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MINIREVIEW

Role and Function of the 26S Proteasome in Proliferation and Apoptosis

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SUMMARY: The 26S proteasome constitutes the central proteolytic machinery of the highly conserved ubiquitin/proteasome system, the cell's major tool for extralysosomal protein degradation. Recently, a plethora of cell proteins implicated in the regulation of basic cellular processes, such as proliferation, differentiation, cell cycling, and apoptosis have been discovered to undergo processing and functional limitation by entering the ubiquitin/proteasome pathway with the final destination to be proteolytically degraded by the 26S proteasome. Because both negative and positive regulators of proliferation and apoptosis undergo proteasomal degradation in a tightly regulated and temporally controlled fashion, the 26S proteasome can play opposite roles in the regulation of proliferation and apoptosis. These roles are apparently defined by the cell's environment and proliferative state. Finally, proteasomal protein degradation is deregulated in a number of human diseases, including cancer and neurodegenerative and myodegenerative diseases, which all exhibit an imbalance of proliferation and apoptosis. An improved understanding of the modes of proteasomal action should lead to the development of beneficial therapeutic and diagnostic strategies in the future. (*Lab Invest 2002, 82:965–980*).

The discovery of the ubiquitin/proteasome pathway and the characterization of its biologic function as the cell's major system for extralysosomal protein degradation have pointed out a new conceptual framework for understanding the regulation of basic cellular processes by selective and temporally controlled proteolytic degradation of regulatory proteins (Ciechanover, 1998; Ciechanover et al, 2000; Ciechanover and Schwarz, 1998; Herschko et al, 2000).

In the past few years, a large number of proteins necessary for cell cycle regulation and control of cell proliferation, cell differentiation, and apoptosis (programmed cell death) have been discovered to undergo processing and functional limitation by entering the ubiquitin/proteasome pathway with the final destination to be degraded by the 26S proteasome, a large multicatalytic protease complex (Grimm and Osborne, 2000; Mann and Hilt, 2000; Yew, 2001; Table 1).

To be processed this way, proteins must be targeted for recognition and subsequent degradation by the 26S proteasome by covalent attachments of multiple monomers of the 76 amino acid polypeptide ubiquitin. This process, called ubiquitination, takes place in a multistep reaction governed by three classes of enzymes, namely ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin-protein ligases (E3) (Ciechanover et al, 2000).

The 26S proteasome, an ATP-dependent multisubunit protease complex found in the cytoplasm and in the nucleus of all eukaryotic cells, constitutes the central proteolytic machinery of the ubiquitin/proteasome system, and is composed of a barrel-shaped 20S catalytic core complex capped at both ends by a 19S regulatory complex (Ferrell et al, 2000; Voges et al, 1999; Fig. 1). The 20S complex has the form of a hollow cylinder composed of four axially stacked rings, each of seven different but related subunits, giving the complex the general stoichiometry of $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ (Groll et al, 1997; Löwe et al, 1995). The two inner rings are formed by β subunits, which carry proteolytic sites formed by amino-terminal threonine residues faced to the central cavity of the 20S complex (Baumeister et al, 1998; Voges et al, 1999). The outer rings consist of nonproteolytic α subunits that allow substrate translocation into the central cavity and conformational interactions between the 20S complex and the 19S regulatory complex (Baumeister et al, 1998). The 19S regulatory complex consists of the base subcomplex containing six AAA-ATPases and the lid subcomplex composed of eight non-ATPase subunits that exhibit sequence homologies to human and *Arabidopsis* COP9-signalosome subunits

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Table 1. Examples of Cellular Regulatory Proteins Degraded by the 26S Proteasome

Protein	Function	Functional effects of degradation/comments	Reference
$p21^{WAF1/Cip1}$	CKI, cell cycle regulation	Cell cycle progression; differentiation	Blagosklonny et al, 1996; Di Cunto et al, 1998
$p27^{Kip1}$	CKI, cell cycle regulation	Cell cycle progression;/enhanced degradation: tumor progression; inhibition of differentiation	Pagano et al, 1995; Sgambato et al, 2000; Baldassarre et al, 2000
$p19^{INK4d}$	CKI, cell cycle regulation	Cell cycle progression	Thullberg et al, 2000
Cyclin A	Cell cycle regulation	Completion of mitosis; entry into S-phase	Yew, 2001
Cyclin D	Cell cycle regulation	Completion of S-phase?/defective degradation in breast cancer	Diehl et al, 1997; Russel et al, 1999
Cyclin E	Cell cycle regulation	Entry into S-phase; progression through S-phase	Singer et al, 1999
E2F	Cell cycle regulation	Completion of G1-to-S transitions	Marti et al, 1999
Cdc6	Initiation of DNA replication	Completion of DNA replication in G1-phase	Peterson et al, 2000
Geminin	Inhibition of DNA replication	Entry into S-phase; completion of mitosis	McGarry and Kirschner, 1998
c-IAP1, XIAP	Inhibition of apoptosis	Promotion of apoptosis;/degradation after autoubiquitination in response to apoptotic stimuli	Yang et al, 2000; Suzuki et al, 2001
Bcl-2	Inhibition of apoptosis	Promotion of apoptosis	Dimmeler et al, 1999; Breitschopf et al, 2000a
IκBα	Inhibitor of NF-κB	Inhibition of apoptosis through activation of NF-κB	Palombella et al, 1994; Chen et al, 1995; Mayo et al, 1997
NF-κB precursor	Maturation of NF-κB	Maintenance of NF-κB abundance	Palombella et al, 1994; Lin et al, 1998
p53	Maintenance of genetic integrity; induction of apoptosis/cell cycle arrest	Posttranslational regulation of p53;/enhanced degradation: promotion of neoplastic proliferation	Maki et al, 1996; Masdehors et al, 2000; Hengstermann et al, 2001
Bax	Promotion of apoptosis	Inhibition of apoptosis;/increased degradation in prostate carcinomas	Chang et al, 1998; Li and Dou, 2000
Bid	Promotion of apoptosis	Inhibition of apoptosis	Breitschopf et al, 2000b
c-Myc	Promotion of proliferation	Inhibition of proliferation;/decreased degradation in lymphomas	Gregory and Hann, 2000
β-Catenin	Signal transduction	Regulation of differentiation?/decreased degradation in inherited colon carcinomas	Aberle et al, 1997; Korink et al, 1997; Morin et al, 1997
$p57^{Kip2}$	CKI, differentiation, cell cycle regulation	Inhibition of differentiation	Urano et al, 1999
Cdc25a	Inhibition of differentiation	Induction of differentiation; cell cycle regulation	Bernardi et al, 2000
CPEB	Translational activation	Completion of meiosis (oocyte maturation)	Reverte et al, 2001

CKI; cyclin-dependent kinase inhibitor; CPEB; cytoplasmic polyadenylation element binding protein.

