Ubiquitination

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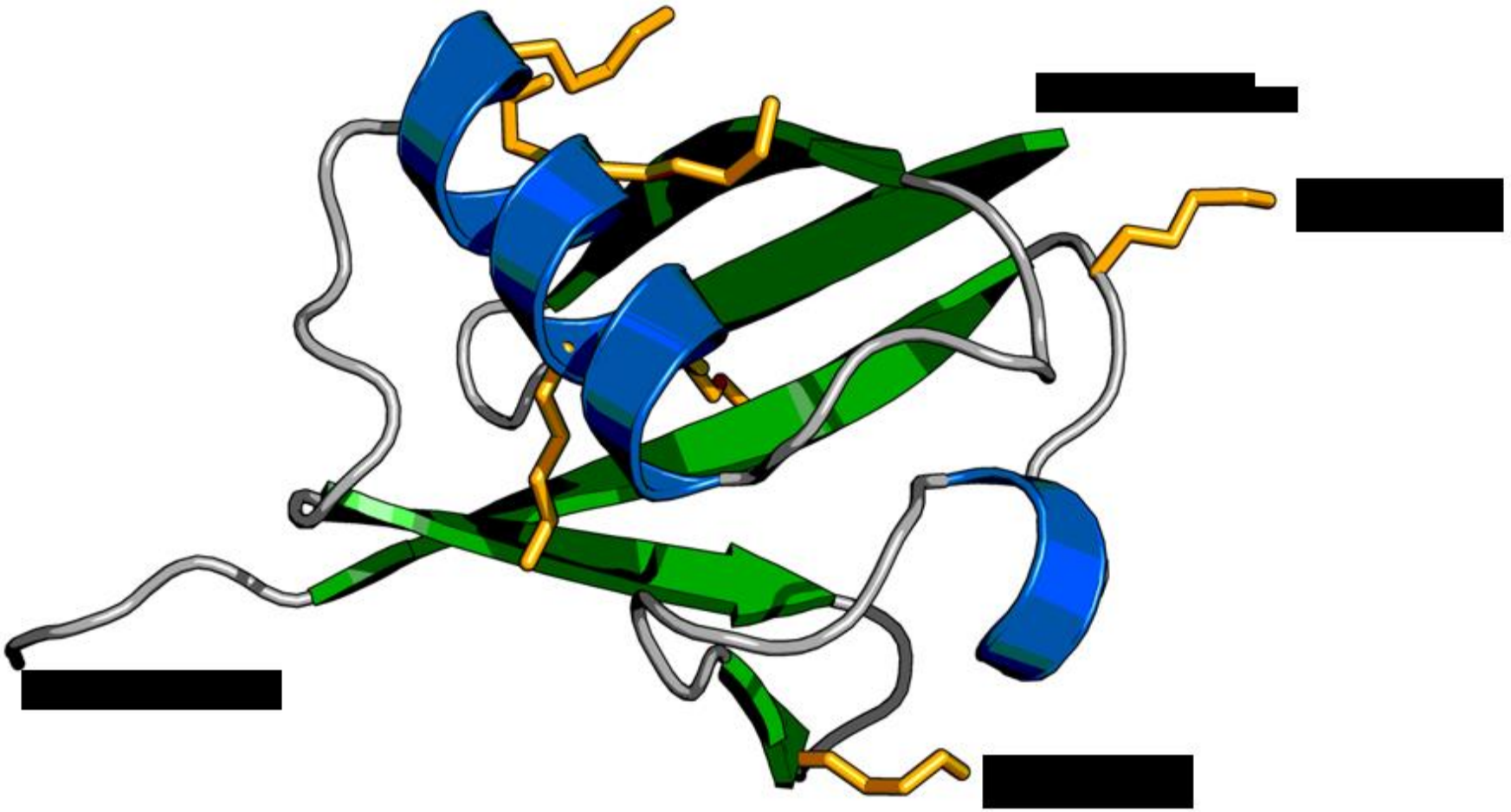
What is Ubiquitin?

Ubiquitin is a small protein(8.5 kDa) that is found in almost all cellular tissues in humans and other eukaryotic organisms, which helps to regulate the processes of other proteins in the body. It was discovered in 1975. Four genes in the human genome code for ubiquitin: UBB, UBC, UBA52 and RPS27A.



Ubiquitination (Ubiquitylation)

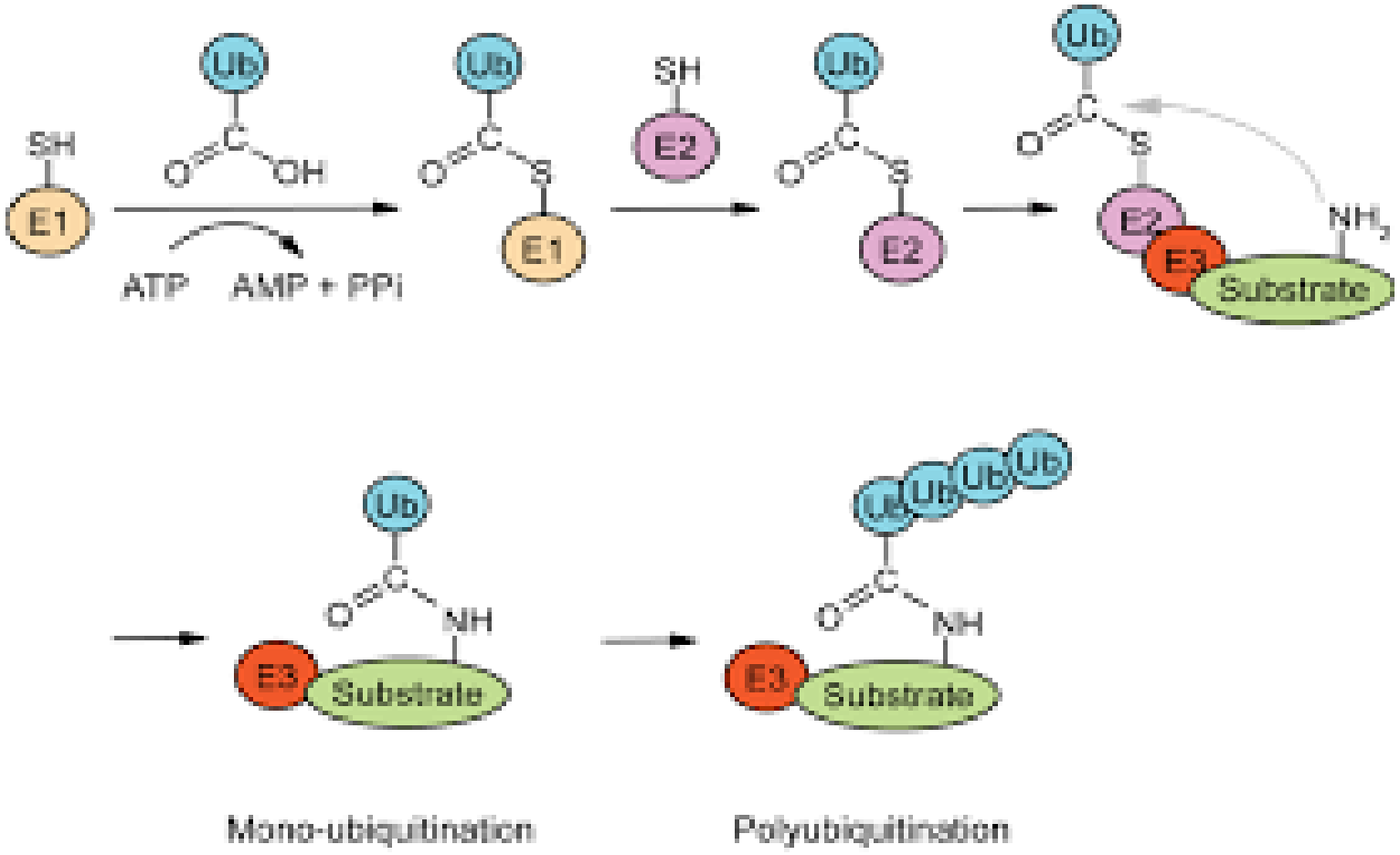
Ubiquitination, also known as ubiquitylation, is an enzymatic process that involves the bonding of an ubiquitin protein to a substrate protein. This has sometimes been referred to as the molecular "kiss of death" for a protein, as the substrate usually becomes inactivated and is tagged for degradation by the proteasome through the attachment of the ubiquitin molecule.

Post-Translation Modification Enzymes

- The process of ubiquitination in regulated by three main types of enzymes to take place in entirety. These include ubiquitin-activating enzymes (E1), ubiquitin conjugating enzymes (E2) and ubiquitin ligases (E3). Each of these enzyme types has an important role to play in ubiquitination and the labeling of proteins to be degraded by the proteasome, which are considered in more detail below

Post-Translation Modification Enzymes

- The first step involves the activation of ubiquitin by the E1 enzyme, which occurs prior to its attachment to the amino acid cysteine, the active site. Energy in the form of ATP is required in order for the ubiquitin molecule to be transferred to the active site and produce an intermediate substance known as ubiquitin-adenylate.
- Following this, the ubiquitin-conjugating enzyme (E2) plays its role to bring the two molecules together, by transferring the ubiquitin from E1 to the active cysteine site. The E2 enzyme has a particular structure that allows it to bond to both the ubiquitin and E1 molecules and allow this step to occur.
- Finally, the ubiquitin protein ligase (E3) is required to recognise and bind the target substrate, subsequently labeling it with the small ubiquitin molecule. This usually occurs by way of an isopeptide bond connecting the last amino acid, glycine 76, of the ubiquitin molecule to a lysine on the substrate protein.
- This enzymatic process is then repeated to form a small chain with several ubiquitin molecules, marking the protein for degradation in the proteasome.


Ub
SH
E1
OH
ATP
AMP + PPi
E2
S
NH
E3
Substrate
NH2
Mono-ubiquitination
Polyubiquitination

Cases of Non-Degradation

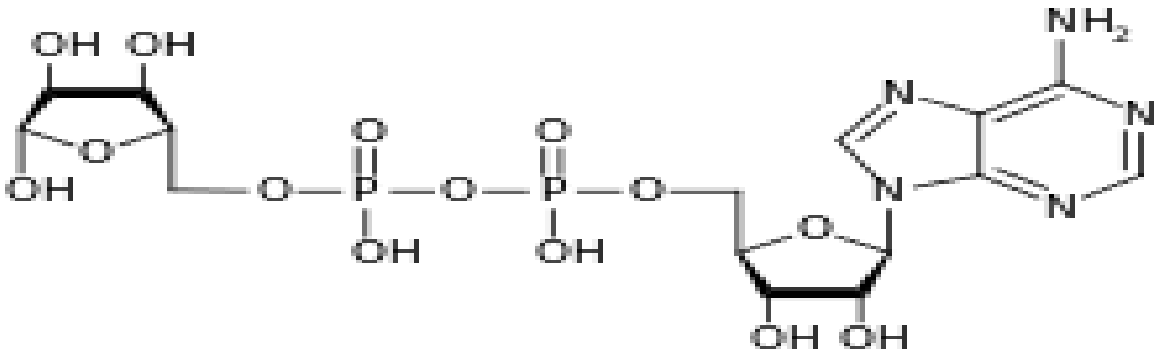
- Although the labeling of a protein via ubiquitination largely results in the degradation of the protein, there are some cases in which it may not prove fatal.

- For example, when a single ubiquitin molecule is bound to a protein without forming a chain of molecules, which is known as mono-ubiquitination, the result can differ significantly. It is common for the protein to instead notice an alteration in function or it may be degraded via lysosomes rather that in the proteasome. This can also occur to some proteins that have undergone poly-ubiquitination, although it is less common.

- Additionally, the process of ubiquitination can be reversed through the action of deubiquitinase enzymes, which break the bond between the ubiquitin molecule and the substrate protein.

ADP-ribosylation

- **ADP-ribosylation** is the addition of one or more ADP-ribose moieties to a protein ·It is a reversible post-translational modification that is involved in many cellular processes, including cell signaling, DNA repair, gene regulation and apoptosis.Improper ADP-ribosylation has been implicated in some forms of cancer. It is also the basis for the toxicity of bacterial compounds such as cholera toxin, diphtheria toxin, and others

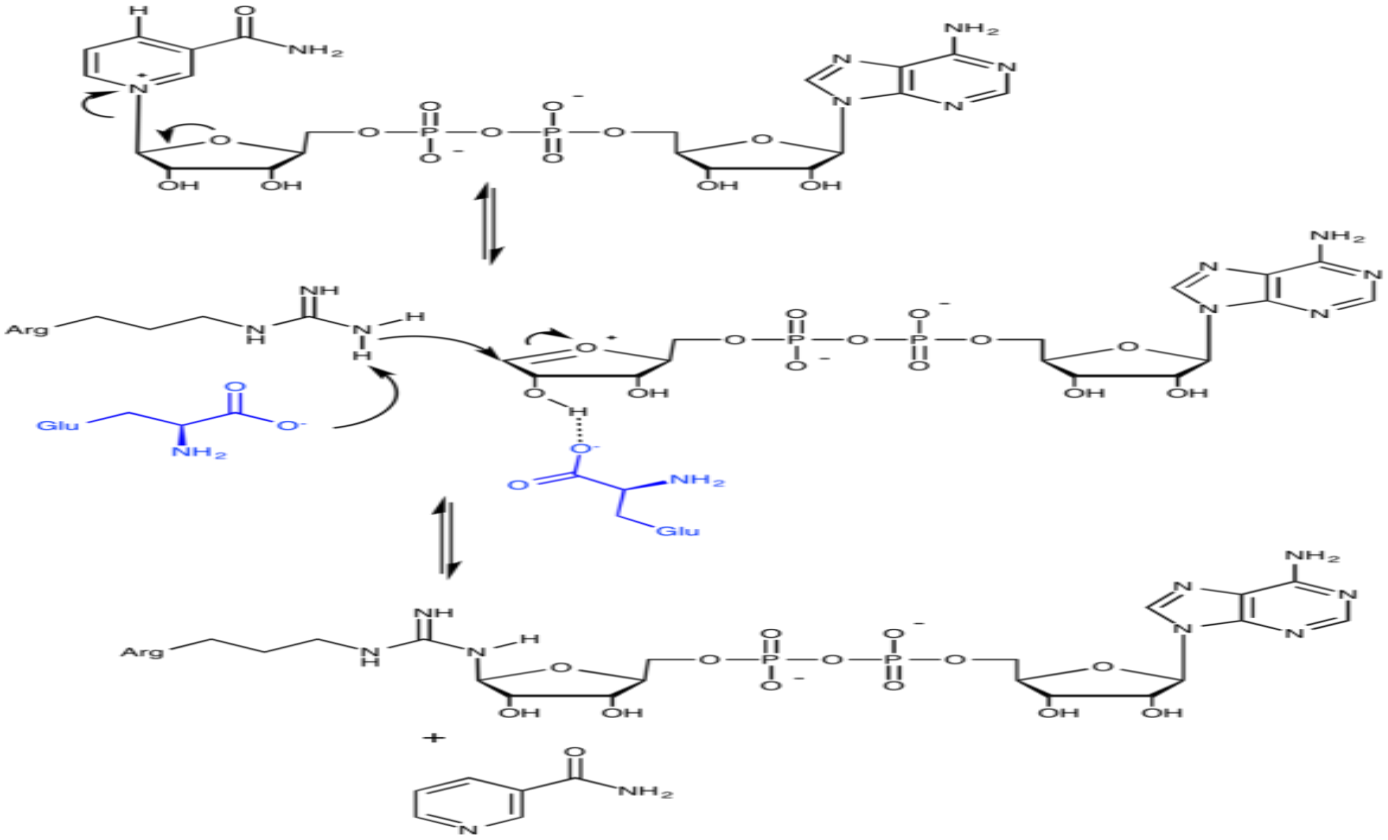


History

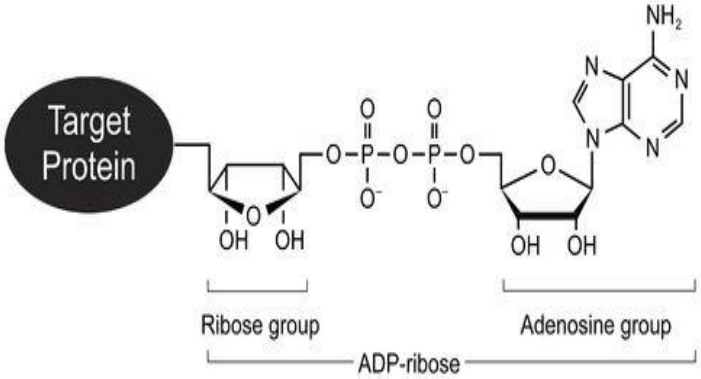
- The first suggestion of ADP-ribosylation surfaced during the early 1960s. At this time, Pierre Chambon and coworkers observed the incorporation of ATP into hen liver nuclei extract. After extensive studies on the acid insoluble fraction, several different research laboratories were able to identify ADP-ribose, derived from NAD+, as the incorporated group. Several years later, the enzymes responsible for this incorporation were identified and given the name poly (ADP-ribose) polymerase. Originally, this group was thought to be a linear sequence of ADP-ribose units covalently bonded through a ribose glycosidic bond. It was later reported that branching can occur every 20 to 30 ADP residues.
- The first appearance of mono-ADP-ribosylation occurred a year later during a study of toxins: corynebacterium diphtheria diphtheria toxin was shown to be dependent on NAD+ in order for it to be completely effective, leading to the discovery of enzymatic conjugation of a single ADP-ribose group by mono-ADP-ribosyl transferase.
- It was initially thought that ADP-ribosylation was a post translational modification involved solely in gene regulation. However, as more enzymes with the ability to ADP-ribosylate proteins were discovered, the multifunctional nature of ADP-ribosylation became apparent. The first mammalian enzyme with poly-ADP-ribose transferase activity was discovered during the late 1980s. For the next 15 years, it was thought to be the only enzyme capable of adding a chain of ADP-ribose in mammalian cells. During the late 1980s, ADP-ribosyl cyclases, which catalyze the addition of cyclic-ADP-ribose groups to proteins, were discovered. Finally, sirtuins, a family of enzymes that also possess NAD+-dependent deacylation activity, were discovered to also possess mono-ADP-ribosyl transferase activity.

Catalytic mechanism

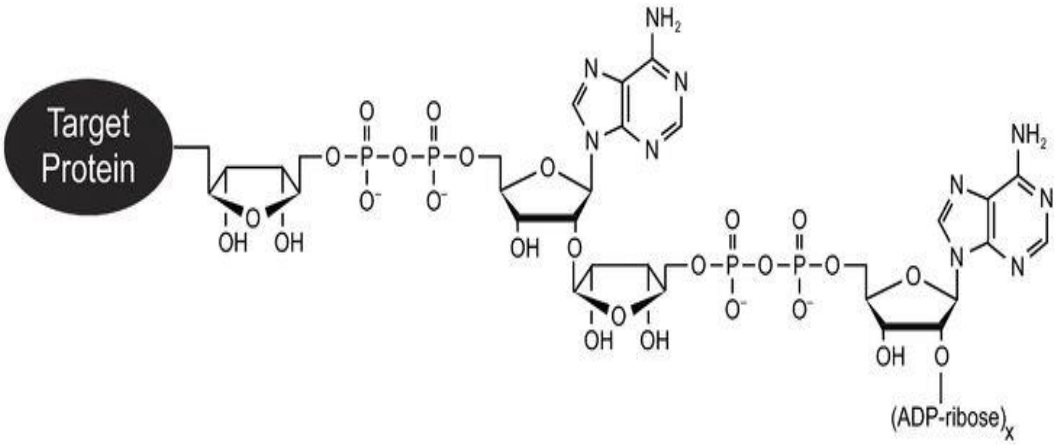
- The source of ADP-ribose for most enzymes that perform this modification is the redox cofactor NAD$^+$. In this transfer reaction, the N-glycosidic bond of NAD$^+$ that bridges the ADP-ribose molecule and the nicotinamide group is cleaved, followed by nucleophilic attack by the target amino acid side chain. ADP-ribosyltransferases can perform two types of modifications: mono-ADP ribosylation and poly-ADP ribosylation.



