Role of Ubiquitination in Regulation of Protein Expression and Degradation in Yeast

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Abstract

Ubiquitin proteasome system (UPS) plays a vital role in cell survivor in degradation of protein aggregations. The efficacy of this system is highly influenced by both the polyubiquitination by E1/E2/E3 mechanism and pretreatment of polyubiquinated substrate by Rps and Rpts and the activities of UPS are under a mutual function of 20S core particle, 19S regulatory particles and different classes of proteasome interacting proteins. Moreover, UPS plays an essential role in regulation of gene expression where in yeast the transcriptional activators Gal4, Ino2, Ino4, pol II and histones recruit the ubiquitin ligases to the transcription site and become ubiquitinated, the later recruits 26S proteasomes that degrade the activator and Gcn4 and rearrange a promoter for additional rounds of transcription, thus stimulates expression of Gcn4-responsive genes. In yeast, the expression of heterologous proteins can be stimulated by regulating the transcriptional elongation through different mechanism such as controlling monoubiquitination of histone (H2B), exploiting the direct interaction between the proteins of 19S regulatory complex especially AAA-ATPases family (S4, S6a, S6b, S7, Sug1, and S10b) with gene promoters and controlling the level of transcription factors through targeted degradation by the UPS. This review highlights different factors which regulates the transcription elongation in yeast, thus gene overexpression.

Keywords: Histone H2B monoubiquitination, transcriptional factor, ubiquitin, proteasome system.

Introduction

Since time immemorial, heterologous proteins were produced by using microorganisms for medical and/or industrial purposes. Bacteria, as the most efficient producers of recombinant proteins, are unable to accomplish some post-translational modifications of eukaryotic proteins. By lacking these capabilities, bacteria often produce inactive, insoluble or misfolded eukaryotic proteins. Yeasts are acceptable for production of recombinant proteins for several reasons. All-important, yeasts provide the ease of rapid microbial growth and genetic manipulation found in bacteria along with the eukaryotic environment and the ability to perform many specific eukaryotic post-translational processing, such as proteolytic maturation of prohormones, oligomerization, disulphide bridge formation, folding, N and O-linked glycosylation (Cereghino and Cregg, 1999; Idris et al., 2010; Celik and Calik, 2012).

Saccharomyces cerevisiae is a yeast cell with a completely studied genetic profile and was used as a host for several heterologous protein production. However, certain limitations to the use of Saccharomyces cerevisiae expression system were found, these include poor plasmid stability, low protein yield, low secretion capacities, hyperglycosylation and difficulties in scaling-up fermentation (Madzak et al., 2004; Celik and Calik, 2012). Several research groups have devoted to search for alternative yeast systems along with designing improved Saccharomyces cerevisiae strains for heterologous protein production.

For this purpose, the Non-Saccharomyces yeasts have took their place in production of heterologous proteins; these are the fission yeast Schizosaccharomyces pombe and different non-conventional yeasts, the budding and dairy yeast Kluyveromyces lactis, the amylyotic yeast Schwanniomyces occidentalis, the methylotrophs Pichia pastoris and Hansenula polymorpha, the dimorphic yeast Arxula adeninivorans and the dimorphic alkane-utilizer yeast Yarrowia lipolytica (Madzak et al., 2004; Van Ooyen et al., 2006; Idris et al., 2010; Celik and Calik, 2012; Vogl et al., 2013). Although researchers from different laboratories continue improving different yeast expression system for heterologous protein expression, there is a need in enhancing protein quality control to eliminate toxic rotamers such as damaged and misfolded proteins, aborted translational products; control metabolic pathways, cell division and signal transduction. Misfolded proteins and protein aggregations are at the basis of several disorders, where in human kind they cause severe diseases like Parkinson’s disease (PD), Alzheimer’s disease (AD), Huntington’s disease (HD), type 2 diabetes, and/or Creutzfeldt-Jakob disease (Paul, 2006; Buchberger, 2013; Amm et al., 2014; Braun, 2015). To reduce harmful effect of misfolded and aggregate proteins to the cell, protein quality control mechanisms undertook by regulated proteolytic systems keep the proteostasis (protein homeostasis), which in eukaryotic cell are mainly regulated by two major systems, the lysosomal and proteasome compartments (He and Klionsky, 2009; Fredrickson and Gardner, 2012; Morimoto and Cuervo, 2014).
The lysosome is involved in autophagy, hydrolyzes insoluble protein-aggregates, pathogen defense against viruses and bacteria, membrane repair, cell death signaling and degradation of extracellular and surface membrane proteins while the proteasome plays a vital role in degradation of most soluble and intracellular proteins through the ubiquitin-proteasome system (UPS). Throughout these two degradation systems, there is a balance in protein expression and degradation to avoid the organism loss of mass (Lecker et al., 2006; Paul, 2006; Buchberger, 2013). Here in review, we focused on mechanisms that keep protein quality control and proteins homeostasis in yeast cells and discussed the role played by ubiquitination and proteasome compartments in protein degradation and transcription regulation.