(Glickman et al, 1998; Henke et al, 1999; Wei et al, 1998). The functions of the 19S regulatory complex seem to be recognition, deubiquitination, unfolding, and translocation of substrate proteins before their proteolytic degradation within the central cavity of the 20S complex (Braun et al, 1999; Glickman et al, 1998; Navon and Goldberg, 2001; Voges et al, 1999; Xie and Varshavsky, 2000).

Although the occurrence of 26S proteasomes as highly elaborated proteolytic machines is restricted to eukaryotic cells, archaea and bacteria possess proteasomes with less complex subunit compositions (as demonstrated for 20S proteasomes from *Thermoplasma acidophilum* or *Rhodococcus erythropolis* and HsIV protease from *E. coli*), and potentially redundant proteasome-related proteolytic complexes (eg, tricorn protease in *T. acidophilum* and *Streptomyces coelicor*) (Baumeister et al, 1998; Tamura et al, 2001; Voges et al, 1999). Thus, protein degradation by the 26S proteasome in eukaryotic cells seems to be a highly

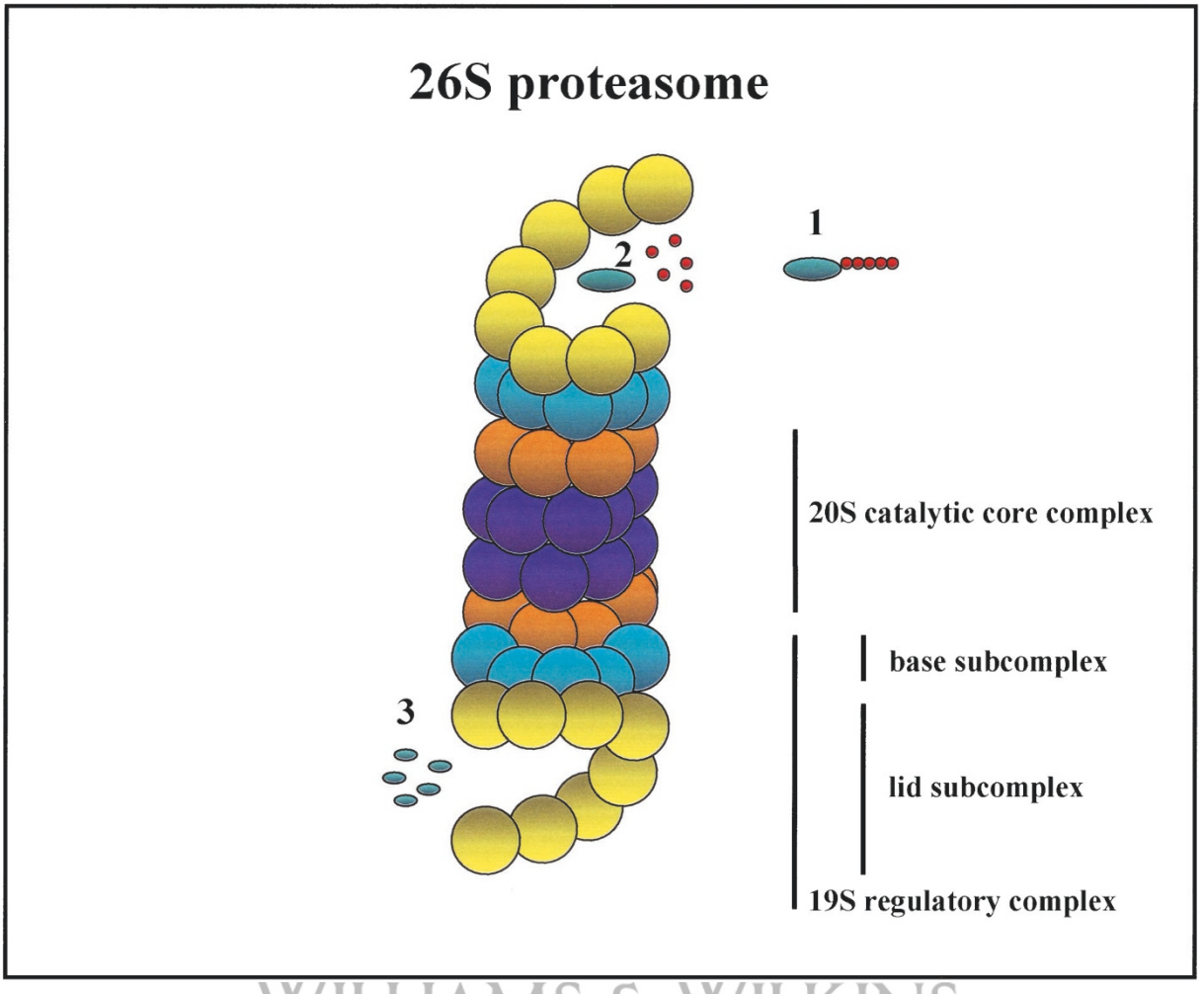

Figure 1.

Subunit composition of and protein degradation by the 26S proteasome (schematic). The 20S catalytic core complex consists of four axially stacked seven-membered rings: two inner rings formed by β subunits (dark blue) carrying proteolytic sites faced to the central cavity of the 20S complex, and two outer rings formed by nonproteolytic α subunits (light brown). The 19S regulatory complex consists of the base subcomplex and the lid subcomplex. The base subcomplex contains six AAA-ATPase subunits (light blue) and two accessory subunits. The lid subcomplex contains eight subunits (yellow). 1, A substrate protein is targeted for proteasomal degradation by the covalent attachment of ubiquitin monomers (red balls). This has been governed previously by ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin-protein ligases (E3). 2, The substrate protein enters the 19S regulatory complex, is recognized, deubiquitinated, unfolded, and translocated into the central cavity of the 20S catalytic core complex, where it is degraded by different hydrolytic activities. 3, Short peptides as a product of degradation are released at the opposite end of the 26S proteasome.

conserved process that might have been evolved to fulfill not only metabolic, but also regulatory functions. In fact, basic cellular processes that regulate cell proliferation and apoptosis in eukaryotes are driven by and depend on controlled protein degradation by the 26S proteasome.

Examples of proteasomal involvement in proliferative processes are the regulation of the mitotic cell cycle by temporally controlled proteasomal degradation of cyclins, cyclin-dependent kinases, and their inhibitors (Koepp et al, 1999; Mann and Hilt, 2000; Yew, 2001), and proteasome-dependent regulatory mechanisms in metazoan development that govern oocyte maturation (Josefsberg et al, 2000; Reverte et al, 2001; Sawada et al, 1999; Tokumoto et al, 1999), embryonic cell cycle progression (Josefsberg et al, 2001; Kawahara et al, 2000a, 2000b; Tokumoto et al, 1999), and postembryonic tissue modelling (Dawson et al, 1995; Jones et al, 1995; Löw et al, 1997). Moreover, the 26S proteasome is involved in induction, but also suppression of apoptosis in eukaryotic cells. These opposite functions in the regulation of apoptosis seem to be determined by the proliferative state of a cell and the cellular environment, and may proceed on the basis of the proteasome's ability to degrade proapoptotic as well as antiapoptotic proteins (Grimm and Osborne, 2000; Orlowski, 1999).

Finally, an increasing body of data provides evidence that deregulation of proteasomal protein degradation contributes to the pathogenesis of human diseases, such as cancer and neurodegenerative and myodegenerative diseases that exhibit an imbalance of proliferation and apoptosis (Chai et al, 1999; Checler et al, 2000; Ciechanover et al, 2000; Cummings et al, 1998; Spataro et al, 1998; Tanaka et al, 2001a). Therefore, the 26S proteasome appears as a regulator

of cell proliferation and apoptosis that may govern the delicate balance between a cell's life and death in dependence on a cell's biologic state and environment.

Role and Function of the 26S Proteasome in Proliferation

Cell Cycle Control

Proliferation and division of cells implies two basic steps finally yielding to a proper genome duplication: (A) the replication of chromosomal DNA, and (B) the separation and division of sister chromosomes. Both of these steps have to ensure a proper distribution of the entire genome into two new cells:

(A) During the tightly regulated G1/S-phase, the sister chromatids are separated, and complemental DNA synthesis and replication takes place. The proper custodial regulation of DNA replication generally refers to the timely ordered progression from G1- to S-phase that constitutes the strict initiation and completion of only one round of DNA replication in each cell cycle. This duplication relies on the coordinated activities of positive regulators, such as cyclins, cyclin-dependent kinases (CDK), CDK-cyclin complexes, E2F and Cdc6, and negative regulators, such as CDK inhibitors (CKI) of the Cip/Kip and INK4 families. The coordinated timely presence and action of these positive and negative regulators is governed by inactivation as a result of proteasomal degradation (Mann and Hilt, 2000; Yew, 2001).

(B) During the G2/M-phase, the doubled chromosome set is separated along kinetochore microtubules and divided into two new cells. The ordered progression of the S- and M-phase also highly depends on the spatial and temporal control of cell cycle regulatory proteins by proteasomal degradation that finally ensures proper cell cycle transitions and adequate frequencies of cell division (Mann and Hilt, 2000; Tatebe and Yanagida, 2000).

Negative Regulators of Cell Cycle Progression

The following negative regulators of cell cycle progression are known substrates of the 26S proteasome. When these proteins undergo proteasomal degradation, the "brake is released" and cell cycle progression may proceed.

$p21^{Cip1/WAF1}$. The CKI $p21^{Cip1/WAF1}$ is proposed to negatively regulate G1- and early S-phase events through binding to CDKs and/or to proliferating cell nuclear antigen (PCNA) (Luo et al, 1995). In response to DNA damage, the level of $p21^{Cip1/WAF1}$ protein is dramatically increased as a result of transcriptional activation of $p21^{Cip1/WAF1}$ by the tumor suppressor protein p53 that finally leads to cell cycle arrest in the G1-phase (El-Deiry et al, 1994). Because selective proteasome inhibitors have been shown to directly induce stabilization of $p21^{Cip1/WAF1}$ followed by cell cycle arrest (Blagosklonny et al, 1996; Naujokat et al, 2000; Sheaff et al, 2000), $p21^{Cip1/WAF1}$ is known to be regulated by proteasomal degradation during the cell cycle. Interestingly, proteasomal degradation of $p21^{Cip1/WAF1}$ in vitro does not strictly require ubiquitination of $p21^{Cip1/WAF1}$ (Sheaff et al, 2000).

$p27^{Kip1}$. Proteasomal degradation of the CKI $p27^{Kip1}$ is thought to be required for G1-to-S-phase progression (Sutterluty et al, 1999) and mainly occurs at the early onset of S-phase (Tyers and Jorgensen, 2000), although $p27^{Kip1}$ degradation also can take place at the G0-to-G1-phase transition (Hara et al, 2001). Consequently, $p27^{kip1}$ protein is abundant in G0 and G1 cells and is down-regulated in proliferating and S-phase cells (Coats et al, 1996). Moreover, ectopic overexpression of mutant $p27^{Kip1}$, but not of wild-type $p27^{Kip1}$, results in cell cycle arrest in the S-phase (Sutterluty et al, 1999), strongly suggesting that proteasomal degradation of $p27^{Kip1}$ is essential for the entry into S-phase. However, inactivation of $p27^{Kip1}$ function may not only occur by proteasomal degradation, but also via alternative pathways such as proteolytic processing (Shirane et al, 1999).

$p19^{INK4d}$. The levels of the CKI $p19^{INK4d}$ have been demonstrated to oscillate periodically during the cell cycle, peaking at S- and G2/M-phase, thereby allowing abundant bindings of $p19^{INK4d}$ to cyclin D-CDK4(6) complexes that result in negative regulation of cell cycle progression (Thullberg et al, 2000). This periodic oscillation of $p19^{INK4d}$ during cell cycle progression is governed by ubiquitination and subsequent proteasomal degradation of $p19^{INK4d}$, demonstrating that cyclin D-CDK4(6)-dependent cell cycle transitions are regulated by the 26S proteasome (Thullberg et al, 2000).

Geminin. Geminin is a 25-kd protein that was originally discovered in fertilized *Xenopus* eggs. Geminin is expressed during the mammalian cell cycle in S-, G2-, and M-phases, but disappears at the time of the metaphase-anaphase transition, where it is ubiquitinated by the anaphase-promoting complex/cyclosome (APC/C), a cell cycle regulated multisubunit ubiquitin-protein ligase, and subsequently degraded by the 26S proteasome (McGarry and Kirschner, 1998). Geminin inhibits DNA replication during S-, G2-, and M-phases by preventing the incorporation of MCM proteins into the prereplication complex. APC/C-triggered proteasomal degradation of geminin at the metaphase-anaphase transition permits DNA replication to proceed in the next cell cycle (McGarry and Kirschner, 1998).

Positive Regulators of Cell Cycle Progression

Positive regulators of cell cycle progression mainly permit the entry into S-phase, and proteasomal degradation of these regulators is thought to tightly control the timely proper entry into S-phase as well as adequate frequencies of S-phase entry.

Cyclin A. Cyclin A is required for the entry into mitosis and for S-phase progression, and is ubiquitinated and targeted for proteasomal degradation by APC/C during late M- to early G1-phase (Bastians et al, 1999). This suggests that proteasomal degradation of cyclin A is required for the completion of mitosis and the entry into the S-phase of the next cell cycle.

Cyclin D. Cyclins of the D-type represent a family of growth factor–regulated cyclins that are capable of binding CKIs such as $p21^{Cip1/WAF1}$ and $p27^{Kip1}$, resulting in the formation of cyclin-CKI complexes (Cheng et al, 1999). D-type cyclins are required for the entry into S-phase and undergo proteasomal degradation after relocalization to the cytoplasm during S-phase (Diehl et al, 1997). It is thought that the function and abundance of D-type cyclins are not only regulated by proteasomal degradation, but also by cellular localization (Yew, 2001). Defective proteasomal degradation of cyclin D leading to cyclin D accumulation is observed in certain breast cancer cells and is thought to contribute to the neoplastic proliferation of these cells (Russel et al, 1999).

Cyclin E. Cyclin E is required for the onset of DNA replication during the late G1- and early S-phase, where it is expressed periodically and bound to CDK2 (Ohtsubo et al, 1995). Additionally, unbound forms of cyclin E are present during G1- and S-phase. Both unbound and CDK2-bound cyclin E is targeted for ubiquitination and subsequent proteasomal degradation after its required function at the G1-to-S-phase transition, demonstrating a role for proteasomal degradation in the regulation of S-phase entry (Singer et al, 1999; Winston et al, 1999).

E2F1. E2F1 is required for the completion of G1-to-S-phase transitions that are thought to be essential for resetting conditions for the next cell cycle (Harper and Elledge, 1999). After its required function at the G1-to-S-phase transition, E2F1 is ubiquitinated and subsequently degraded by the 26S proteasome at S-to-G2-phase (Marti et al, 1999) that is dependent on dissociation of E2F1 from the retinoblastoma tumor suppressor protein Rb (Campanero and Flemington, 1997).

Cdc6. The Cdc6 protein is a component of the prereplication complex required for initiation of DNA unwinding and replication before S-phase (DePamphilis, 2000). During completion of DNA replication in G1-phase, Cdc6 dissociates from DNA-bound replication complexes and is ubiquitinated and targeted for proteasomal degradation by the APC/C (Peterson et al, 2000).

Development and Differentiation

Development and cellular differentiation in metazoans rely on precise intrinsic programs based on a tightly regulated control of both gene expression and posttranslational protein modification (McGhee, 1995; Morisato and Anderson, 1995; Pahl and Baeuerle, 1996; Zhang et al, 1998). More and more experimental evidence is being collected that the basic molecular events operative in development and differentiation are regulated, at least in part, by the 26S proteasome. For example, early developmental studies in insect model organisms, including *Drosophila* and *Manduca sexta,* have suggested that early embryogenesis and metamorphosis in insects depend on proteasome accumulation and tightly regulated proteasomal degradation of certain target proteins (Dawson et al, 1995; Jones et al, 1995; Klein et al, 1990; Löw et al, 1997). Subsequent studies in diverse multicellular organisms, such as sea urchin (*Lytechinus pictus*), *Xenopus laevis*, mouse, and rat have demonstrated that developmentally regulated expression of distinct subunits of the proteasomal regulatory 19S subcomplex, as well as proteasomal degradation of cell cycle regulatory proteins, is essential for the initiation of early embryonal mitosis and development (Josefsberg et al, 2001; Kawahara et al, 2000a, 2000b; Tokumoto et al, 1999). Moreover, recent studies in vertebrates reveal that the molecular assembly and the proteolytic activity of certain 26S proteasome subunits undergo changes during the completion of meiosis (oocyte maturation) (Sawada et al, 1999; Tokumoto et al, 1999) and, very recently, it has been demonstrated that the proteolytic activity of the 26S proteasome is absolutely essential for rat and *Xenopus* oocyte maturation (Josefsberg et al, 2000; Reverte et al, 2001).

Terminal differentiation of eukaryotic cells requires withdrawal from the cell cycle that is accomplished by the down-regulation of CDK activities during G1-phase (Zavitz and Zipursky, 1997). This mainly proceeds, as shown in mammalian cells, through binding of CDKs to the CKIs of the INK4 family ($p16^{Ink4a}$, $p15^{Ink4b}$, $p18^{Ink4c}$, and $p19^{INK4d}$) and the Cip/Kip family ($p21^{WAF1/Cip1}$, $p27^{Kip1}$, and $p57^{Kip2}$) (Sherr and Roberts, 1999). Because most of these CKIs, including $p21^{WAF1/Cip1}$, $p27^{Kip1}$, $p57^{Kip2}$, and $p19^{INK4d}$, undergo proteasomal degradation (Blagosklonny et al, 1996; Pagano et al, 1995; Sheaff et al, 2000; Shirane et al, 1999; Thullberg et al, 2000; Urano et al, 1999), the 26S proteasome appears as a negative regulator of terminal differentiation. In fact, this was originally demonstrated in rodent neuronal cells induced to undergo terminal neurite formation by treatment with compounds subsequently identified as specific proteasome inhibitors (Fenteany et al, 1994, 1995; Saito and Kawashima, 1989; Tsubuki et al, 1993, 1996). More recent studies, however, have attempted to define the inhibitory role of the 26S proteasome in differentiation by its degradation of CKIs such as $p27^{Kip1}$ and $p57^{Kip2}$. For example, retinoid acid–induced neuronal differentiation of human multipotent embryonal stem cells has been shown to depend on the down-regulation of proteasomal degradation of $p27^{Kip1}$ (Baldassarre et al, 2000), and transforming growth factor β-induced inhibition of rat osteoblast differentiation has been demonstrated to occur mainly via proteasomal degradation of $p57^{Kip2}$ (Urano et al, 1999).

By contrast, proteasomal degradation of $p21^{WAF1/Cip1}$ and other cell cycle regulators, such as cyclin D1 and Cdc25A phosphatase, however, can promote differentiation of certain mouse and human cells (Bernardi et al, 2000; Di Cunto et al, 1998; Negishi et al, 2001; Spinella et al, 1999), ultimately pointing out a complex function of the 26S proteasome in cellular differentiation.

Role and Function of the 26S Proteasome in Apoptosis

Apoptosis is a distinct form of cell death that ensures the control of cell numbers and tissue homeostasis during embryogenesis and postembryonal development and proliferation. The mechanisms of apoptosis are highly conserved from lower eukaryotes to mammals and exhibit a sophisticated network of tightly ordered molecular events that finally converge into the enzymatic fragmentation of chromosomal DNA, thereby driving a cell into death (Hengartner, 2000).

Proapoptotic Functions

First indications of a proapoptotic function of the 26S proteasome came from studies focusing on the developmental regression of abdominal intersegmental muscles of the tobacco hawkmoth, *Manduca sexta*. Shortly after the emergence of the adult insect from its pupal cuticle, the intersegmental muscles undergo apoptotic regression in response to declining levels of the steroid molting hormone 20-hydroxyecdysone (Schwartz and Truman, 1983). This is accompanied by a dramatic increase in both ubiquitin expression and proteasomal proteolytic activity as evidenced by the 5- to 9-fold enhanced tryptic, chymotryptic, caseinolytic, and peptidylglutamyl-hydrolizing activity of the 26S proteasome (Dawson et al, 1995; Jones et al, 1995). This increase in proteasomal proteolytic activity is correlated with the 8-fold increase in the absolute cellular amounts of the 26S proteasome, the incorporation of four new subunits into the 20S complex, and the 20-hydroxyecdysone–dependent exchange of ATPases within the 19S regulatory complex (Dawson et al, 1995; Löw et al, 1997). Such changes in both proteasomal proteolytic activity and subunit composition during development of *Manduca sexta* show a reprogramming of the 26S proteasome that might result in an enhanced proteasomal degradation of certain ubiquitinated cell proteins, leading to apoptosis of intersegmental muscle cells.

The requirement of proteasomal activity for the progression of apoptosis has been demonstrated originally in two nonproliferating mammalian cell types, resting thymocytes and differentiated neuronal cells (Grimm et al, 1996; Sadoul et al, 1996). Grimm et al were able to demonstrate that primary mouse thymocytes induced to undergo apoptosis by treatment with phorbol 12-myristate 13-acetate, dexamethasone or γ-radiation were rescued from apoptosis when treated with proteasome inhibitors up to 1 hour after the initiation of the apoptotic stimulus. This inhibition of apoptosis was accompanied by prevention of the cleavage of poly(ADP-ribose) polymerase (PARP), which is a biochemical hallmark of caspase activity leading to apoptotic cell death (Hengartner, 2000). By contrast, later addition (3 to 5 hours) of proteasome inhibitors failed to rescue the cells from apoptosis, suggesting that proteasomal activity promotes apoptosis only at upstream points of apoptotic signal transduction pathways. This has been confirmed in later studies showing that proteasome activation occurs upstream of central apoptotic signaling events, such as disruption of mitochondrial transmembrane potential, mitochondrial release of cytochrome *c*, and activation of caspases in rodent thymocytes induced to undergo apoptosis by various stimuli (Dallaporta et al, 2000; Hirsch et al, 1998; Stefanelli et al, 1998). Similar findings were obtained in studies with differentiated neuronal cells. Sympathetic neurons from rat superior cervical ganglia (Sadoul et al, 1996) and rat cerebellar neurons (Canu et al, 2000) undergoing apoptosis in response to deprivation of nerve growth factor and potassium, respectively, are rescued from apoptosis, and fail to exhibit caspase activation and mitochondrial release of cytochrome *c* when treated with proteasome inhibitors early after the initiation of the apoptotic stimulus.

One candidate mechanism of how proteasomal activity promotes apoptosis at an upstream point of apoptotic signal transduction has been uncovered recently in primary mouse thymocytes: XIAP and c-IAP1, members of the highly conserved family of inhibitors of apoptosis proteins (IAPs) (Duckett et al, 1996) that exert their antiapoptotic activity, at least in part, by inhibiting the activation and enzymatic activity of caspases (Devereaux et al, 1997, 1998), and by ubiquitination and targeting of caspase-3 for proteasomal degradation (Suzuki et al, 2001), are autoubiquitinated and subsequently degraded by the 26S proteasome in response to various apoptotic stimuli (Yang et al, 2000). This autoubiquitination and subsequent proteasomal degradation of XIAP and c-IAP1 depends on an intact RING finger domain of the proteins, and appears to be highly operative in transducing apoptosis, because cells expressing XIAP and c-IAP1 with mutant RING finger domains display a lack of proteasomal degradation of the mutant proteins and fail to undergo apoptosis induced by diverse stimuli (Yang et al, 2000).

Another candidate mechanism of providing proapoptotic signals by proteasomal activity has been demonstrated in HUVECs induced to undergo apoptosis by treatment with TNF-α. Early after the initiation of TNF-α treatment of HUVECs, Bcl-2, a mitochondrial membrane-anchored protein capable of blocking apoptosis induced by diverse stimuli, was shown to be specifically degraded by the 26S proteasome (Breitschopf et al, 2000a; Dimmeler et al, 1999). This event was demonstrated to be operative in inducing apoptosis, because pretreatment of HUVECs with specific proteasome inhibitors reversed both TNF-α–induced Bcl-2 degradation and induction of apoptosis (Breitschopf et al, 2000a; Dimmeler et al, 1999).

Antiapoptotic and Survival Functions

Because early studies revealed that proteasomes are abnormally highly expressed in rapidly growing metazoan embryonic and human neoplastic cells, but not in their well differentiated and normally growing counterparts (Ichihara et al, 1993; Kanayama et al, 1991; Klein et al, 1990; Kumatori et al, 1990; Shimbara

et al, 1992), the 26S proteasome has been suggested to play a pivotal role in maintaining survival and proliferation of rapidly and somehow abnormally growing cells (Ichihara and Tanaka, 1995). This hypothesis was supported at the same time by studies in yeast that have shown that disruption of any one of 13 of the 14 genes encoding the subunits of the 20S catalytic core complex leads to complete suppression of cell growth followed by cell death (Fujiwara et al, 1990; Heinemeyer et al, 1991).

The recent identification and availability of selective synthetic and biologic proteasome inhibitors (Fenteany and Schreiber, 1998; Lee and Goldberg, 1998) have allowed a more detailed definition of the roles of the 26S proteasome in fundamental cellular processes, such as apoptosis and proliferation. The stereotyped effects of such proteasome inhibitors observed in neoplastic and rapidly growing cells have finally confirmed the "survival hypothesis" deduced from the early findings of abnormally high proteasome expression in neoplastic and rapidly growing cells: proteasome inhibitors have been found to abundantly induce apoptosis in neoplastic and rapidly growing mammalian cells of hematopoietic (Drexler, 1997; Naujokat et al, 2000; Shinohara et al, 1996; Tanimoto et al, 1997), neuronal (Kitagawa et al, 1999; Lopes et al, 1997; Qiu et al, 2000), mesenchymal (Drexler et al, 2000; Lopes et al, 1997), and epithelial origin (Adams et al, 1999; Herrmann et al, 1998). Intriguingly, human myelogenous leukemic cells abundantly undergoing apoptosis when treated with proteasome inhibitors become refractory to proteasome inhibitor–induced apoptosis after the experimental induction of terminal differentiation leading to noncycling and respectively mature myelogenous cells (Chen et al, 2000; Drexler, 1997). This clearly demonstrates that the proteolytic activity of the 26S proteasome exerts survival functions only in rapidly growing and cycling cells, and also reflects the essential role of the 26S proteasome for maintaining neoplastic proliferation, as already being anticipated in the early studies, which have demonstrated an abnormally high proteasome expression in neoplastic cells, but not in normally growing respective cells (Kumatori et al, 1990; Shimbara et al, 1992).

Numerous cell proteins involved in signal transduction pathways that control proliferation and apoptosis have been discovered to be degraded by the 26S proteasome. Such proteins are supposed to provide a delicate balance of apoptotic and survival signals as a result of their properly regulated levels in normal cells, but might be inappropriately limited or activated in neoplastic cells by enhanced or deregulated proteasomal degradation, thereby allowing neoplastic proliferation.

One such candidate protein is NF-κB, a member of a large family of transcription factors found in the cytoplasm (Verma et al, 1995). NF-κB is sequestered in the cytoplasm by inhibitory IκB proteins and can translocate into the nucleus to act as a transactivator of target genes only after phosphorylation, ubiquitination, and subsequent proteasomal degradation of its inhibitory IκB proteins (Chen et al, 1995; Palombella et al, 1994). NF-κB has been demonstrated to provide antiapoptotic and survival signals in neoplastic cells by transactivation of antiapoptotic genes (Mayo et al, 1997; Wang et al, 1998) and by its high level of activity as a result of deregulated proteasomal degradation of its inhibitory IκB proteins (Besancon et al, 1998; Guzman et al, 2001; Izban et al, 2001; Kordes et al, 2000; Ni et al, 2001). In contrast to this scenario that permits survival signals and suppression of apoptosis as a result of an abundant release of an antiapoptotic protein from its inhibitor, the 26S proteasome is also believed to directly generate antiapoptotic and survival signals in neoplastic cells by degrading proapoptotic proteins such as Bax and Bid (Breitschopf et al, 2000b; Chang et al, 1998; Li and Dou, 2000). Bax and Bid are members of the Bcl-2 family of apoptosis-regulating proteins located in the outer mitochondrial membrane and are involved in the regulation of mitochondrial release of cytochrome *c*, which is a prerequisite for the execution of apoptosis (Adams and Cory, 1998; Hengartner, 2000).

Another protein found to undergo enhanced and deregulated proteasomal degradation in neoplastic cells is the CKI $p27^{Kip1}$. $p27^{Kip1}$, a negative regulator of cell cycle progression involved in differentiation-associated growth arrest (Durand et al, 1997; Wang et al, 1996), is known to be regulated by proteasomal degradation (Pagano et al, 1995; Shirane et al, 1999), which is promoted by binding of $p27^{Kip1}$ to Jab1, a component of the COP9 signalosome complex homologous to certain subunits of the 19S regulatory proteasomal lid subcomplex (Tomoda et al, 1999). High levels of $p27^{Kip1}$ as a result of $p27^{Kip1}$ overexpression or proteasome inhibition can induce apoptosis in tumor cells, ultimately pointing out a proapoptotic and tumor suppressor function of $p27^{Kip1}$ under certain proliferative conditions (Katayose et al, 1997; Kudo et al, 2000; Schreiber et al, 1999; Wang et al, 1997). Because proteasomal degradation of $p27^{Kip1}$ is enhanced in several human tumors of epithelial and mesenchymal origin (Chiarle et al, 2000; Esposito et al, 1997; Kawana et al, 1998; Loda et al, 1997; Piva et al, 1999), and low levels of $p27^{Kip1}$ correlate with tumor progression and poor prognosis (Catzavelos et al, 1997; Chiarle et al, 2000; Sgambato et al, 2000; Slingerland and Pagano, 2000), the loss of $p27^{Kip1}$ due to deregulated proteasomal degradation may promote the neoplastic proliferation of certain tumor cells.

Other cell cycle regulatory and tumor suppressor proteins, such as $p21^{WAF1/Cip1}$ and p53, are potential targets for deregulated proteasomal degradation in neoplastic cells. The CKI $p21^{WAF1/Cip1}$ displays similar properties and functions to those of $p27^{Kip1}$ (Sherr and Roberts, 1999) and is a known target of proteasomal degradation (Blagosklonny et al, 1996; Sheaff et al, 2000). As in the case of $p27^{Kip1}$, overexpression of $p21^{WAF1/Cip1}$ causes induction of apoptosis in tumor cells (Katayose et al, 1995; Schreiber et al, 1999; Sheikh et al, 1995), and accumulation of $p21^{WAF1/Cip1}$ induced by proteasome inhibition has been shown to precede G2/M arrest and apoptosis in rapidly growing

cells (Naujokat et al, 2000; Rieber and Rieber, 2000), suggesting, however, a role for a deregulated proteasomal degradation of $p21^{WAF1/Cip1}$ in maintaining neoplastic proliferation.

The tumor suppressor protein p53 is a short-lived nuclear protein capable of inducing both growth arrest and apoptosis after its stabilization in response to genotoxic stress and DNA damage (Levine, 1997; Sionov and Haupt, 1999). This stabilization of p53 leads to growth arrest or apoptosis (with the final outcome of tumor suppression) and is thought to occur mainly via the down-regulation of its proteasomal degradation, because inhibition of the ubiquitin/proteasome pathway by various means has been demonstrated to result in p53 stabilization followed by apoptosis (Chowdary et al, 1994; Lopes et al, 1997; Maki et al, 1996; Monney et al, 1998). Apoptosis in such case could well be mediated by a combination of up-regulation of both the genes of Bax and $p21^{WAF1/Cip1}$, proapoptotic targets of p53 (El-Deiry et al, 1994; Miyashita and Reed, 1995), and by down-regulation of the antiapoptotic protein Bcl-2, which is repressed by p53 (Agarwal et al, 1998). An example of enhanced proteasomal degradation of p53 leading to neoplastic proliferation is well documented in human papilloma virus (HPV)–related cervical carcinomas: the E6 oncoprotein encoded by the high risk HPV-16 and -18 subtypes binds to p53 and promotes its rapid degradation by the 26S proteasome, a feature that is essential for the neoplastic growth of HPV-positive carcinoma cells (Hengstermann et al, 2001; Scheffner et al, 1990). Moreover, it has been demonstrated recently that human chronic lymphocytic leukemia cells exhibit a constitutively up-regulated proteasomal proteolytic activity leading to an enhanced degradation of p53, which is thought to allow survival and neoplastic proliferation of these cells (Masdehors et al, 2000).

Finally, there is increasing evidence for a functional cooperation of the COP9 signalosome, a multimeric protein complex homologous to the 19S regulatory proteasomal lid subcomplex (Henke et al, 1999), with the 26S proteasome in degradation of p53, $p27^{Kip1}$, $I\kappa B\alpha$, and c-Jun (Bech-Otschir et al, 2001; Seeger et al, 1998; Tomoda et al, 1999). This cooperation may also contribute to deregulated proteasomal protein degradation in neoplastic cells.

Role of the 26S Proteasome in the Pathogenesis of Human Diseases

An increasing body of recent data provides evidence that deregulation of the ubiquitin/proteasome system contributes to the pathogenesis of several human diseases, such as cancer and neurodegenerative and myodegenerative diseases that exhibit an imbalance of proliferation and apoptosis.

Cancer

In various entities of human cancer, a deregulated proteasomal proteolysis of tumor suppressors and oncoproteins has been associated with initiation and maintenance of neoplastic proliferation as well as tumor progression and poor prognosis. This has been widely demonstrated in the case of enhanced proteasomal degradation of $p27^{Kip1}$ in lung carcinomas (Catzavelos et al, 1999; Esposito et al, 1997; Kawana et al, 1998), colorectal carcinomas (Loda et al, 1997), breast carcinomas (Catzavelos et al, 1997; Porter et al, 1997), gliomas (Piva et al, 1999), and lymphomas (Chiarle et al, 2000). Moreover, enhanced proteasomal degradation of the tumor suppressor protein p53 is clearly linked to the development and neoplastic proliferation of HPV-related cervical carcinomas (Hengstermann et al, 2001; Scheffner et al, 1990), and is thought to confer resistance to apoptosis in chronic lymphocytic leukemia (Masdehors et al, 2000).

A more indirect function of the 26S proteasome in the permission of cancer might be operative in the multistep carcinogenesis of inherited colorectal carcinomas: the oncoprotein β-catenin, a central component of signaling and differentiation pathways of the colorectal epithelium, is targeted for proteasomal degradation by the tumor suppressor APC (adenomatous polyposis coli), which is mutated or lost in inherited colorectal carcinomas. The consequent accumulation of β-catenin in APC-defective colorectal carcinomas leads to the activation of signaling pathways that promote carcinogenesis and neoplastic proliferation (Aberle et al, 1997; Inomata et al, 1996; Korink et al, 1997; Morin et al, 1997; Powell et al, 1992; Sellin et al, 2001).

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the selective cell death of dopaminergic neurons located in the substantia nigra of the midbrain. By its clinical definition, PD represents a heterogenous disorder that encompasses a small proportion of individuals with an autosomal recessive juvenile form of PD (AR-JP) and a larger population with an idiopathic form of PD with the neuropathologic stigma of Lewy bodies, cytoplasmic inclusion bodies consisting of aggregates of abnormally accumulated proteins that are not observed in AR-JP (Hattori et al, 2000; Kitada et al, 2000). α-synuclein, a protein that forms the major fibrillary component of Lewy bodies, has been shown to interact with the 19S proteasomal regulatory complex and to undergo proteasomal degradation (Bennett et al, 1999; Ghee et al, 2000). Intriguingly, proteasomal proteolytic activity is dramatically impaired in dopaminergic neurons in idiopathic PD (McNaught and Jenner, 2001), and recent experimental data provide evidence that proteasome inhibition in α-synuclein–expressing neuronal cells results in accumulation and aggregation of α-synuclein, leading to apoptosis (Rideout et al, 2001; Tanaka et al, 2001b; Tofaris et al, 2001).

The parkin gene, which is implicated in the onset and progression of AR-JP (Kitada et al, 1998), encodes the 53-kd parkin protein that functions as a

ubiquitin-protein ligase for targeting proteins for proteasomal degradation (Shimura et al, 2000). Parkin mutations that are frequently found in AR-JP patients (Kitada et al, 2000) lead to the functional loss of the parkin protein as a ubiquitin-protein ligase. This failure causes accumulation of several proteins destined to be degraded by the 26S proteasome, and finally results in apoptosis (Imai et al, 2001; Tanaka et al, 2001a).

Alzheimer's Disease

Alzheimer's disease (AD), the most common form of senile dementia, is characterized by the progressive degeneration and loss of cortical and limbic neurons that is associated with the extracellular deposition of amyloid β-peptides in senile plaques and the occurrence of intracellular neurofibrillary tangles composed of paired helical filaments of hyperphosphorylated tau proteins. Because the presence of ubiquitin and distinct proteasomal subunits has been detected immunohistochemically in both senile plaques and neurofibrillary tangles (Fergusson et al, 1996; Ii et al, 1997), it has been postulated that defective proteolytic functions of the 26S proteasome contribute to the pathogenesis of AD. However, subtle biochemical studies have revealed that amyloid β selectively inhibits the proteasomal chymotrypsin-like proteolytic activity in a noncompetitive fashion (Gregori et al, 1995, 1997) and, in turn, proteasomal inhibition leads to an increased α-secretase–dependent production of amyloid β and its precursors (Marambaud et al, 1997, 1998). This multimodal amplification of proteasome inhibition in the context of a pathologic amyloid β metabolism is likely to explain the global impairment of proteasome activity that has been detected postmortally in cortical neurons of AD patients (Keller et al, 2000; Salon et al, 2000). Moreover, a mutant form of ubiquitin, termed Ub(+1), is selectively expressed in AD neurons and competes for ubiquitination of proteasomal target proteins with normal ubiquitin, thereby inhibiting proteasomal protein degradation (Lam et al, 2000). Thus, defective proteasome function may cause, at least in part, the accumulation of aberrant protein aggregates leading to neuronal degeneration and cell death in AD.

Polyglutamine Diseases and Muscle Dystrophy

Polyglutamine diseases encompass an increasing number of inherited neurodegenerative diseases characterized by the expansion of CAG trinucleotide repeats in the causative genes that leads to the translation of polyglutamine stretches abundantly found in the encoded proteins. These abnormal proteins are thought to be involved in the induction of apoptotic mechanisms causing neuronal degeneration in several polyglutamine diseases, such as spinocerebellar ataxias (SCAs) and Huntington's disease (Evert et al, 2000). Immunohistochemical studies in neuronal tissues from patients with SCA type 1 and SCA type 3 have demonstrated that the 26S proteasome colocalizes with intranuclear inclusions formed by aggregates of the abnormal polyglutamine proteins ataxin-1 and ataxin-3, respectively (Chai et al, 1999; Cummings et al, 1998). These findings have been confirmed recently in a more detailed study showing that an ATPase subunit of the 19S proteasomal regulatory complex specifically interacts with ataxin-7, the abnormal polyglutamine protein found in SCA type 7 (Matilla et al, 2001). Moreover, in ataxin-3–transfected neurons, the 26S proteasome has been shown to redistribute into nuclear inclusions formed by ataxin-3 (Chai et al, 1999). Interestingly, proteasome inhibitors have been found to increase nuclear aggregate formation of polyglutamine proteins in SCA type 3 and Huntington's disease neurons (Chai et al, 1999; Martin-Aparicio et al, 2001), suggesting a rather protective role of proteasomal activity in polyglutamine diseases.

Catabolic muscle wasting during pathologic conditions associated with cancer cachexia, muscular dystrophies, amyotrophic lateral sclerosis, and peripheral neuropathies has been shown to occur as a result of muscle fiber degradation mainly by the 26S proteasome (Hasselgren et al, 2002; Kumamoto et al, 2000; Williams et al, 1999). However, several accessory mechanisms, including calpain-dependent proteolysis and deregulated activities of tripeptidyl peptidase II and COP9 signalosome, might be involved in the process of proteolytic muscle wasting (Hasselgren et al, 2002).

Conclusions

Proteolytic degradation of cell proteins by the 26S proteasome is a highly complex and tightly regulated process that plays pivotal roles in the regulation of basic cellular processes, including differentiation, proliferation, cell cycling, apoptosis, gene expression, and signal transduction. The mechanisms that underlie the regulation of both proteasomal protein degradation and polyubiquitination of target proteins as a prerequisite for degradation are still largely unknown, and the considerable number of proteasomal target proteins discovered to date may represent only a minor part of the myriad proteins awaiting the identification as proteasomal substrates.

From a mechanistic view, the 26S proteasome is capable of governing strictly opposite biologic features that crucially determine the fate of a cell, proliferation, and apoptosis. Moreover, also within the regulation of proliferation and apoptosis, the 26S proteasome can play opposite roles as revealed by its ability to contribute to either inhibition or promotion of each of these basic cellular processes, apparently in dependence on a cell's environment and proliferative state. