The nuclear ubiquitin-proteasome system

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Summary

In eukaryotes, thousands of genes have to be organized and expressed in the cell nucleus. Conformational and kinetic instability of nuclear structure and components appear to enable cells to use the encoded information selectively. The ubiquitin-proteasome system is active in distinct nuclear domains and plays a major role controlling the initial steps of gene expression, DNA repair and nuclear quality-control mechanisms. Recent work indicates that a tuned balance of ubiquitylation and proteasome-dependent protein degradation of nuclear proteins is instrumental in nuclear function and, when deregulated, leads to the development of diseases such as polyQ disorders and other neurodegenerative conditions.

Key words: Cell nucleus, Proteasome, Protein aggregation, Proteolysis, Transcription, Ubiquitin

Introduction

Within the nucleus, DNA is packed into differentially condensed chromatin, in which it is repaired, replicated, and transcribed into RNA that has to be spliced and transported to the cytoplasm for protein synthesis. Current research is focusing on spatial organization and regulation of gene expression by epigenetic mechanisms, including DNA and histone modifications. The interphase nucleus contains highly dynamic subcompartments (Misteli, 2001), such as chromosome territories (Cremer et al., 2004), the nuclear envelope (Wilson, 2000), nucleoli (Scheer and Hock, 1999; Dundr et al., 2002), speckles (Spector, 1993), PML bodies (Zhong et al., 2000) and Cajal bodies (Gall, 2000; Cioce and Lamond, 2005). The nucleolus represents a paradigm for the dynamic form and function of these components. Basically all events of ribosome biogenesis – such as transcription of the ribosomal RNA (rRNA) genes, processing of their primary transcripts into mature rRNAs and assembly with ribosomal and non-ribosomal proteins to integrate into pre-ribosomes – are confined to the nucleolus. In most eukaryotic cells, the entire structure breaks down and reforms during each mitotic cycle. The nucleolus is reassembled around the ribosomal DNA (rDNA) repeats (Angelier et al., 2005; Mais et al., 2005), which occupy specific chromosomal loci, the nucleolar organizer regions (NOR).

Dynamic nuclear processes have to be regulated and controlled to maintain the function of the nucleus throughout interphase. More than twenty years ago, Varshavsky and co-workers discovered that ubiquitin-dependent pathways play a role in cell-cycle control and suggested that protein degradation is instrumental in regulation of gene expression (Finley et al., 1984). Prior to that, Franke and colleagues had shown that proteasomes localize to the nuclei of *Xenopus laevis* oocytes and HeLa cells (Hugle et al., 1983; Kleinschmidt et al., 1983). Subsequent work confirmed that components of the ubiquitin-proteasome system (UPS) indeed reside in the cell nucleus and that nuclear proteins are substrates for proteasomal degradation. However, research predominantly concentrated on cytoplasmic protein breakdown, showing that this generates peptides for antigen presentation (Rock et al., 1994) and removes newly synthesized, misfolded proteins (Reits et al., 2000; Schubert et al., 2000). The importance of localization of proteolysis in distinct cellular compartments is exemplified by endoplasmatic reticulum (ER)-associated degradation (ERAD), in which substrates are polyubiquitylated within the ER and degraded by proteasomes in the cytosol (Ahner and Brodsky, 2004; Pines and Lindon, 2005). Similarly, export to the cytoplasm is considered to be required for efficient degradation of nuclear substrates such as the tumor suppressor p53 and cyclin-dependent kinase (CDK) inhibitor p27 (Freedman et al., 1998; Kamura et al., 2004). However, growing evidence implicates direct engagement of a nuclear UPS (nUPS) in DNA repair, replication, transcription and nuclear quality-control (Mendez et al., 2002; Baker and Grant, 2005; Chen and von Mikecz, 2005a; Gardner et al., 2005; Pfander et al., 2005; Rockel et al., 2005). Here, I discuss ubiquitylation pathways and proteasomal proteolysis in the cell nucleus as likely players that help control nuclear structure and function.

