Cell Biology: Cellular Organization, Division and Processes

Prof. Shikha Laloraya
Department of Biochemistry
Indian Institute of Science – Bangalore

Lecture 08
The Ubiquitin Proteasome System

Hello everyone, I am Shikha Laloraya, Professor of Biochemistry at IISc. Today's lecture is on the ubiquitin proteosome system that is an intracellular protein quality control system present in cells. It is involved in the degradation of proteins in the cell. We will also discuss the complexity of the system and about ubiquitin and ubiquitin-like proteins and how the post-translational modification by ubiquitin and ubiquitin related proteins impacts several cellular processes.

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The ubiquitin proteasome system is a highly complex system for the post-translational modification of protein targets by ubiquitin, and for their controlled degradation. This complexity allows for possibilities of regulation at multiple levels depending on the cellular conditions and also for specifically modulating the pathway for the desired outcomes. It is one of the main cellular quality control system for proteins inside the cell.

The requirements for this system to function are: a poly Ubiquitin tag has to be attached to the proteins that are destined for degradation, there is a complex multi-component nanomachine, the proteasome, that degrades these tagged proteins. There are also many other regulators, enzymes and adapters for bringing about the specific recognition and modification of the target protein at the appropriate time and also for its recognition by the proteasome and for degradation. And of course, this process requires energy in the form of ATP.

In addition, ubiquitylation, which is also often referred to as ubiquitination, can result in effects other than degradation. Sometimes the poly-ubiquitin chain is not assembled but the protein can either be mono or multi-ubiquitylated. And such modifications by ubiquitin and the ubiquitin-like proteins can alter the properties of the target protein in terms of its activity or its interactions with other proteins or its localization in the cell or impact its function in various ways. So, this provides additional levels of complexity and regulation.

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Ubiquitin is a short 76 amino acid polypeptide that is covalently conjugated to the target proteins. Ubiquitin is attached via its own C terminus to the amino group of the side chain of lysine residues of the target substrate protein via an isopeptide linkage. The carboxylic group of ubiquitin is attached to the amino group of lysine side chain rather than to the alpha amino group. The conjugation with

Ubiquitin can alter the fate or function of the modified protein as I mentioned, it may get targeted for degradation or it may also alter its properties and interactions.

There are many other Ubiquitin-like polypeptides and these can also be conjugated to the target proteins resulting in diverse outcomes and ubiquitin though is the founding member of this family of modifiers, which bear some structural resemblance to each other.

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So, the Ubiquitin-like protein families are family of small proteins, which are related to ubiquitin. So, these are all known as ubiquitin-like proteins or UBLs/Ubls and they share a similarity in 3D structure. They all have a characteristic ubiquitin fold also known as a beta grasp fold. It consists of a five stranded beta sheet, an alpha helix and a short 3_{10} helix. This 3_{10} helix, it is a variation from the alpha helix, where it has a repeat unit consisting of three amino acids in one turn.

The mature form of the Ubiquitin-like polypeptide ends with the signature diglycine sequence and this is often exposed by processing of a ubiquitin precursor polypeptide. The carboxyl group of the C-terminal glycine is the site of attachment to the substrates and the lysine side chains are the most common target sites within substrate proteins resulting in an amide or isopeptide bond between the ubiquitin like protein and the substrate.

Of course, there are always exceptions in biology. So, there are rare examples of conjugation of the ubiquitin C-terminus to a free alpha amino group of an N-terminal residue of a protein, or even via a Cysteine side chain for example in the MHC class 1 heavy chain in a reaction that is catalyzed by a viral E3 known as MIR1. So, exceptions do occur, but mostly the attachment is to the epsilon amino group of a lysine side chain.

The Ubiquitin like proteins are highly conserved, that is, for example there are only three differences in amino acid sequence out of 76 positions between yeast ubiquitin if you compare it to the human Ubiquitin.

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Ubiquitylation is a very complex multi-step process and here I am trying to show a generalized Ubiquitin conjugation pathway. Now of course the first step, which is not shown here is that there are precursor ubiquitin-like proteins. And they are processed by DUBs that are deubiquitinylating enzymes or ULP, Ubiquitin like protein-specific proteases, to expose the C-terminal glycine in the mature ubiquitin. That is not shown here but by that cleavage you would get a ubiquitin polypeptide whose C terminus is a glycine. Now this processed ubiquitin can be conjugated and it is activated with ATP by E1 or a Ubiquitin activating enzyme shown here. And this step of course, is energy requiring and it requires ATP. So, it gets attached to the E1 enzyme and then it is transferred to another enzyme known as the Ubiquitin conjugating enzyme or E2.

And then it is finally transferred to the target with the help of a complex between this E2 and an E3 ligase. The E3s mediate substrate selectivity by binding the substrate as well as the E2 and then

finally the Ubiquitin is attached to the substrate shown here, and it can also be removed from the target by de-Ubiquitylating enzymes. So, this is a reversible modification. Another point is that an E2 enzyme cannot bind an E1 as well as in E3 simultaneously, only after transfer of the Ubiquitin from the E1 to the E2. The E1 should be released before it can bind to the E3 enzyme.

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So, another point, a little bit about complexity: this is a multi-enzyme process anyway, there are these three main enzymes E1, E2 and E3. But within cells, you have multiple ones of these. For example in mammalian cells there are two E1s and about 40 or so E2 conjugating enzymes and approximately 600 E3 ligases. So, there are lots of E3 ligases as you can see and this is important because they need to recognize different substrates at different times and so you need to have a large number of them.

So, this process of course of modification because it has important consequences for the modified protein, it is a highly selective process and the selectivity comes about because of the E3 which has got the specificity determinants for recognition of the correct target proteins. These Ubiquitin E3s in general are classified into 4 types. They have two main types, which we are discussing today the RING and the HECT types. But there is also U-box family and a PHD finger, which is sometimes also referred to as a Ring type. So, the RING type E3s, they are sort of like adapters; they catalyze modification by binding simultaneously to the Ubiquitin like E2 thioester complex and the substrate, which has to be modified. And it positions the amino group of the substrate lysine near the E2 Ubiquitin thioester and catalyzes the transfer of this ubiquitin to the substrate.

So, but there is no covalent bond formation here between the ubiquitin and the E3 in this case. However, in the HECT family the mechanism is different from the ring E3s. HECT stands for homologous to the E6 AP Carboxy Terminus; it refers to a domain which is conserved in these proteins. And in this case, in the HECT domain Ubiquitin ligases, first the Ubiquitin is transferred to a catalytic cysteine within the HECT domain via a trans thiolation reaction. And then this E3 Ubiquitin like thioester complex transfers the UBL to the substrate. Now the U-box family has this E2 binding domain. It is a particular type of domain referred to as a U-box, it is a relatively small family of E3 ligases. And the PHD finger, these are domains which resemble the RING domains. And here also the folding relies on coordination of two zinc ions in a cross-brace arrangement. And overall conformations of these two domains, PHD and RING finger, are quite similar.

And finally, as I already mentioned, the deubiquitylating enzymes as well as the Ubiquitin like protein proteases can remove the ubiquitin-like proteins attachments from the substrates.

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So, let us try to understand the structure of ubiquitin. Here is shown the structure of ubiquitin and it has got a characteristic ubiquitin-fold also referred to as a beta grasp fold. And this consists of a five stranded beta sheet, which has both parallel and anti-parallel beta elements, and it also has an alpha

helix and also a short 3_{10} helix. So, this is a tightly packed globular structure and it is got this mixed parallel antiparallel beta sheet and this packs against the alpha helix to form this hydrophobic core.

And it is actually common in many protein families. The different lysine side chains are shown here. So, this is actually the sequence of one of the ubiquitins that I got from PDB. And you can see the 2 diglycine motif at the end C-terminus, which would be here in the structure. And there are 7 lysine residues in this sequence, which are shown here as these yellow sticks- the side chains. And two of the most commonly used and well characterized ones are labelled lysine 48 and lysine 63, and they are used for formation of the poly-ubiquitin chain. So, they are the sites of conjugation of Ubiquitin for formation of poly-ubiquitin chains.

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So, this is shown here, how this might happen. This slide shows two of these common linkages used in making the poly-ubiquitin chains. So, there is K48, lysine 48 and lysine 63. The linkage is shown over here where the C-terminal carboxylate group of the second ubiquitin here is attached to the epsilon amino group of the side chain of lysine 48. And in this case, you can see of course the orientation has to be slightly different but here the C- terminus of the second ubiquitin is attached again to the amino group of the lysine 63.

These are di-ubiquitins shown here; but with the help of the respective E3 enzymes, this process of conjugation can repeat to build up a poly-ubiquitin chain.

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So, here is a summary of the key process of ubiquitin mediated protein degradation. First the poly ubiquitin tag is attached by the ubiquitylation machinery, the multi-enzyme system consisting of the E1, E2 and E3 enzymes. These polyubiquitylated proteins are recognized by the proteasome, shown in this cartoon here. So, this is a very large multi-subunit complex and it has actually got protease activity that degrades the proteins, which are tagged by ubiquitin.

And the end product of this is that the protein gets degraded into small segments or polypeptides; it could be 3 or up to 23 or 25 amino acids, and then these are released and then these can be further degraded and these amino acids could be recycled. And also ubiquitin is released and it is available.

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The proteasome depicted here can be thought of as a protein shredder, sort of like your paper shredder machine or even some kind of garbage disposer. It destroys the tagged proteins, it chops it up into small pieces and it spits it out. And this destructive activity actually is of course a very harmful activity. So, it is very carefully protected inside. It cannot attack all of the normal proteins in the cell only those, which were tagged by ubiquitin, are targeted and recognized by this proteasome. And these proteins are recognized and then the polypeptide enters inside the chamber inside this

cylindrical subunit or the core of the proteasome. And there are active sites, which have protease activity inside the chamber where it is cleaved into the small peptide segments and then those are released from the proteasome. Of course, before it enters a cavity inside, the ubiquitin is removed before it enters the chamber.

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Here is shown the structure of the yeast proteasome, which has been deduced by using a combination of multiple methods to solve the structures of proteins and I got it from the protein database 'molecule of the month' site. So, you can actually check it out also if you wish. The proteasome, it is a complex nanomachine: it is very big, it is about 2.5 megadaltons and it is composed of two copies each of 33 basic subunits and also some proteasome interacting proteins as well.

This is a 26S holo-complex and it has got two types of sub complexes. So, one is this cylindrical 20S core particle referred to as CP which harbors this proteolytic chamber inside. And there are two 19S regulatory particles which are attached to the opposite ends of this core particle cylinder. And there is also a deubiquitylating enzyme near the mouth of this entry channel, which removes the Ubiquitin.

The regulatory particles, they recognize the proteins that have to be degraded. So, they recognize and bind polyubiquitylated proteins and then these proteins are deubiquitylated and then unfolded And these also control the opening of the gate which gives access to the interior of the core particle. So, the access of the protein substrates to this chamber is restricted and it depends on a class of enzymes referred to as triple-A ATPases (AAA-ATPases).

These enzymes, they generate mechanical force through cycles of ATP binding and hydrolysis and this is used for unfolding the substrates and then the gate is opened leading into the proteolytic chamber. And the substrate is translocated into the active chamber inside which has got proteases which are facing inside the cavity. So, there are some 6 triple A ATPases, which are present at the base of the ring of the 19S regulatory particle that perform all these functions while they also interact with this 20S catalytic chamber. And near those at the same time there is the deubiquitylating enzyme which is also near the mouth of this hexamer of the triple A ATPase and it removes the ubiquitin which is released. So, the Ubiquitin part does not enter into the cylinder chamber. The proteases inside, there are three types of proteolytic activities, which have got different cleavage specificities-so, they can actually cleave the protein inside at many different sites.

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So, here are some of the processes of interest to us, which are affected by the proteasome mediated degradation. Most important examples are there in cell cycle regulation, we referred to some of them in the previous lectures. For example, there are the two main E3 ligase complexes, which are involved in cell cycle regulation. One is referred to as the SCF, SCF-E3 ligase: this is a modular E3 ligase and is named after its subunits Skp1, Cdc53 cullin and F-box containing protein. It is associated with an E2 referred to as Cdc34 and some of its substrates include the G1 cyclins, Sic1, which is a Cdk

inhibitor, Wee1 and other regulators such as E2f and I-kappa B. Another important E3 in the mitotic cell cycle is APC. APC stands for Anaphase Promoting Complex. It is an E3 with 12 subunits and recognition and destruction by APC requires the presence of a specific nine amino acid motif called the destruction box. Example of its substrate is Pds1. Pds1 is an important regulatory protein, which regulates the metaphase to anaphase transition. It forms a complex with an enzyme separase called Esp1 in yeast, which basically when Pds1 is degraded separase is released and it cleaves cohesin triggering the metaphase to anaphase transition. Another target of APC is a mitotic cyclin. So, they have multiple targets; I am just giving a few examples.