Impact of Ubiquitin-Proteasome system on protein quality control and proteostasis

The cell survival depends on several factors relying on different homeostatic functions. Proteins, either intracellular or extracellular should be maintained in balance by degradation to their constituent amino acids and replacement by new synthesis. The rate of protein degradation process differ from protein to protein and it also depends on degradation system (e.g. ubiquitin-proteasome system and autophagy) and degradation site, i.e. in the nucleus, endoplasmic reticulum, cytosol and mitochondria; it may vary to minutes (e.g. regulatory enzymes), days or weeks (e.g. actin and myosin) up to months (e.g. hemoglobin) (Lecker et al., 2006; Rothman, 2010). Ubiquitin-proteasome pathway (UPP) (Fig. 1) is one of proteolytic processes in eukaryotes. This pathway is a cohort work of three enzymatic modules which begins by activation of ubiquitin molecule by ubiquitin-activating enzyme (E1 or Uba), the active ubiquitin molecule is then transferred and prepared for conjugation by ubiquitin-carrier protein (E2 or Ubc), the conjugation of the active ubiquitin molecule to the protein substrate is catalyzed by ubiquitin-protein ligase (E3) which first recognizes the defective protein substrate specific for degradation. Once ubiquitin molecule tagged the protein substrate, other ligases attach additional ubiquitin molecules which results in a polyubiquitin chain. The substrates with polyubiquitin chains are recognized and degraded by 26S proteasome which is a protein complex consists of 20S core particle that shelter inside the protease active sites, and two 19S regulatory particles at the ends that regulate the entry of protein substrate into destruction chamber. 20S core particle is composed of two copies each of fourteen subunits, α (outer part, inactive) and β (inner part, β1, β2 and β5 are active) subunits, organized into four arranged rings each of seven subunits (α5β3δ7), while each regulatory particle contains two sub-complexes, lid and base. Lid consists of nine non-ATPase subunits, Rpn3, 5-9, 11-12 and 15 which perform deubiquitination (Dub) and recruitment of the polyubiquinated substrate whereas base consists of six AAA-ATPase subunits (ATPases Associated with various cellular Activities), Rpt1-6 (S4, S6a, S6b, S7, Sug1, and S10b) and three non-ATPase subunits, Rpn1, 2, 13 (S1, S2 and S5a), which unfold the protein substrate and translocate the unfolded substrate into 20S core particle which is the catalytic chamber. The associative function of base-lid is stabilized by Rpn10 subunit (Verma et al., 2000; Hellmann and Estelle, 2002; Lecker et al., 2006; Hochstrasser, 2007; Guerrero et al., 2008; Saeki et al., 2009; Bhat et al., 2010; Kaake et al., 2010; Chen et al., 2011; Förster et al., 2014; Aufderheide et al., 2015). The efficacy of proteosomal degradation system is highly influenced by both the polyubiquitination by E1/E2/E3 mechanism and pretreatment of polyubiquinated substrate by Rpn6 and Rpts. However, the 26S proteasome is associated with certain cofactors (referred to as proteasome-interacting proteins, PIPs) such as E3 ligases (Hul5 in yeast), shutting ubiquitin receptors (Rad23, Dsk2 and Ddi1 in yeast) and deubiquitinating enzymes Ubp6 (timing device) which regulate its function in the cell, and the proteosomal degradation is expedited by the cell division cycle protein 48 (Cdc48) in yeast and p97 in mammals this is an AAA-ATPase complex (Guerrero et al., 2008; Saeki et al., 2009; Förster et al., 2014; Aufderheide et al., 2015).

Ubiquitin (Ub) as a small globular protein, 76 amino acids, contains a C-terminus with an essential glycine (G76) and an N-terminus with lysine, the isopeptide bond in polyubiquitin chain is formed between these two terminal residues, glycine and lysine and at least a four ubiquitin chain is required for being efficiently detected by 26S proteasome. Only seven lysines are count in ubiquitin, K6, K11, K27, K29, K33, K48 and K63; the inter-ubiquitin linkages in polyubiquitin chain are formed at K48 prior to degradation of the target protein by 26S...
proteasome, although endocytosis, kinase activation, signal transduction and tolerance to DNA damage are regulated by ubiquitin K63 linkages. Moreover, studies have suggested that under some situations, polyubiquitin chain based on K6, K11, K27 and K29, and a tetraubiquitin chain based on K63 linkage which bound to K48 of the fused ubiquitin can involve in proteasomal degradation (Kulkarni and Smith, 2008; David et al., 2010; Kravtsova-Ivantsiv and Ciechanover, 2012; Sadanandom et al., 2012; Sadowski and Sarcevic, 2010). Proteasomal degradation system is a ladder network of three groups of enzymes E1s, E2s and E3s. The efficacy of proteasomal degradation mechanism relies on the specificity and selectivity of these three enzymes; the isofrom number of each of these enzymes in eukaryotes vary from one class to another; studies have shown a limited number of E2s, 11 in yeast and 60 in human, a big number of E3s, 42 in yeast and about 600 in human, whereas only 1E1 has found. In additional to these enzymes, 20 deubiquitination enzymes (DUb) have reported in yeast. This variation plays a crucial role in targeting different specific substrates (Wang and Dohlman, 2006; Lee et al., 2008).

**Regulation of UPP by proteasomal-interacting proteins and Cdc48 in yeast**

The ubiquitin-proteasome degradation plays a vital role in regulation of intracellular proteins, thus the ubiquitin-proteasome system regulates the degradation of proteins located in endoplasmic reticulum, nucleus and cytosol. The intracellular proteins take in several cellular functions, such as, cell cycle, DNA repair, chromosome maintenance, growth, stress response, transcription and apoptosis. The activities of ubiquitin-proteasome system are under a mutual function of 20S core particle, 19S regulatory particles and different classes of proteasome interacting proteins. The proteasome is composed of 33 subunits in total, with two key subunits in yeast, Rpn10 and Rpn13, which possess distinct ubiquitin binding domain (Guerrero et al., 2008; Gomez et al., 2011). In addition to the ubiquitin binding activity of these two subunits, the budding yeast Rad23, Dsk2, and Ddi1 proteins which hold an N-terminal ubiquitin like domain (UBL) that binds to the proteasome and a C-terminal ubiquitin association domain (UBA) that binds to ubiquitin chains, help as shuttle receptor by binding substrates in the nucleus and cytoplasm and convey them to the proteasome. These shuttle receptors bind to Rpn1 subunit of the proteasome, though some studies suggest that in yeast, Dsk2 can also bind to Rpn10 and Rpn13 whereas yeast Rad23 could also interact with Rpt6 (Husnjak et al., 2008; Zhang et al., 2009; Wade and Auble, 2010; Gomez et al., 2011). Yeast proteasomes contain two deubiquitinating enzymes, Ubp6 and Rpn11. Rpn11 is a metalloprotease which is a core structural component of the lid. It promotes protein breakdown through its degradation-coupled activity. In contrast, Ubp6 is a cysteine protease associated with the base and has the ability to play a double role; it has both deubiquitinating activity and proteasome-inhibitory activity (delaying the degradation). However, delay of degradation by Ubp6 does not require its catalytic activity and this provides a time space (timing device) allowing gradual deubiquitination of the substrate by Ubp6 (Hanna et al., 2006; Peth et al., 2013). Cell division cycle protein 48 (Cdc48) in yeast and p97 in mammals (in the past, also often referred to as VCP) is an AAA-ATPase complex which has a central role as a facilitator of the ubiquitin-proteasome system (UPS). In interaction with several cofactors, Cdc48/p97 promotes poly-Ubiquitination chain elongation, segregates substrates from interactors and chaperons them to the 26S proteasome. Thus, Cdc48 is thought to act as a molecular chaperone in the UPS and enhance the degradation of unfolded proteins during endoplasmic reticulum (ER)-associated degradation (ERAD) (Förster et al., 2014). Most studies connect Cdc48 to ubiquitin-dependent processes where Cdc48 complexes bind to ubiquitinated proteins and then catalyze remodeling of the substrate structure to facilitate downstream steps. The remodeling of ubiquitinated protein by the core complex Cdc48–Npl4–Ufd1 (Npl4: Nuclear protein localization homolog 4, Ufd1: Ubiquitin fusion degradation 1) triggers removal of the proteins from complexes or cellular surfaces, frequently to enable degradation by the proteasome. This mechanism is found in the handling of membrane-bound transcription factor precursors and in ER-associated degradation, where Cdc48–Npl4–Ufd1 removes polyubiquitinated proteins from the membrane into the cytosol for transport to the proteasome (Meyer et al., 2012).

**Ubiquitin-Proteasome system in transcription regulation**

Studies have revealed a direct impact of proteasome-dependent proteolysis on transcription, therefore on target gene expression. And several laboratories have remarked an opposite correlation between the effectiveness of transcriptional activators and their levels in the cell, suggesting a mechanistic link between transcriptional activation and the turnover of the activator by the UPS. Many transcription factors, including yeast Gcn4 and Gal4 as well as mammalian Myc, Jun, E2-F and p53, are unstable proteins. Their protein levels are usually maintained low through targeted degradation by the UPS to allow rapid response to environmental stimuli (Lonard et al., 2000; Ouni et al., 2011). The phosphorylation of the yeast transcription factor Gcn4 by the cyclin-dependent kinases (CDKs) Srb10 and Pho85 leads to its proteasome-dependent degradation. The Srb10 cyclin-dependent kinases, a component of the RNA polymerase II (pol II) holoenzyme allows Gcn4 to bind to the promoter region which activates Gcn4-responsive genes and is then marked for destruction by SCF-Cdc4, the ubiquitin ligase for Gcn4. The transcriptional activators Gal4, Ino2, Ino4, pol II and histones recruit the ubiquitin ligases to the transcription site and become ubiquitinated, then ubiquitinated
activator recruits 26S proteasomes that degrade the activator and Gcn4 and rearrange a promoter for additional rounds of transcription (transcription elongation), therefore, stimulates expression of Gcn4-responsive genes (Von, 2006). Furthermore, the change in location can regulate the transcription factors: in the case for the pro-inflammatory transcriptional activator, NF-κB, which is kept into the cytosol by binding to its inhibitor IkB. The phosphorylation of IkB triggers its degradation, which causes it to be recognized by the E3 β-transducin repeat containing protein (β-TRCP). IkB then is ubiquitinated and rapidly degraded leading to its dissociation from the NF-κB DNA-binding subunits, and the unbound NF-κB translocates to the nucleus where it activates a multitude of target genes (Page et al., 2001; Lecker et al., 2006). Another case is the yeast transcription factor Spt23 which is attached to the outer membrane of the endoplasmic reticulum and activation in response to fatty acid limitation is initiated by Spt23 ubiquitylation followed by incomplete proteolysis to fasten off the membrane anchor. The mature Spt23 is released from the membrane and can translocate to the nucleus to activate expression of genes involved in fatty acid biosynthesis (Ouni et al., 2011).

