \quad \text{GS} \quad \text{ADP} + \text{glutamine} + P_1$$

Glutamine synthetase is converted from its relatively active form to the less active form by the transfer of 12 mol of AMP from ATP to specific tyrosine residues in each of the 12 sub-units of the enzyme, to yield covalently adenylyl derivatives of the phenolic OH groups of the tyrosines. The enzyme may also be enzymatically de-adenylated to its active form.

Importance of PO_4lation/De-PO_4 lation.

A typical mammalian cell possesses thousands of PO_4lated proteins and several hundred protein Kinases and protein phosphatases that catalyse their inter-conversion. The abundance of many protein Kinases in cells is an indication of the great importance of protein PO_4lation in cellular regulation. Exactly 113 protein Kinase genes have been recognized in yeast while 868 putative protein Kinae genes have been identified in the human genome. The case of inter-conversion of enzymes between their phosphor- and dephospho-forms accounts for the frequency of PO_4lation – dePO_4-lation as a mechanism for the control of metabolic pathways. PO_4lation-de $P0_4$lation permits the functional properties of the affected enzyme to be altered only for as long as it serves a specific need. Once the need has passed, the enzyme can be converted back to its original form, ready to respond to the next stimulatory event.

Another factor underlying the widespread use of protein PO_4lation-de-PO_4lation lies in the chemical properties of the phosphoryl group itself. In order to alter an enzyme functional properties, any modification of its chemical structure must influence the protein's 3-dimensional structure. The high charge density of protein-bound phosphoryl group – generally -2 at physiologic pH – and their propensity to form salt bridges with arginy residues make their potent agents for modifying protein structure and

function PO_4lation generally targets amino acids distant from the catalytic site itself. Consequent conformational changes then influence an enzyme's intrinsic catalytic efficiency or other properties.

PO_4lation and de-PO_4lation provide for short-term readily reversible regulation of metabolite flow in response to specific physiologic signals. They are both under direct neural and hormonal control.

PO_4lation can also alter the location of an enzyme within the cell. It can alter the enzyme's susceptibility to proteolytic degradation, or responsiveness to regulation by allosteric ligands.

The third level at which metabolic regulation is exerted is through the genetic control of the rate of enzyme synthesis or degradation. The rate of a given metabolic sequence must depend on the conc. of the vetuse form of the enzyme in a sequence which in turn is the result of a balance between the rate of its synthesis and the rate of its breakdown. Body proteins are continuously synthesized and degraded – a process referred to as Protein Turnover. While the steady state concs of some enzymes and other proteins remain essentially, or constitutive, over time, the concs of many enzymes are influenced by a wide range of physiologic, hormonal or dietary factors.

Control of Enzyme Synthesis.

The amounts of enzyme synthesized by a cell are determined by transcription regulation if the gene encoding a particular enzyme protein is turned on or off, changes in the amount of enzyme activity soon follow. Induction – activation of enzyme synthesis and repression which is the shut-down of enzyme synthesis, are important mechanisms for the regulation of metabolism. By controlling the amount of an enzyme that is present at any moment, cells can either activate or terminate various metabolic routes.

Inducible enzymes of humans include tryptophan pyrrolase, threonine dehydrase tyrosine-α-Ketoglutarate amino transferase, enzymes of the urea cycle, HMG-CoA reductase and cytochrome P450. Both induction and repression involve cis elements, specific DNA sequences located upstream of regulated genes (Operons) and trans-acting regulation proteins, e.g. Dietary Cholesterol decreases hepatic synthesis of cholesterol. This feedback regulation does not involve feedback inhibition, rather, HMG-CoA reductase, the rate-limiting enzyme of cholesterogenesis is affected.

Regulation in response to dietary cholesterol involves curtailment by cholesterol or a cholesterol metabolite of the expression of the gene that encodes HMG-CoA reductase.

Control of Enzyme Degradation

Enzyme/protein degradation poses a real hazard to cellular processes. To control this hazard, protein degradation is compartmentalized, either in macromolecular structures known as Proteasomes or in degradative organelles such as lysosomes. Protein degradation within lysosomes is largely non-selective, selection occurs during lysosomal uptake. Proteasomes are found in encaryotic as well as prokaryotic cells. Regulation of protein levels through degradation is an essential cellular mechanism. It is both rapid and irreversible.

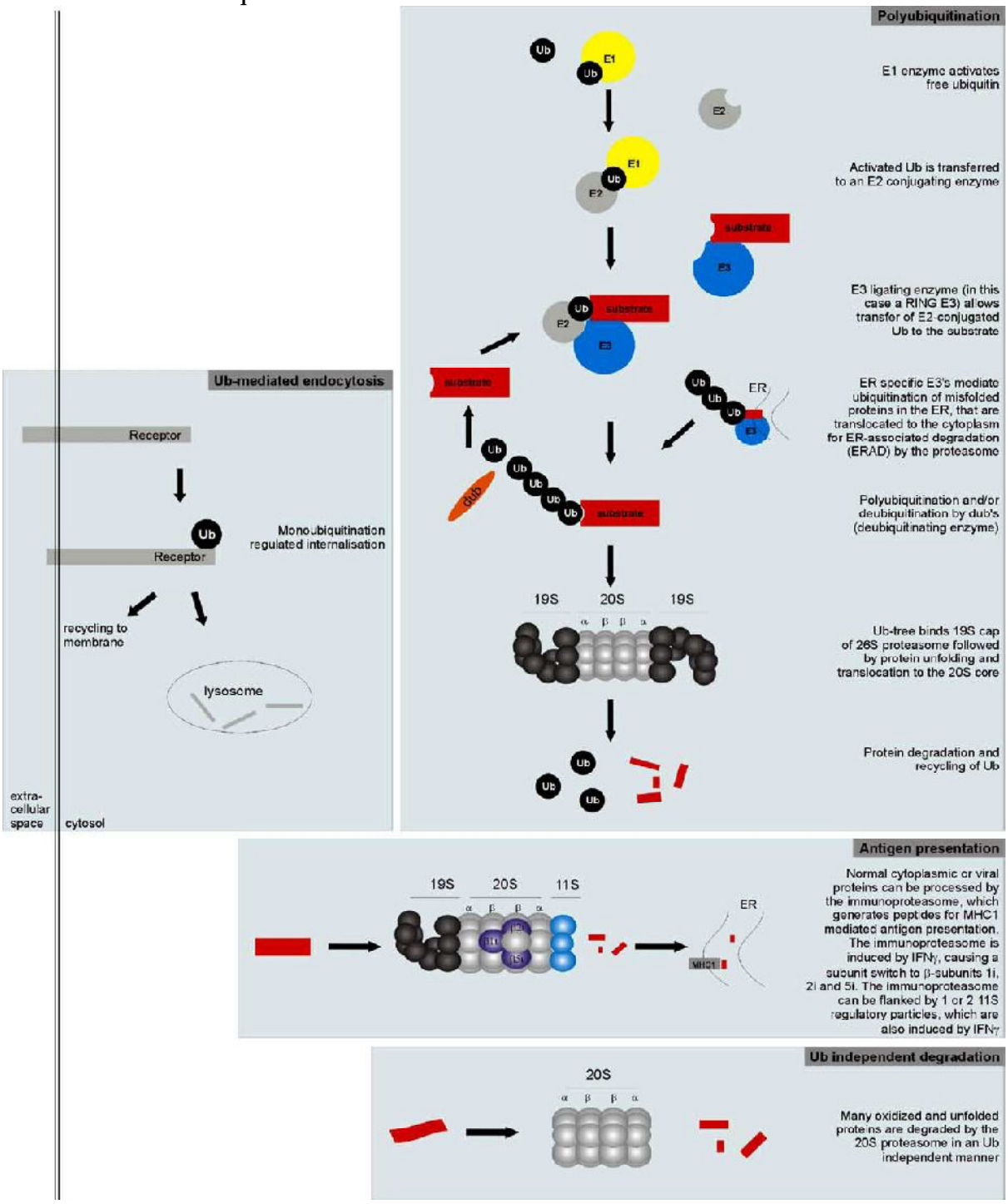
In many animals, many proteins are degraded by the ubiquitin-proteasome pathway. Eukaryotic cells contain 2 forms of proteasomes – the 20S proteasomes and its larger counterpart, the 26S proteasome. The eukaryotic 26S proteasome is made up of a 20S proteasome and 2 additional substructures known as 19S regulators (also called 19S caps or PA 700). Overall the 26S proteasomes has 2 copies each of 32 to 34 distinct sub-units, 14 in the 20S core and 18 to 20 in the cap structures. The 20S are contains 7 different kinds of α-subunits and 7 different kinds of β-sub units. The sub-units are arranged in the form of a hollow cylinders.

19S 20S 19S

The active sites of the proteolytic sub-units face the interior of the cylinder, thus preventing indiscriminate degradation of cellular proteins.

The most common mechanism by which proteins are labeled for proteasome degradation is Ubiquitination – the covalent attachment/ligation of one or more ubiquitin molecules to the protein. Ubiquitin is a small, approximately 75 residue protein that is highly conserved among eukaryotes. Three additional proteins are involved in ubiquitination. They are E_1, E_2, and E_3 ligases.

Mechanism of Ubiquitination



(1) E_1 Ubiquitin – activating enzyme

E_1 (Ubiquiting-activating enzyme) becomes attached through a thioester bond to the C-terminal Glu residue of ubiquitin through an ATP-driven formation of an activated ubiquitin-adenylate intermediate. Ubiquitin is then transferred from E_1 to an SH group on E_2, the ubiquitin carrier protein, a family of at least 7 different small proteins, many of which are heat shock proteins. In protein degradation, E_2-S-ubiquinin transfers ubiquitin to free amino groups on proteins selected by E_3 , the ubiquitin-protein ligase. Upon binding a protein substrate, F_3 catalyses the transfer of ubiquitin from E_2S-ubiquitin to free amino groups (usually Lys-E-NH_2) on the protein. More than one ubiquitin may be attached to a protein substrate. Ubiquitin has 7 Lysine residues at positions 6, 11, 27, 29, 33, 48 and 63. E_3 plays a central role in recognizing and selecting proteins for degradation. E_3 selects proteins by the nature of the N-terminal amino acid. Proteins must have a free α-amino acid terminus to be susceptible. Proteins having either Met, Ser, Ala, Thr, Val, Gly or Cys at the amino terminus are resistant to the ubiquitin-mediated degradation pathway. However, proteins, yaving Arg, Lys, His, Phe, Lyr, Try, Leu, Asn, Gln, Asp or Glu – N terminal are susceptible. Most proteins with susceptible N-terminal residues are not normal intra-cellular proteins but tend to be secreted proteins in which the susceptible residue has been exposed by action of a signal peptidase.

The ubiquitin-proteasone pathway is thus responsible both for the regulated degradation of selected cellular proteins, e.g;. cyclins, in response to specific intra or extra-cellular signals and for the removal of defective or aberrant protein species. The key to the versatility and selectivity of the ubiquitin proteasome system resides in both the variety of intra-cellular E_3 ligases and their ability to discriminate between different physical or conformational states of a target protein. Thus, the ubiquitin – proteasome pathway can selectively degrade proteins whose physical integrity and functional competency has been compromised by the loss of or damage to a prosthetic group, oxidation of systems or histidine residues, or deamidation of asparagines or glutamine residues. Recognition by proteolytic enzymes also can be regulated by covalent modifications such as PO_4lation, binding of substrates or allosteric effectors or association with membranes oligonucleotides or other proteins. A growing body of evidence suggests that dysanctions of the ubiquitin-proteasome pathway contribute to the accumulation of aberrantly folded protein species xteristic of several neurodegenerative diseases.

(4) Metabolic regulation can also be effected by hormones. Hormones are chemical messengers elaborated by endocrine glands that pass via the blood to certain target tissues where they stimulate or inhibit specific metabolic activities. For instance, both insulin and glucagon regulate the metabolism of glucose.

Other ways of Regulating Metabolic pathways

(1) Covalent activation of zymogens.

Most proteins become fully active when their synthesis is completed and they spontaneously fold into their nature, 3-dimensional conformation/structures. Some proteins, however, are synthesized and secreted as in active precursors known as Proproteins. The proteins of enzymes are termed Proenzymes or Zymogens. They acquire full activity only upon specific proteolytic cleavage of one or several of their peptide bonds. Unlike allosteric regulation or covalent modification, zymogen activation is an irreversible process. Activation of enzymes and other physiologically proteins by specific proteolysis is a strategy frequently exploited by biological systems to switch on processes at the appro-priate time and place. Proteins synthesized as proproteins include the hormone insulin, the digestive enzymes pepsintrypsin and chymotrypsin, several factors of the blood clatting and blood clot dissolution cascades and the connective tissue protein collagen

Origin	Zymogen	Active Protein
Pancreas	Trypsinogen	Trypsin
Pancreas	Chymotrypsinogen	Chymo trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelestase	Elastase
Stomach	Pepsinogen	Pepsin
	Pnansulin	Insulin
	Procollagen	Collagen

Insulin – Secreted as Proinsulin – 86 residue. Proteolytic removal of residues 31 to 65 yields insulin.