The ubiquitin-proteasome system (UPS)

Intracellular proteolysis plays a crucial role in the cell cycle, signal transduction, gene expression, development, maintenance of proper protein folding, antigen processing and other cellular processes (Kirschner, 1999). Eukaryotic cells have two major mechanisms for protein degradation: lysosomes and proteasomes. The majority of intracellular proteins are proteolyzed by the proteasomes. In most cultured mammalian cells, these account for 80-90% of protein breakdown (Lee and Goldberg, 1998) and the substrates include transcriptional regulators (e.g. NF-κB and IκB) (Fan and Maniatis, 1991; Palombella et al., 1994; Alvarez-Castelao and Castano, 2005), cell-cycle proteins (e.g. cyclins, cyclin-dependent kinase inhibitors) (Glotzer et al., 1991; Hershko et al., 1991; Pagano et al., 1995), oncogene products (Ciechanover et al., 1991; Stancovski et al., 1995) and the

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tumor suppressor p53 (Scheffner et al., 1990; Maki et al., 1996; Kubbutat et al., 1997). The 'housekeeping' 26S proteasomes are ATP-driven, multisubunit proteolytic machines that preferentially degrade proteins tagged with polyubiquitin chains (Hershko et al., 1982; Pickart and Rose, 1985; Voges et al., 1999; Hershko and Ciechanover, 1998; Elsasser and Finley, 2005). The covalent attachment of ubiquitin to substrates involves the formation of an isopeptide bond between the ϵ-amino group of a lysine residue of the substrate and the C-terminal carboxylate group of ubiquitin. Conjugation is accomplished in three sequential reactions, which are catalyzed by a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase (E3). After the formation of the ubiquitin-substrate isopeptide bond, a second ubiquitin can be conjugated to a specific lysine residue, typically Lys48, of the first ubiquitin. Repetition of this reaction leads to assembly of a polyubiquitin chain on the proteolytic substrate that targets the protein for degradation by the 26S proteasome.

Elegant studies of structurally defined, polyubiquitylated model substrates showed that conjugation of a tetra-ubiquitin chain represents the minimum signal for efficient proteasomal targeting (Thrower et al., 2000). However, ubiquitylation does not always lead to proteolysis. Non-degradative functions of ubiquitin have emerged in recent years, including the regulation of protein location, protein function and protein-protein interactions (Schnell and Hicke, 2003). Mono-ubiquitylation regulates the activity of proteins located at the plasma membrane. Most of these proteins require ubiquitylation of their cytoplasmic domains to be internalized into the endocytic pathway. Similarly, evidence is accumulating that mono-ubiquitin is an essential part of virus budding in infected cells (Strack et al., 2000). Finally, histones are post-translationally modified by ubiquitylation at their N-terminal tails, which contributes to the 'histone-code'. For example, in yeast nuclei, Rad6-mediated mono-ubiquitylation of histone H2B regulates H3 methylation, which, in turn, mediates gene silencing (Sun and Allis, 2002).

The 26S proteasome comprises a 20S core component and two flanking 19S complexes that regulate substrate specificity (Fig. 1). The 20S core is an abundant particle that has been highly conserved from yeast to humans; simpler prototypes are found in prokaryotes (Löwe et al., 1995). Four α- and β-rings surround a barrel-shaped cavity in the 20S core (Groll et al., 1997) (Fig. 1). The two inner β-rings form a central chamber that harbors the proteolytic centers containing chymotryptic, tryptic and caspase-like activities. According to the current 'two substrate' model, ubiquitylated or denatured proteins are first recognized by the ATPase subunit S6′ (Rpt5) of the 19S regulatory complex (Lam et al., 2002), unfolded and channeled via a central passageway into the degradation chamber, then degraded into peptides, and finally released through the entry channel (Finley, 2002; Hutschenreiter et al., 2004).

