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Section I. Enzymology of Control by Phosphorylation. 1 The Enzymology of Control by Phosphorylation Original research article Pages Edwin G. Krebs. Download PDF.

It performs its myriad functions through conjugation to a large range of target proteins. A variety of different modifications can occur. The ubiquitin protein itself consists of 76 amino acids and has a molecular mass of about 8. Key features include its C-terminal tail and the 7 lysine residues. It is highly conserved among eukaryotic species: Genes[edit] Ubiquitin is encoded in mammals by 4 different genes. These proteins also share sulfur chemistry with ubiquitin. MoaD, which is involved in molybdenum cofactor biosynthesis, interacts with MoeB, which acts like an E1 ubiquitin-activating enzyme for MoaD, strengthening the link between these prokaryotic proteins and the ubiquitin system. It is also believed that the Saccharomyces cerevisiae protein Urm-1, a ubiquitin-related modifier, is a "molecular fossil" that connects the evolutionary relation with the prokaryotic ubiquitin-like molecules and ubiquitin. Ubiquitination or ubiquitylation is an enzymatic post-translational modification in which a ubiquitin protein is attached to a substrate protein. This process most commonly binds the last amino acid of ubiquitin glycine 76 to a lysine residue on the substrate. The process consists of three main steps: Ubiquitin is activated in a two-step reaction by an E1 ubiquitin-activating enzyme , which is dependent on ATP. The initial step involves production of a ubiquitin-adenylate intermediate. The E1 binds both ATP and ubiquitin and catalyses the acyl-adenylation of the C-terminus of the ubiquitin molecule. The second step transfers ubiquitin to an active site cysteine residue, with release of AMP. This step results in a thioester linkage between the C-terminal carboxyl group of ubiquitin and the E1 cysteine sulfhydryl group. E2 ubiquitin-conjugating enzymes catalyse the transfer of ubiquitin from E1 to the active site cysteine of the E2 via a trans thio esterification reaction. In order to perform this reaction, the E2 binds to both activated ubiquitin and the E1 enzyme. Humans possess 35 different E2 enzymes, whereas other eukaryotic organisms have between 16 and They are characterised by their highly conserved structure, known as the ubiquitin-conjugating catalytic UBC fold. The isopeptide bond is highlighted yellow. E3 ubiquitin ligases catalyse the final step of the ubiquitination cascade. Most commonly, they create an isopeptide bond between a lysine of the target protein and the C-terminal glycine of ubiquitin. In general, this step requires the activity of one of the hundreds of E3s. E3 enzymes function as the substrate recognition modules of the system and are capable of interaction with both E2 and substrate. Some E3 enzymes also activate the E2 enzymes. E3 enzymes possess one of two domains: HECT domain E3s transiently bind ubiquitin in this process an obligate thioester intermediate is formed with the active-site cysteine of the E3 , whereas RING domain E3s catalyse the direct transfer from the E2 enzyme to the substrate. Having levels within the cascade allows tight regulation of the ubiquitination machinery. Multi-monoubiquitination is the addition of one ubiquitin molecule to multiple substrate residues. The monoubiquitination of a protein can have different effects to the polyubiquitination of the same protein. The addition of a single ubiquitin molecule is thought to be required prior to the formation of polyubiquitin chains. The linkage between the two ubiquitin chains is shown in orange. Diagram of lysine linked diubiquitin. Polyubiquitination is the formation of a ubiquitin chain on a single lysine residue on the substrate protein. Following addition of a single ubiquitin moiety to a protein substrate, further ubiquitin molecules can be added to the first, yielding a polyubiquitin chain. K63 chains have also been well-characterised, whereas the function of other lysine chains, mixed chains, branched chains, M1-linked linear chains, and heterologous chains mixtures of ubiquitin and other ubiquitin-like proteins remains more unclear. At least four ubiquitin molecules must be attached to a lysine residue on the condemned protein in order for it to be recognised by the 26S proteasome. Once inside, the proteins are rapidly degraded into small peptides usually 3â€”25 amino acid residues in length. Ubiquitin molecules are cleaved off the protein immediately prior to destruction and are recycled for further use. Instead, they allow the coordination of other processes such as endocytic trafficking ,

inflammation , translation , and DNA repair. K, K, [75] K and M1-linked chains have a fairly linear conformation; they are known as open-conformation chains. K6-, K, and Klinked chains form closed conformations. The ubiquitin molecules in open-conformation chains do not interact with each other, except for the covalent isopeptide bonds linking them together. In contrast, the closed conformation chains have interfaces with interacting residues. Altering the chain conformations exposes and conceals different parts of the ubiquitin protein, and the different linkages are recognized by proteins that are specific for the unique topologies that are intrinsic to the linkage. Proteins can specifically bind to ubiquitin via ubiquitin-binding domains UBDs. The distances between individual ubiquitin units in chains differ between lysine and linked chains. The UBDs exploit this by having small spacers between ubiquitin-interacting motifs that bind lysine linked chains compact ubiquitin chains and larger spacers for lysine linked chains. The machinery involved in recognising polyubiquitin chains can also differentiate between Klinked chains and M1-linked chains, demonstrated by the fact that the latter can induce proteasomal degradation of the substrate.