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This figure shows an important step in cell cycle regulation that is regulated by the ubiquitin proteasome system. It shows the process of cyclin degradation. Cyclin is a key regulator of the cyclin-dependent kinase. And its levels fluctuate during the cell cycle and it its levels fall by ubiquitin mediated protein degradation as was mentioned earlier. So, at the right time which is monitored by a mitotic checkpoint, in this case the activating subunit Cdc20 becomes available and it can associate with an E3 known as APC. APC stands for anaphase promoting complex. So, Cdc20 can bind only a phosphorylated state of APC and this activates APC and APC can now go and target its substrates; it can ubiquitylate its various substrates. One of these substrates is the protein Pds1, which is not shown here. So, Pds1 is a regulator of the metaphase to anaphase transition. It gets degraded upon ubiquitylation by APC and when it is degraded it releases separase with which it was forming a complex. Separase is an enzyme that cleaves cohesin. Cohesin is a complex that holds sister chromatids together. And upon cleavage of cohesin, the sister chromatids can separate from each other and hence the metaphase to anaphase transition is facilitated.

Another important target of APC is the M-cyclin, which is present in this complex with the mitotic Cdk. So, the activated form of APC polyubiquitylates the M-cyclin, and attachment of this polyubiquitin tail to this cyclin targets it for recognition by the proteasome and also for its degradation within the proteasome. Hence cyclin is no longer available and the M-Cdk is inactivated because its partner cyclin which activates it is no longer present. Upon inactivation of the mitotic cyclin protein phosphorylation reduces; it can no longer phosphorylate its targets. And this also allows for certain phosphatases present in the cell to dephosphorylate the targets of M-Cdk, which is also required for the exit from mitosis and also for cytokinesis.

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Here is another example of the importance of the proteasome; this is in immune cells in the immune system. So, as you know the cell surface display of peptides by MHC class 1 molecules to lymphocytes is very important for protection against pathogens such as viruses. The Proteasome is required for formation of these peptides for the MHC class 1 antigen presentation pathway. So, when a cell gets infected with a virus then it chops up the viral proteins and then it displays them on its surface along with the MHC so that the immune system can recognize it. For this purpose, there is a specialized proteasome, which is referred to as the immunoproteasome. And it brings about these

cleavages to cleave the viral protein into smaller peptides. It is similar to a normal proteasome but it has got three different catalytic subunits. And one of these subunits cleaves the polypeptide chains next to hydrophobic amino acids thus creating peptides that can anchor very well into the MHC protein complex.

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There are also other processes which are affected by modification by ubiquitin-like proteins. We already discussed the ubiquitylation of cyclin B targets it for proteasome mediated degradation and then inactivation of Cdk-1 happens. And cells can then exit from mitosis and progress to the interphase. Ubiquitylation of cyclin requires this destruction box sequence in the substrate proteins.

Another important Ubiquitin-like protein is SUMO, Small Ubiquitin related Modifier. It can also be attached to target proteins by SUMO E3 ligases along with the E1 and E2 enzymes of the same pathway. So, sumoylation of proteins of course modifies their properties as well and it is also becoming a very common and popular modification, which impacts many cellular processes.

An example is the sumoylation of Ran-GAP, which a GTPase activating protein; it aids in its association with the nuclear pore complex , which is present in the nuclear membrane. This is required for import of proteins from the cytosol into the nucleus via the nuclear pore complex.

Another example of ubiquitin-like proteins is ATG8 and 12 and this is essential for autophagy. Autophagy is a process in eukaryotes where the cytoplasmic constituents get sequestered into small vesicles and they are then deposited into the vacuole or sometimes a lysosome for breakdown by hydrolysis. So, there are many other examples, also many processes which are impacted by modification by these Ubiquitin-like proteins, you name it: transcription, repair, stress resistance and many other pathways are regulated by ubiquitylation.

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Listed here are some of the important references and reading list for this course, thank you.