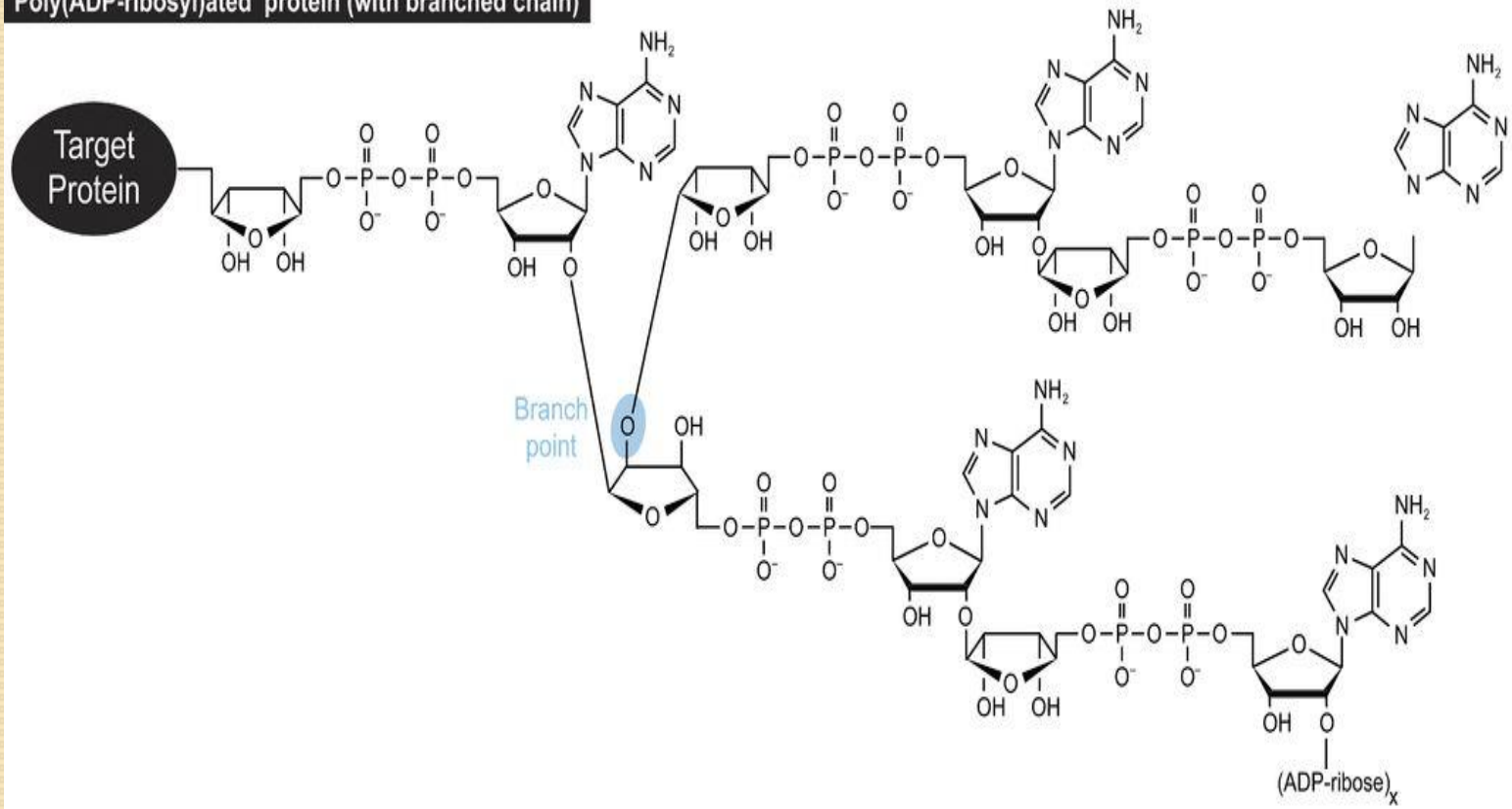
Mono(ADP-ribosyl)ated protein



Poly(ADP-ribosyl)ated protein (with linear chain)



Poly(ADP-ribosyl)ated protein (with branched chain)



- **Mono ADP-ribosylation**
- Mono-ADP ribosyltransferases commonly catalyze the addition of ADP-ribose to arginine side chains using a highly conserved R-S-EXE motif.[12] The reaction proceeds by breaking the bond between nicotinamide and ribose to form an oxonium ion. Next, the arginine side chain of the target protein then acts a nucleophile, attacking the electrophilic carbon adjacent to the oxonium ion. In order for this step to occur, the arginine nucleophile is deprotonated by a glutamate residue on the catalyzing enzyme. Another conserved glutamate residue forms a hydrogen bond with one of the hydroxyl groups on the ribose chain to further facilitate this nucleophilic attack. As a result of the cleavage reaction, nicotinamide is released. The modification can be reversed by ADP-ribosylhydrolases, which cleave the N-glycosidic bond between arginine and ribose to release ADP-ribose and unmodified protein; NAD+ is not restored by the reverse reaction.
- **Poly ADP-ribosylation**
- Poly-(ADP-ribose) polymerases (PARPs) are found mostly in eukaryotes and catalyze the transfer of multiple ADP-ribose molecules to target proteins. As with mono-ADP ribosylation, the source of ADP-ribose is NAD^+. PARPs use a catalytic triad of His-Tyr-Glu to facilitate binding of NAD^+ and positioning of the end of the existing poly-ADP ribose chain on the target protein; the Glu facilitates catalysis and formation of a (1->2) O-glycosidic linkage between two ribose molecules. There are several other enzymes that recognize poly-ADP ribose chains, hydrolyse them or form branches; over 800 proteins have been annotated to contain the loosely defined poly ADP-ribose binding motif; therefore, in addition to this modification altering target protein conformation and structure, it may also be used as a tag to recruit other proteins or for regulation of the target protein.

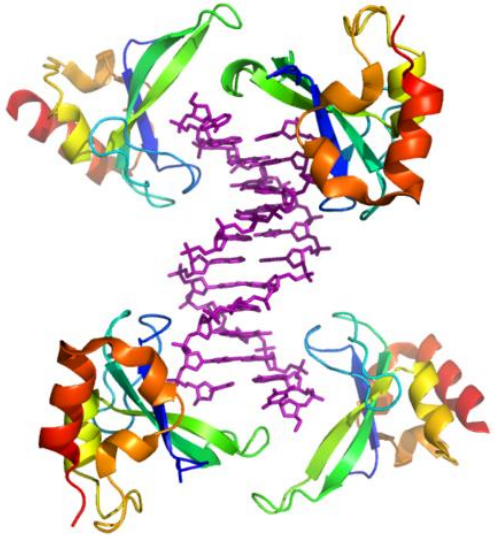
- **Apoptosis**
- During DNA damage or cellular stress PARPs are activated, leading to an increase in the amount of poly-ADP-ribose and a decrease in the amount of NAD+.For over a decade it was thought that PARP1 was the only poly-ADP-ribose polymerase in mammalian cells, therefore this enzyme has been the most studied. Caspases are a family of cysteine proteases that are known to play an essential role in programmed cell death. This protease cleaves PARP-1 into two fragments, leaving it completely inactive, to limit poly-ADP-ribose production. One of its fragments migrates from the nucleus to the cytoplasm and is thought to become a target of autoimmunity.
- During caspase-independent apoptosis, also called parthanatos, poly-ADP-ribose accumulation can occur due to activation of PARPs or inactivation of poly(ADP-ribose) glycohydrolase, an enzyme that hydrolyses poly(ADP-ribose) to produce free ADP-ribose. Studies have shown poly-ADP-ribose drives the translocation of the apoptosis inducing factor protein to the nucleus where it will mediate DNA fragmentation. It has been suggested that if a failure of caspase activation under stress conditions were to occur, necroptosis would take place. Overactivation of PARPs has led to a necrotic cell death regulated by the tumor necrosis factor protein. Though the mechanism is not yet understood, PARP inhibitors have been shown to affect necroptosis.