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Chapter 2 : Methods in enzymology. Volume X. Oxidation and phosphorylation.

The Enzymology o Control f by Phosphorylation EDWIN G. KREBS Howard Hughes Medical Institute and Department of Pharmacology University of Washington Seattle, Washington 1.

W H Freeman ; Search term Section In these instances, a donor molecule provides a functional moiety that modifies the properties of the enzyme. Most modifications are reversible. Phosphorylation and dephosphorylation are the most common but not the only means of covalent modification. Histonesâ€”proteins that assist in the packaging of DNA into chromosomes as well as in gene regulationâ€”are rapidly acetylated and deacetylated in vivo Section More heavily acetylated histones are associated with genes that are being actively transcribed. The acetyltransferase and deacetylase enzymes are themselves regulated by phosphorylation, showing that the covalent modification of histones may be controlled by the covalent modification of the modifying enzymes. Modification is not readily reversible in some cases. Some proteins in signal-transduction pathways, such as Ras and Src a protein tyrosine kinase , are localized to the cytoplasmic face of the plasma membrane by the irreversible attachment of a lipid group Section Fixed in this location, the proteins are better able to receive and transmit information that is being passed along their signaling pathways Chapter The attachment of ubiquitin, a protein comprising 72 amino acids, is a signal that a protein is to be destroyed, the ultimate means of regulation Chapter Cyclin, an important protein in cell-cycle regulation, must be ubiquitinated and destroyed before a cell can enter anaphase and proceed through the cell cycle Table Virtually all the metabolic processes that we will examine are regulated in part by covalent modification. Indeed, the allosteric properties of many enzymes are modified by covalent modification. Phosphorylation Is a Highly Effective Means of Regulating the Activities of Target Proteins The activities of many enzymes, membrane channels, and other target proteins are regulated by phosphorylation, the most prevalent reversible covalent modification. Indeed, we will see this regulatory mechanism in virtually every metabolic process in eukaryotic cells. The enzymes catalyzing phosphorylation reactions are called protein kinases, which constitute one of the largest protein families known, with more than homologous enzymes in yeast and more than in human beings. This multiplicity of enzymes allows regulation to be fine-tuned according to a specific tissue, time, or substrate. The acceptors in protein phosphorylation reactions are located inside cells, where the phosphoryl-group donor ATP is abundant. Proteins that are entirely extracellular are not regulated by reversible phosphorylation. Protein phosphatases reverse the effects of kinases by catalyzing the hydrolytic removal of phosphoryl groups attached to proteins. Examples of serine and threonine kinases and their activating signals. The unmodified hydroxyl-containing side chain is regenerated and orthophosphate Pi is produced. It is important to note that phosphorylation and dephosphorylation are not the reverse of one another; each is essentially irreversible under physiological conditions. Furthermore, both reactions take place at negligible rates in the absence of enzymes. Thus, phosphorylation of a protein substrate will take place only through the action of a specific protein kinase and at the expense of ATP cleavage, and dephosphorylation will result only through the action of a phosphatase. The rate of cycling between the phosphorylated and the dephosphorylated states depends on the relative activities of kinases and phosphatases. This highly favorable free-energy change ensures that target proteins cycle unidirectionally between unphosphorylated and phosphorylated forms. Phosphorylation is a highly effective means of controlling the activity of proteins for structural, thermodynamic, kinetic, and regulatory reasons: A phosphoryl group adds two negative charges to a modified protein. Electrostatic interactions in the unmodified protein can be disrupted and new electrostatic interactions can be formed. Such structural changes can markedly alter substrate binding and catalytic activity. A phosphate group can form three or more hydrogen bonds. The tetrahedral geometry of the phosphoryl group makes these hydrogen bonds highly directional, allowing for specific interactions with hydrogen-bond donors. The free energy of phosphorylation is large. Of the kcal mol-1 kJ mol-1 provided by ATP , about half is consumed in making phosphorylation irreversible; the other half is conserved in the phosphorylated protein.