Proteasomes cleave their substrates to give 3≅20-residue peptides. These are further degraded into single amino acids by downstream endo- and amino-peptidases (Tamura et al., 1998) or escape complete proteolysis and are subjected to antigen presentation by MHC class I molecules (Rock et al., 1994). Inhibition of proteasomal protein degradation by specific inhibitors such as the steptomyces-metabolite lactacystin results in accumulation of substrates within the cell, and these can be measured by immunoblotting,

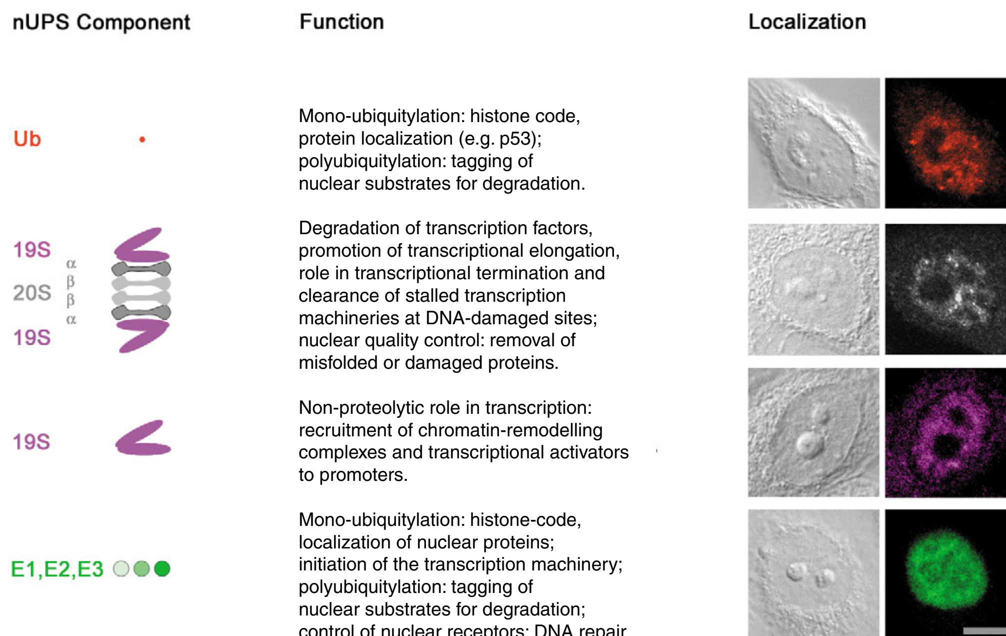

Fig. 1. Function and localization of nuclear UPS components. Micrographs on the right show confocal immunofluorescence of HEp-2 cells with antibodies against components of the UPS: rabbit polyclonal anti-ubiquitin (Ub FL-76; red), mouse monoclonal anti-20S proteasome core subunits (20S core; gray), mouse monoclonal anti-19S regulator (19S Rpt6, purple) and rabbit anti-ubiquitin-conjugating enzyme E2 (UbcH6, green). Micrographs in the left show corresponding differential interference contrast (DIC) images. Ub, ubiquitin; 19S-20S-19S, 26S proteasome; 19S, 19S regulator complex; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase. Bar, 5 μm.

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immunofluorescence or metabolic labeling techniques (Ciechanover, 1998; Lee and Goldberg, 1998).

Components of the UPS in the cell nucleus

Strong evidence now indicates that proteasomes occur in the cytoplasm and the cell nucleus (Hugle et al., 1983; Kleinschmidt et al., 1983; Reits et al., 1997; Lafarga et al., 2002; Adori et al., 2006). Nuclear localization of proteasomes greatly depends on cell type, cell density and growth conditions, and the relative proportion of nuclear versus cytoplasmic proteasomes can vary between 17% and 50% (Rivett et al., 1992). Proteasomes have been localized to PML nuclear bodies (Fabunmi et al., 2001; Rockel and von Mikecz, 2002), nucleoplasmic speckles (Chen et al., 2002) and focal clusters throughout the nucleoplasm (Rockel et al., 2005). In particular conditions, such as when Myc expression is elevated, proteasomes also appear in nucleoli (Arabi et al., 2003); however, proteomic analyses have corroborated earlier observations that nucleoli do not contain proteasomes under normal conditions (Andersen et al., 2002; Andersen et al., 2005).