Proteolytic enzymes of the digestive tracts. Enzymes of the digestive tract that hydrolyse dietary proteins are synthesized in the stomach and pancreas

as zymogens. When these enzymes are secreted into the GIT, they are coverted into their active forms by the selective hydrolytic cleavage of one or more specific peptide bonds in the zymogen molecule.

pepsin

Pepsiinogen ---------- pepsin + peptides free pepsin at low pH removes

H^+

42 amino acid residues as a mixture of peptides from the N-terminal end of pepsinogen.

Entero

Trypsinogen ---------- Trypsin + hexapeptide. Entero Kinase converts

Kinase

Trypsinogen to trypsin by removal of a hexapeptide from the N-terminal end. The activation of chymotrypsin however reps. a peculiar and interesting example. Chymotrypsinogen is a 245 residue polypeptide chain X-linked by 5 disulphide bonds.

Chymotrypsinogen is converted to an enzymatically active form called II-Chymotrypsin when trypsin cleaves the peptide bond joining Arg^{15} and Ile^{16}. The enzymatically active II-Chymotrypsin acts upon other II – Chymotrysin molecules, excising 2 dipeptides; Ser^{14} Arg^{15} and Thr^{14} Asn^{148}. The end-product of this series of proteolysis is the nature protease α-Chymotrypsin, in which the 3 peptide chains, A (residues 1-13) B (residues 16-146), and C (residues 149 – 245), remain together because they are linked by 2 disulphide bonds one from A to B and one from B to C. It is interesting to note that the coversion of inactive chymotrypsinogen to active II-Chymotrypsin requires the cleavage of just one particular peptide bond. The Zymogens are kept from exerting proteolytic activity on intracellular proteins so long as they remain within the cells in which they are made. They are turned on to generate the active form only after they are secreted into the GIT. As mentioned earlier, this type of covalent regulation is one-way: there are no known enzymatic reactions which can transform these 3-enzymes back into their respective zymogens.

Blood Clotting

The formation of blood clots is the result of a series of zymogen activations. The amplification achieved by this cascade of enzymatic activations allows blood clotting to occur rapidly in response to injury.

Thrombin cleaves peptides rich in –ve charge from fibrinogen, converting II to fibrin. Fibrin aggregates into ordered fibrous arrays that are subsequently stabilized by covalent X-links. Thrombin specifically cleaves Arg-Gly peptide bonds.

2. Isozymes

Anlther type of regulation of metabolic activity is through the participation of isozymes. Isozymes are multiple forms of the same enzyme that occur in a single species of organism or even in a single cell. A classic, e.g. is mammalian lactate DH, which exists as 5 different isozymes in the tissues of rat and other vertebrates. They all catalyse the same overall reaction.

Lactate + NAD + Pyruvate + NADA + HT

(1) All 5 isozymes have the same mut, about 134,000

(2) All contain 4 polypeptide chains eachof mut 33,500

(3) The 5 isozymes consist of 5 different combinations of 2 different kinds of polypeptide chains designated A and B. The isozyme predominating in skeletal muscle has 4 identical A chains and is designated A4. Another which predominates in heart has 4 identical B chains and is designated B4. The other 3 isozomes have the composition A_3B,A_2B_2 and AB_3.

(4) Although tye all catalyse the same reaction, they differe in their dependence on substrate conc., particularly pyruvate, as well as their Vimax values when pyruvate is the substrate. The isozyme A4, xteristic of skeletal muscle and embryonic tissues, reduces pyruvate to lactate at a relatively high rate. The B4 isozyme on the other hand, Xteristic of the heart and other red muscles, reduces pyruvate at a relatively low rate. Mroeover, the dehydrogen nation of lactate catalysed by the B4 isozyme is strongly inhibited by pyruvate. The other LDH isozymes have kinetic properties intermediate between those of the A4 and B4 isozymes in proportion to their relative content of A and B chains.

(5) If se then compare these kinetic xteristics with the metabolic features of the tissues in which the A4 and B4 isozymes predominate, the function of LDH isozymes becomes clear. Skeletal muscle and embryonic tissue have anaerobic metabolism, thus can convert glucose to lactate via glycolysis. A4 isozyme is thus adapted for this role and has a high Vmax for pyruvate. The heart muscle on the other hand has aerobic metabolism and does not form lactate from glucose. Rather, it oxidizes pyruvate to $C0_2$ without intermediate formation of lactate. The reasons are not also far fetched. Heart muscle cells are rich in mitochondria whereas most skeletal muscles contain relatively few mitochondrin. Free fatty acids carried by serum albumen from the adipose tissue are the major fuel of the heart. The heart uses glycolysis as a source of extra energy.