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Recall that a free-energy change of 1. Hence, phosphorylation can change the conformational equilibrium between different functional states by a large factor, of the order of Phosphorylation and dephosphorylation can take place in less than a second or over a span of hours. The kinetics can be adjusted to meet the timing needs of a physiological process. Phosphorylation often evokes highly amplified effects. A single activated kinase can phosphorylate hundreds of target proteins in a short interval. Further amplification can take place because the target proteins may be enzymes, each of which can then transform a large number of substrate molecules. ATP is the cellular energy currency Chapter The use of this compound as a phosphoryl group donor links the energy status of the cell to the regulation of metabolism. Protein kinases vary in their degree of specificity. Dedicated protein kinases phosphorylate a single protein or several closely related ones. Multifunctional protein kinases modify many different targets; they have a wide reach and can coordinate diverse processes. Comparisons of amino acid sequences of many phosphorylation sites show that a multifunctional kinase recognizes related sequences. It should be noted that this sequence is not absolutely required. Lysine, for example, can substitute for one of the arginine residues but with some loss of affinity. Short synthetic peptides containing a consensus motif are nearly always phosphorylated by serine- threonine protein kinases. Thus, the primary determinant of specificity is the amino acid sequence surrounding the serine or threonine phosphorylation site. However, distant residues can contribute to specificity. For instance, changes in protein conformation may alter the accessibility of a possible phosphorylation site. Activation is often a multistep process initiated by hormones Chapter Cyclic AMP serves as an intracellular messenger in mediating the physiological actions of hormones, as will be discussed in Chapter The striking finding is that most effects of cAMP in eukaryotic cells are achieved through the activation by cAMP of a single protein kinase. This key enzyme is called protein kinase A or PKA. The kinase alters the activities of target proteins by phosphorylating specific serine or threonine residues. As we shall see, PKA provides a clear example of the integration of allosteric regulation and phosphorylation. The activation mechanism is reminiscent of that of aspartate transcarbamoylase. Like that enzyme, PKA in muscle consists of two kinds of subunits: In the absence of cAMP, the regulatory and catalytic subunits form an R2C2 complex that is enzymatically inactive Figure The binding of two molecules of cAMP to each of the regulatory subunits leads to the dissociation of R2C2 into an R2 subunit and two C subunits. These free catalytic subunits are then enzymatically active. Thus, the binding of cAMP to the regulatory subunit relieves its inhibition of the catalytic subunit. PKA and most other kinases exist in isozymic forms for finetuning regulation to meet the needs of a specific cell or developmental stage. The binding of four molecules of cAMP activates protein kinase A by dissociating the inhibited holoenzyme R2C2 into a regulatory subunit R2 and two catalytically active subunits C. How does the binding of cAMP activate the kinase? Each R chain contains the sequence Arg -Arg- Gly - Ala - Ile , which matches the consensus sequence for phosphorylation except for the presence of alanine in place of serine. In the R2 C 2 complex, this pseudosubstrate sequence of R occupies the catalytic site of C, thereby preventing the entry of protein substrates see Figure The binding of cAMP to the R chains allosterically moves the pseudosubstrate sequences out of the catalytic sites. The released C chains are then free to bind and phosphorylate substrate proteins. The residue catalytic subunit has two lobes Figure ATP and part of the inhibitor fill a deep cleft between the lobes. Like other kinases Section The PKA structure has broad significance because residues 40 to constitute a conserved catalytic core that is common to essentially all known protein kinases. We see here an example of a successful biochemical solution to the problem of protein phosphorylation being employed many times in the course of evolution. Three-dimensional structure of a complex of the catalytic subunit of protein kinase A and an inhibitor bearing a pseudosubstrate sequence. The inhibitor yellow binds in a cleft between the domains of the enzyme. The bound peptide in this crystal occupies the active site because it contains the pseudosubstrate sequence Arg -Arg- Asn - Ala - Ile Figure The structure of the complex reveals the basis for the consensus sequence. The guanidinium group of the first arginine residue forms an ion pair with the carboxylate side chain of a glutamate residue Glu of the enzyme. The second arginine likewise interacts with two other carboxylates. The nonpolar side chain of isoleucine,

which matches Z in the consensus sequence, fits snugly in a hydrophobic groove formed by two leucine residues of the enzyme. Binding of Pseudosubstrate to Protein Kinase A. The two arginine side chains of the pseudosubstrate form salt bridges with three glutamate carboxylates. Hydrophobic interactions are also important in the recognition of substrate. The isoleucine residue more By agreement with the publisher, this book is accessible by the search feature, but cannot be browsed.