**The impact of 19S regulatory particle on transcription regulation**

The 19S regulatory particle of the proteasome plays a vital role in cellular proteolytic. In addition to this role, 19S regulatory complex might also have a positive non-proteolytic role in transcriptional elongation. This complex comprises around 18 different proteins, including six highly related adenosine triphosphatases (ATPases) of the AAA-ATPases family (S4, S6a, S6b, S7, Sug1, and S10b). Five of these ATPases have been linked to transcription either biochemically or genetically. It has been suggested that the 19S regulatory subunit of the proteasome can act freely of the 26S proteasome. Direct proteasome-DNA connections have been witnessed at some yeast promoters, including GAL1-10 and CDC20, and in one of these cases, this interaction has been implicated in transcriptional activation. In yeast, ATPase components of the 19S proteasome associate with actively transcribing genes and regulate the elongation process carried out by RNA polymerase II. Subunits of the 19S, but not the 20S, have been shown to associate with Gal4 activators and Gal responsive promoters (GAL1-10). Specific inhibition of the 19S leads to a decrease in RNA pol II-mediated elongation, but inhibition of the 20S increases RNA pol II-mediated elongation, indicating that these sub-complexes have a function in transcription independent of both each other and of proteolytic activity (Fernando et al., 2002; Von, 2006; Bhat et al., 2010). Another form of transcriptional regulation by the yeast proteasome involves cleavage of the membrane bound, partially redundant transcription factors Spt23 and Mga2. The activated non-membrane bound form of these transcription factors regulates the transcription of OLE1, an enzyme essential for unsaturated fatty acid synthesis. The activating cleavage of Spt23 and Mga2 is dependent on both the proteasome and the highly conserved Cdc48-Npl4-Ufd1 complex. This complex serves as an adaptor for proteasomal function, coupling proteasome-dependent cleavage with OLE1 regulation as well as ER-associated degradation of misfolded proteins (Fig. 2). Spt23 and Mga2 are thought to modulate local chromatin environments, rather than acting as classical transcription factors. They do not contain canonical DNA binding domains but are able to activate transcription when fused to a DNA binding domain. They have also been shown to affect the silencing of mating type loci. In combination with genetic interactions with the SWI/SNF transcriptional complex, Spt23 and Mga2 can regulate transcription through modulating chromatin accessibility (Auld et al., 2006).

**Ubiquitination of histone in transcription control**

In the eukaryotic cells, the DNA is enveloped in the nuclei as chromatin, the basic unit of which is the nucleosome that is composed of 146 base pairs of DNA wrapped around a core histone octamer (containing two copies each of the histone proteins H2A, H2B, H3, and H4). The structure of chromatin accomplishes vital functions, not only by condensing and protecting DNA, but also in preserving genetic information and controlling gene expression. However, given its compacted structure, chromatin hinders several important cellular processes including, transcription, replication, and the detection/repair of DNA breaks (Zdenko and Rabib, 2011). Recent studies have proved that modifications to DNA or histones, or modifications in chromatin structure basically affect gene expression. For e.g., covalent histone modifications, including the site-specific acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation of histone tails, can directly change nucleosome or chromatin structure, or change the accessibility of certain transcriptional regulators to DNA, thus activating or repressing transcription (CAO and MA, 2011; Cao and Qin Yan, 2012).
A number of families of chromatin-modifying factors have been linked with gene activation, especially the histone methyl-transferases (e.g., CARM1 and the MLL-related family), histone acetyl-transferases (HATs, e.g., p300), and ATP-dependent remodeling enzymes (SWI/SNF and NURF). These factors are believed to decondense chromatin therefore allowing the subsequent binding of factors crucial for transcription. Furthermore, gene activation includes factors working freely of chromatin including the Mediator complex, which interacts with the activator and several components of the basal transcriptional machinery, such as TFII D and RNA polymerase II (Pavi et al., 2006). Histone ubiquitination happens mainly on histones H2A and H2B and it is a reversible process; the ubiquitin-specific proteases (UBPs in yeast) can remove attached ubiquitin from substrates. H2A is first ubiquitinated protein to be recognized, about 5–15% of the total H2A in mammalian cells is monoubiquitinated at Lys-119. However, the monoubiquitinated H2A (H2Aub1) has not been detected in yeast or higher plants. Histone H2B monoubiquitination (H2Bub1) was discovered in mouse cells around the same time as the identification of H2A as a target for ubiquitination. Though H2Bub1 is less abundant than H2Aub1, 1–2% compared with 5–15% for H2Aub1 in vertebrate cells, while about 10% of H2B are ubiquitinated in yeast cells. H2B is widely distributed throughout eukaryotic organisms from yeast to humans and plants. The ubiquitination residue in S. cerevisiae H2B is Lys-123, which corresponds to Lys-119 in Schizosaccharomyces pombe, Lys-120 in humans and Lys-146 in Arabidopsis (Weake and Workman, 2008; CAO and MA, 2011; Cao and Qin Yan, 2012).

Monoubiquitination of H2A and H2B have been obviously involved in transcriptional regulation. H2Aub work is more frequently associated with gene silencing, while H2Bub is mostly connected with transcription activation. H2A ubiquitin ligases (RING1A/RING1B/8M11 complex is the major E3 in mammalian cells) were found in transcription repressor complexes, such as the PRC1, BCoR, E2F6.com-1 and 2A-HUB complexes. RING1B mediated H2Aub is required for polycomb targeted gene silencing. Moreover, in an in vitro assay, H2Aub represses transcriptional initiation and inhibits the formation of transcriptional active markers H3K4me2 and me3 (Cao and Qin Yan, 2012). In contrast, H2Bub work is strongly correlated with active gene expression in most cases, possibly through several mechanisms, including promoting other active histone modifications and Pol II elongation and is necessary for reassembly of nucleosomes and restoration of the chromatin structure during the transcription elongation, thus influencing the kinetic properties of elongating Pol II (Xiao et al., 2005; Cao and Qin Yan, 2012). In a ChIP-on-Chip (Chromatin Immunoprecipitation) experiment, H2B monoubiquitination was found in the transcribed regions of highly expressed genes. As this mark is not linked to distal gene promoters but fairly to transcription start site (TSS) and more to gene bodies of active genes, it was suggested that, it is associated to transcription elongation rather than initiation. In yeast, monoubiquitinated H2B is required for the COMPASS complex for di- and tri-methylation of H3 at lysine 4, which are active markers for transcription (Zdenko and Rabih, 2011; Cao and Qin Yan, 2012). The effect of ubiquitilation on histone methylation can explain its role in both activation and inhibition of transcription. For example, it has been suggested that ubiquitilation of H2B occurs frequently in euchromatin leading to H3K4 and H3K79 methylation, which would prevent SIR proteins (Silent Information Regulator) from association with active euchromatic regions, thus restricting SIR proteins to heterochromatic regions to mediate silencing. At the same time in euchromatin, the ubiquitilation would activate the transcription by methylating H3K4 and by facilitating the transcriptional elongation (Zdenko and Rabih, 2011).

**Conclusion**

Since long ago heterologous proteins were produced by using microorganisms for medical and/or industrial purposes. Yeasts are preferable for production of recombinant proteins for their ease of rapid microbial growth and genetic manipulation found in bacteria along with the eukaryotic environment and the ability to perform many specific eukaryotic post-translational processing. Several research groups have devoted to search for competent yeast systems along with designing improved strains for heterologous protein production. Although there is a need in enhancing protein quality control to eliminate protein aggregations which are at the basis of several disorders, where in human kind they cause severe diseases like Parkinson’s, and Alzheimer’s disease. The protein quality control mechanisms is undertaken by regulated proteolytic systems which in eukaryotic cell are two major systems, the lysosomal and proteasome compartments. The efficacy of proteasomal degradation system is highly influenced by both the polyubiquitination by E1/E2/E3 mechanism and pretreatment of polyubiquitinated substrate by Rns and Rpts. And the activities of ubiquitin-proteasome system are under a mutual function of 20S core particle, 19S regulatory particles and different classes of proteasome interacting proteins. Moreover, UPS plays an essential role in regulation of gene expression where in yeast, the transcriptional activators like Gal4, Ino2, Ino4, pol II and histones can recruit the ubiquitin ligases to the transcription site and become ubiquitinated, then ubiquitinated activator recruits 26S proteasomes that degrade the activator and Gcn4 and rearrange a promoter for additional rounds of transcription, thus stimulates expression of Gcn4-responsive genes. In yeast, the expression of heterologous proteins can be stimulated by regulating the transcriptional elongation through different mechanism such as controlling monoubiquitination of histone (H2B), exploiting the direct interaction between the proteins of 19S regulatory...
complex especially AAA-ATPases family (S4, S6a, S6b, S7, Sug1, and S10b) with gene promoters and controlling the level of transcription factors through targeted degradation by the UPS.

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