Gene regulation

- ADP-ribosylation can affect gene expression at nearly every level of regulation, including chromatin organization, transcription factor recruitment and binding, and mRNA processing.
- The organization of nucleosomes is key to regulation of gene expression: the spacing and organization of nucleosomes changes what regions of DNA are available for transcription machinery to bind and transcribe DNA. PARP1, a poly-ADP ribose polymerase, has been shown to affect chromatin structure and promote changes in the organization of nucleosomes through modification of histones.
- PARPs have been shown to affect transcription factor structure and cause recruitment of many transcription factors to form complexes at DNA and elicit transcription.Mono ADP-ribosyltransferases are also shown to affect transcription factor binding at promoters. For example, PARP14, a mono ADP-ribosyltransferase, has been shown to affect STAT transcription factor binding.
- Other ADP-ribosyltransferases have been shown to modify proteins that bind mRNA, which can cause silencing of that gene transcrip

DNA repair

- Poly-ADP-ribose polymerases (PARPs) can function in DNA repair of single strand breaks as well as double strand breaks. In single-strand break repair (base excision repair) the PARP can either facilitate removal of an oxidized sugar or strand cleavage. PARP1 binds the single-strand breaks and pulls any nearby base excision repair intermediates close. These intermediates include XRCC1 and APLF and they can be recruited directly or through the PBZ domain of the APLF.[26] This leads to the synthesis of poly-ADP ribose. The PBZ domain is present in many proteins involved in DNA repair and allows for the binding of the PARP and thus ADP-ribosylation which recruits repair factors to interact at the break site. PARP2 is a secondary responder to DNA damage but serves to provide functional redundancy in DNA repair



Protein degradation

- The ubiquitin-proteasome system (UPS) figures prominently in protein degradation. The 26S proteasome consists of a catalytic subunit (the 20S core particle), and a regulatory subunit (the 19S cap).[28] Poly-ubiquitin chains tag proteins for degradation by the proteasome, which causes hydrolysis of tagged proteins into smaller peptides
- **Cancer**
- PARP1 is involved in base excision repair (BER), single- and double-strand break repair, and chromosomal stability. It is also involved in transcriptional regulation through its facilitation of protein–protein interactions. PARP1 uses NAD+ in order to perform its function in apoptosis. If a PARP becomes overactive the cell will have decreased levels of NAD+ cofactor as well as decreased levels of ATP and thus will undergo necrosis. This is important in carcinogenesis because it could lead to the selection of PARP1 deficient cells (but not depleted) due to their survival advantage during cancer growth.

Bacterial toxins

- Bacterial ADP-ribosylating exotoxins (bAREs) covalently transfer an ADP-Ribose moiety of NAD+ to target proteins of infected eukaryotes, to yield nicotinamide and a free hydrogen ion. bAREs are produced as enzyme precursors, consisting of a "A" and "B" domains: the "A" domain is responsible for ADP-Ribosylation activity; and, the "B" domain for translocation of the enzyme across the membrane of the cell. These domains can exist in concert in three forms: first, as single polypeptide chains with A and B domains covalently linked; second, in multi-protein complexes with A and B domains bound by non-covalent interactions; and, third, in multi-protein complexes with A and B domains not directly interacting, prior to processing