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Chapter 3 : Faculty | Department of Biochemistry | Virginia Tech

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At the age of 2, he and his parents moved to Vienna, where Racker grew up. After finishing high school, he went to the University of Vienna to study medicine. Because his graduation from medical school in was around the time Hitler marched into Austria, Racker decided to leave while it was still possible and fled to Great Britain where J. There, Racker tried to find biochemical causes for mental diseases. When Great Britain entered the war, Racker lost his job at Cardiff and was interned on the Isle of Man where he practiced medicine for the first time in his life. Although he enjoyed being a doctor, he decided to try his luck as a researcher in the United States. His first appointment was as a research associate in physiology at the University of Minnesota, Minneapolis, from to There, Racker carried on his search for a biochemical basis for brain diseases and showed that polio virus inhibited glycolysis in the mouse brain. Despite spending a year doing research, Racker once again found himself working as a physician when he accepted a position at the Harlem Hospital in New York City. His career in biochemistry began in earnest in when he was appointed assistant professor of microbiology at the New York University Medical School. During his time in New York, Racker continued his glycolysis studies and found that the inhibition could be overcome by the addition of glutathione. This led to his discovery that glyoxylase converts glyoxal to glycolic acid via a carboxyl-S-glutathione intermediate 1. Similarly, Racker and his technician Isidore Krimsky showed that glyceraldehyde 3-phosphate oxidation occurred through a thiol ester enzyme intermediate 2. In , Racker was offered the position of associate professor at Yale Medical School, which he accepted. There, he continued to work on carbohydrate metabolism and discovered and purified trans-ketolase, a key enzyme in the pentose phosphate pathway. At first, Racker continued to work on the mechanism of glycolysis and the pentose phosphate pathway but then turned to the regulation of glycolysis. Pullman joined his department. Pullman had just earned a Ph. Upon his arrival at the Public Health Research Institute he decided he wanted to determine the mechanism of ATP synthesis in mitochondria and chloroplasts. Penefsky, started by attempting to isolate the enzymes involved in ATP synthesis. They obtained fresh bovine hearts and, using a mechanical blender, isolated several grams of mitochondrial membrane fragments, which catalyzed oxidative phosphorylation. These submitochondrial particles were then vigorously shaken with tiny glass beads in a shaker built by Peter M. The shaker was considered so dangerous that Nossal screwed it to the floor of a separate room, operated it by remote control, and allowed nobody else to touch it. When the mitochondrial fragments were sedimented in an ultracentrifuge, they still respired but no longer synthesized ATP. However, Racker and his co-workers discovered that oxidative phosphorylation could be restored if the supernatant was added back to the fragments. While attempting to isolate the soluble component that made oxidative phosphorylation possible, Racker and co-workers discovered that an ATPase was purified together with the phosphorylation activity. Eventually, the researchers realized that this ATPase was in fact the coupling factor that restored oxidative phosphorylation. They named this first enzyme of oxidative phosphorylation Factor 1 or F1. After the publication of these papers, Pullman remained at the Public Health Research Institute and was eventually promoted to associate director in Racker continued to work on ATP synthesis and together with Vida Vambutas he purified a similar coupling factor from spinach chloroplasts 3. Later, with Yasuo Kagawa, Racker subfractionated submitochondrial particles with cholate and salt and identified a membrane factor that anchored F1 to the membrane and rendered it cold-stable and sensitive to the toxic antibiotic oligomycin 4. In , Racker left the Public Health Research Institute to help create and lead the biochemistry department of a new biology unit at Cornell University. By this time, Racker was convinced that oxidative phosphorylation was not mediated by a high energy chemical intermediate but by a transmembrane proton gradient as proposed by

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Peter Mitchell 5. Joined by Walther Stoeckenius, Racker incorporated bacteriorhodopsin, a protein that functioned as a light-driven proton pump, and the F1Fo-ATPase into liposomes 6. In the years that followed, Racker and his colleagues reconstituted a variety of different membrane enzymes into liposomes and established reconstitution as a powerful approach for determining the mechanics of pumps, transporters, and receptors.

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In this volume, a section is included on the preparar tion of guineapig mammary gland mitochondria by W. L. Nelson & R. A. Butow (pp.). (See also DSA29[].).

Chapter 5 : CGI/ABHD5 is phosphorylated on Ser by protein kinase A: control of subcellular localization

While attempting to isolate the soluble component that made oxidative phosphorylation possible, Racker and co-workers discovered that an ATPase was purified together with the phosphorylation activity.

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Phosphorylation Is a Highly Effective Means of Regulating the Activities of Target Proteins. The activities of many enzymes, membrane channels, and other target proteins are regulated by phosphorylation, the most prevalent reversible covalent modification.

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