Several subunits of the 20S proteasome have nuclear localization signals (Nederlof et al., 1995), and tyrosine phosphorylation of these might play a role in the nucleo-cytoplasmic transfer of proteasomes (Tanaka et al., 1990). In yeast, 20S proteasomes and the 19S regulator seem to be imported into the nucleus as inactive precursor complexes by karyopherin $\alpha\beta$ (Lehmann et al., 2002; Wendler et al., 2004). Ubiquitin-conjugating (Rhp6/Ubc2/Rad6) and -ligating enzymes (Ubr1) concentrate nuclear proteasomes by linking them to the nuclear envelope protein Cut8, which has been shown to anchor 26S proteasomes at the inner nuclear membrane of fission yeast (Takeda and Yanagida, 2005). In contrast to nuclear import, there is no experimental evidence for export of proteasomes or proteasomal subunits from the cell nucleus to the cytoplasm. These might therefore be transported unidirectionally from the cytoplasm to the nucleus during interphase (Reits et al., 1997), and the cytoplasmic and nuclear proteasome pools could then equilibrate during mitosis. However, comparative analysis of 20S proteasomes purified from HeLaS3 cells has shown that nuclear proteasomes have a clearly different subtype pattern compared with cytoplasmic proteasomes and a significantly higher content of the immunosubunits beta1i and beta5i, which are incorporated into the 20S core under certain conditions, such as immunostimulation by interferon γ (N. Klare and B. Dahlmann, personal communication). This suggests preferential nuclear import of selected subunits or 20S proteasomes that have a particular subunit composition.

The nucleus also contains other components of the UPS, such as ubiquitin (Schwartz et al., 1988) (Fig. 1), the regulatory subunit PA28 (Fabunmi et al., 2001), the 19S regulatory complex (Peters et al., 1994) (Fig. 1), the proteasome activator Blm10 (Schmidt et al., 2005), the ubiquitin-specific protease HAUSP (Everett et al., 1997) and E1-E3 enzymes (Roth et al., 1998; Plafker et al., 2004) (Fig. 1). These may be regulated by changes in their intracellular distribution. The E3 ligase Nedd4, for example, contains a Crm-dependent nuclear export signal that maintains the cytoplasmic localization of this enzyme (Hamilton et al., 2001), whereas the E3 ligase Mdm2 that regulates the turnover of p53 changes its intracellular distribution through nuclear import, export or nucleolar localization in response to environmental stimuli (Roth et al., 1998; Bernardi et al., 2004). Under certain physiological conditions the Mdm2 and the E3 ligase von Hippel-Lindau tumor suppressor (VHL) become immobilized in the nucleolus; such nucleolar segregation inhibits their ubiquitin ligase function and stabilizes their respective substrates p53 and hypoxia-inducible factor (HIF) (Mekhail et al., 2005). Nuclear import of the class III E2 enzymes UbcM2, UbcH6 and UBE2E2 involves the karyopherin importin 11 and is triggered by covalent attachment of ubiquitin to the active site cysteine residues of these enzymes (Plafker et al., 2004).

With all the players in place, the obvious question is whether the nUPS is active; are nuclear proteins ubiquitylated and degraded within the nucleus or are they transported to the cytoplasm in order to be hydrolyzed?

Substrates of the nUPS

Substrates reported to be degraded by nuclear proteolysis include the yeast cyclin-dependent kinase (CDK) inhibitor Far1 (Blondel et al., 2000); the transcriptional repressor

Table 1. Substrates of the nuclear ubiquitin-proteasome system (nUPS)

Substrate	Nuclear structure and/or function
Far1	Cyclin-dependent kinase (CDK) inhibitor (Blondel et al., 2000)
Estrogen receptor alpha	Nuclear hormone receptor (Nawaz et al., 1999)
E1A	Nuclear oncoprotein (Ciechanover et al., 1991)
Fos	Nuclear oncoprotein (Ciechanover et al., 1991)
Jun	Nuclear oncoprotein (Ciechanover et al., 1991)
Myc	Nuclear oncoprotein (Ciechanover et al., 1991)
p53	Tumor suppressor (Ciechanover et al., 1991; Shirangi et al., 2002)
STAT1	Transcription factor (Kim and Maniatis, 1996)
GCN4	Transcription factor (Mayor et al., 2005)
Matα2	Transcriptional repressor (Lenk and Sommer, 2000)
MyoD	Transcription factor (Floyd et al., 2001)
RNA polymerase II	Transcription (Beaudenon et al., 1999)
CREB-binding protein (CBP)	Histone acetylase/transcriptional co-activator (Jiang et al., 2003)
Histone H2A	Chromatin structure (Rockel and von Mikecz, 2002)
SmB/B′	Spliceosomal component (Rockel and von Mikecz, 2002)
U1-70k	Spliceosomal component (Rockel and von Mikecz, 2002)
SC-35	Nucleoplasmic speckles/ splicing factor (Rockel and von Mikecz, 2002)
PML	PML body component/tumor suppressor (Rockel and von Mikecz, 2002)
DNA topoisomerase I	DNA topology (Desai et al., 1997; Chen et al., 2005)

Matα2, whose rapid degradation is observed only when the protein is efficiently imported into the nucleus (Lenk and Sommer, 2000); the transcription factor MyoD (Floyd et al., 2001); and p53, which is degraded by nuclear proteasomes during downregulation of the stress response after DNA damage (Shirangi et al., 2002). The principal regulator of p53 is Mdm2/Hdm2, an E3 ubiquitin ligase that induces degradation of p53 by the UPS. Numerous studies indicated that p53 is degraded by cytoplasmic proteasomes and must be exported first. However, in the aftermath of stress responses that do not result in cell death, addition of leptomycin B, an inhibitor of nuclear export, induces re-accumulation and proteasome-dependent proteolysis of endogenous p53 in the nucleus. Subsequent work indicates that the level of Mdm2 activity differentially controls the fate of p53: low-level Mdm2 activity induces mono-ubiquitylation and nuclear export of p53, whereas high Mdm2 activity promotes polyubiquitylation of p53 and its degradation in the nucleus (Li et al., 2003).

Consistent with the idea of nuclear proteolysis is the observation that mutated forms of influenza virus nucleoprotein (NP) misfold and rapidly cluster with proteasomes, ubiquitin and the chaperone HSC70 in PML nuclear bodies (Anton et al., 1999). PML bodies might represent proteolytic centers in the nucleus because interferon γ and virus infection induce recruitment of proteasomes, regulator 11S and misfolded proteins to this location (Fabunmi et al., 2001). Direct evidence that they function as proteolytic sites comes from microinjection experiments that localize degradation of ectopic protein DQ-ovalbumin to distinct nucleoplasmic foci that overlap with a subpopulation of PML bodies (Rockel et al., 2005).

In mammalian cells, specific inhibition of proteasomal degradation by lactacystin stabilizes nuclear proteins such as histone protein H2A, splicing factor SC35, spliceosomal components U1-70k and Sm-B/B′, and PML protein (Rockel and von Mikecz, 2002). These accumulate in the subnuclear compartments in which they normally reside upon inhibition of proteasomes, which suggests that they represent substrates of the nUPS. Such substrates can be located in splicing speckles, PML bodies and additional foci within the nucleus; by contrast, nucleolar and nuclear envelope proteins such as fibrillarin and lamin A/C do not appear to represent proteasome substrates under normal conditions (Chen et al., 2002; Rockel and von Mikecz, 2002). Biochemical fractionation revealed that the nucleoplasm (but not nucleolar or nuclear envelope fractions) contains proteasomal activity (Chen et al., 2005; Rockel et al., 2005). Furthermore, microinjection of the fluorogenic model substrates DQ-ovalbumin and DQ-BSA shows that proteasome-dependent proteolysis occurs in transient foci in the nucleoplasm, which disappear when proteasomal degradation is inhibited (Rockel et al., 2005). These results establish proteasomal proteolysis as an intrinsic function of the nucleus.

The role of the nUPS in gene expression

The obvious advantage of a nUPS is tight regulation of nuclear function. A typical mammalian nucleus harbors ~25,000 genes (International Human Genome Sequencing Consortium, 2001 and 2002) (Venter et al., 2001; Lindblad-Toh et al., 2005). Only a fraction are expressed at a given time point, allowing the cell to respond to environmental stimuli, proliferate and develop. It is generally anticipated that gene expression in eukaryotes is organized as an assembly line enabling the sequential ordering of RNA synthesis, maturation and transport (Sims et al., 2004). Hundreds of macromolecules including chromatin modulators, transcription factors and ribonucleoprotein particles (RNPs), cluster to form the molecular machineries for replication, DNA repair, transcription, RNA splicing and ribosome biogenesis. How are these clusters disassembled and turned off to enable the sequential steps in gene expression to proceed?

Several laboratories have noticed an inverse correlation between the potency 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. Consistent with this idea is the observation that proteasome inhibitors block estrogen-receptor-mediated transcription, and specifically inhibit cycling of estrogen receptor α and other transcription factors onto and off estrogen-responsive promoters (Lonard et al., 2000; Reid et al., 2003). Several steroid-receptor-interacting proteins have been identified as components of the UPS, including Sug1/Trip1 (Lee et al., 1995; vom Baur et al., 1996), Rsp5/Rpf1 (Imhof and McDonnell, 1996), E6-AP (Nawaz et al., 1999) and Ubc9 (Göttlicher et al., 1996). Transcriptional output may thus depend on tight control of the exchange rate of ligand-bound nuclear receptors by the nUPS.

A direct influence of proteasome-dependent proteolysis on transcription has also been demonstrated by Deshaies and co-workers. They have shown that phosphorylation of the yeast transcription factor Gcn4 by the cyclin-dependent kinases (CDKs) Srb10 and Pho85 leads to its proteasome-dependent degradation (Chi et al., 2001). Since Srb10 is a component of the RNA polymerase II (pol II) holoenzyme, they concluded that Gcn4 binds to the promoter region, activates Gcn4-responsive genes and is subsequently marked for destruction by SCF^{Cdc4}, the ubiquitin ligase for Gcn4, and proposed the hypothesis that proteolysis is required to remove 'spent' activators to reset the promoter. Indeed, degradation of Gcn4 and the transcriptional activators Gal4 and Ino2 and Ino4 by the UPS stimulates expression of their targets. Moreover, studies have shown that mutations in SCF^{Cdc4} and mutations in ubiquitin that prevent proteolysis also impair the transcription of Gcn4 targets (Lipford et al., 2005), confirming this idea. Muratani and Tansey have proposed a model in which interaction of a transcriptional activator with the general transcription machinery recruits ubiquitin ligases to the transcription site. They suggest that the activator, pol II and histones are ubiquitylated and this recruits 26S proteasomes that degrade the activator and promote transcriptional elongation (Muratani and Tansey, 2003).

Subunits of the 19S regulatory complex of the proteasome might also have a positive role in transcription that is independent of their proteolytic function. This complex contains approximately 18 distinct proteins, including six highly related adenosine triphosphatases (ATPases) of the ATPases associated with various cellular activities (AAA) family. Five of these ATPases have been linked to transcription either biochemically or genetically. In yeast, proteins from the 19S regulatory complex are recruited to the *GAL1-10* promoter by Gal4 upon induction with galactose (Gonzalez et al., 2002). The ATPases recruit the chromatin-remodelling complex Spt-Ada-Gcn5-acetyltransferase (SAGA) to the *GAL1-GAL10*

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promoter and stimulate interactions between SAGA and sequence-specific, DNA-binding transcriptional activators (Lee et al., 2005). This targeting induces increased histone H3 acetylation, chromatin decondensation and *GAL1* transcription.

The nUPS has also been connected with DNA repair. DNA double-strand breaks (DSBs) occasionally occur during DNA replication and, more frequently, when cells are exposed to ionizing radiation or other DNA-damaging agents. Checkpoint pathways that delay the cell cycle to allow the repair of the damage sustain the integrity of the genetic material. In 1987, Jentsch et al. showed that a classic DNA repair gene in yeast, *RAD6*, encodes a ubiquitin-conjugating enzyme (Jentsch et al., 1987). Another protein, Rad23, which is involved in nucleotide excision repair (NER), interacts with the 26S proteasome through an N-terminal ubiquitin-like domain (UbL^{R23}), and its C-terminus binds to the Rad4 DNA-repair protein. Thus, Rad23 creates a link between DNA repair and the UPS (Schauber et al., 1998). The UbL of Rad23 is required for optimal activity of an in vitro NER system, because inhibition of 19S regulatory complex ATPases diminishes NER activity and increases UV sensitivity in vivo. Consistent with the idea of proteolysis-independent functions of the complex is the observation that inhibition of protein degradation by the proteasome has no effect on the efficiency of NER mediated by Rad23 (Russell et al., 1999), which suggests that NER does not require the proteolytic activity of the proteasome but depends on the functional integrity of the 19S cap. Damage-induced ubiquitylation of proliferating cell nuclear antigen (PCNA) at K164 is linked to *RAD6*-dependent DNA repair. Mono-ubiquitylation of PCNA by Rad6 and Rad18, modification by K63-linked multi-ubiquitylation and conjugation of PCNA to the small ubiquitin-related modifier (SUMO) by the ubiquitin-conjugating enzyme Ubc9 differentially modulates resistance to DNA damage (Hoege et al., 2002).

Recent reports reveal a direct role of the proteasome in DSB repair. Yeast Sem1 and its human orthologue DSS1, which are instrumental in repair of DSBs, together with tumor suppressor protein BRCA2, co-purify with the 19S regulatory complex (Krogan et al., 2004). Sem1 is required for efficient repair of DSBs by homologous recombination (HR) and nonhomologous end joining (NHEJ) and is recruited to sites of DNA damage together with the 19S regulatory complex and the 20S proteasome. Additionally, 26S proteasomes have been shown to physically associate with transcriptionally active genes at sites of DNA damage and the 3′ ends of these genes. Thus, proteasomes play an important role in transcription termination and the clearance of stalled transcription machinery that is blocked by DNA damage (Gillette et al., 2004).

The role of the nUPS in nuclear quality-control

Proteasomes serve as a quality-control system that rapidly eliminates the ~30% of proteins that are mal-folded or damaged and would interfere with normal cell function if allowed to accumulate (Schubert et al., 2000). Gardner et al. have identified a nuclear quality-control system in yeast. It involves the ubiquitin ligase San1p, which in conjunction with the ubiquitin conjugating enzymes Cdc34p and Ubc1p, targets four distinct mutant nuclear proteins for ubiquitin-dependent degradation (Gardner et al., 2005). Analogous systems should exist in higher eukaryotes, because quality-control is particularly important for protection of cells against harsh conditions, such as heat shock and oxidative stress, and in a variety of diseases – for example, degenerative diseases characterized by intranuclear accumulation of insoluble polyglutamine (polyQ)-containing proteins and other proteins (Goldberg, 2003). Nine neurodegenerative disorders are caused by expansion of CAG repeats encoding polyQ streches. The mutant proteins undergo a conformational change and aggregate to form characteristic inclusion bodies in both the cytoplasm and the nucleus (Forster and Lewy, 1912). Ubiquitin is a major component of such inclusions (Lowe et al., 1988).

Another key component of inclusions associated with neurodegeneration is mal-folded proteins, which can be a consequence of mis-sense mutations, aberrant modification or post-translational damage of proteins, as well as expansion of polyQ repeats. Moreover, changes in transcriptional activity have been linked to protein aggregation and perturbation of the UPS. In several polyQ diseases, the transcriptional co-activator CREB-binding protein (CBP) and other nuclear factors become sequestered in nuclear inclusions (Jiang et al., 2003; Chen and von Mikecz, 2005b), and polyQ-mediated toxicity can be tempered in cell culture by overexpression of CBP (Nucifora et al., 2001). Additionally, mutant huntingtin, the signature polyQ protein of Huntington's disease (HD), induces proteasome-dependent degradation of CBP (Jiang et al., 2003) and inhibition of CBP-induced transcription. CBP molecules might acquire an abnormal structure through interactions with mutant polyQ proteins and thus be targeted for degradation by the nUPS. Protein aggregation could therefore lead to proteasomal degradation that depletes essential proteins within the cell nucleus, thereby causing toxicity (Ross and Pickart, 2004). However, the molecular interplay between the nUPS and protein aggregation, as well as the role of intranuclear inclusions in pathogenesis of protein aggregation diseases, remains controversial. It is not clear whether nuclear protein aggregates and/or inclusions represent active proteolysis centers, staging areas or cellular junkyards (Kopito, 2000). Recent work shows that uptake of silica nanoparticles (nano-SiO_2) by the cell nucleus induces formation of inclusion bodies in the nucleoplasm that contain proteins characteristic of particular nuclear domains, the transcriptional co-activator CBP, components of the nUPS and polyQ proteins (Chen and von Mikecz, 2005b). Since formation of these bodies correlates with inhibition of gene expression and appearance of features typical of cellular senescence, the use of nano-SiO_2 in cell culture could serve as a valuable model for studies of the role of the nUPS in nuclear protein aggregation.

Intranuclear inclusions can be grouped according to their pathology. Marinesco bodies (MBs) are intranuclear inclusions detectable in nigral pigmented neurons from patients with polyQ diseases and in hepatic encephalopathy. MBs increase in number during aging, and their formation is considered to represent a cellular reaction to stress. Unique clusters of nuclear proteins and components of the nUPS have also been detected in dendritic cells from patients with systemic autoimmune diseases such as scleroderma that are linked to xenobiotics (Chen et al., 2005; Chen and von Mikecz, 2005a). One idea is that xenobiotic-induced autoimmunity and protein-aggregation diseases share a

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common subnuclear pathology involving the nUPS (von Mikecz and Hemmerich, 2004; von Mikecz, 2005). Indeed, in dendritic cells, polyubiquitylated proteins are sorted to transient cytosolic aggregates named dendritic cell aggresome-like induced structures (DALIS) that accumulate misfolded proteins and contain a ubiquitin-activating enzyme, the ubiquitin-conjugating enzyme $E2_{25K}$, and the ubiquitin ligase C-terminus of Hsp70 interacting protein (CHIP) (Lelouard et al., 2004). I propose that similar ubiquitylation and proteolysis centers exist in nuclei of dendritic cells, and that such centers are instrumental in regulation of cell maturation and/or presentation of nuclear antigens. Clusters of nuclear proteins with components of the nUPS in dendritic cells from patients with scleroderma might represent nuclear DALIS.

Concluding remarks and perspectives

Compartmentalized proteolysis allows cells to control degradation of specific proteins by regulating their subcellular localization. Considering the central role of the UPS in cellular processes, detailed knowledge of the time and place a substrate is ubiquitylated and degraded will prove to be essential to our understanding of the molecular mechanisms that regulate cell structure, function, development and disease pathology. It is well established that the UPS is not only present in, but an active component of the cell nucleus. Studies of epigenetic factors that control gene expression are revealing both non-proteolytic functions of UPS components and proteasomal degradation in the cell nucleus, and accumulating evidence suggests that the nUPS is involved in regulation of chromatin structure. In *S. pombe*, clustering of ubiquitin ligase component Cul4 with histone methyltransferase Clr4 and Rik1 protein is instrumental in heterochromatin formation that mediates silencing at heterochromatic gene loci (Jia et al., 2005). A major challenge of future research will be to characterize further nucleoplasmic protein clusters that contain components of the UPS, and determine their protein composition, ubiquitylation capacity and proteasomal activity to distinguish functional from pathological protein clusters or aggregates. The tight balance of ubiquitylation and proteasomal proteolysis within or near such nucleoplasmic clusters may decide whether the cell nucleus walks the line or succumbs to disease.

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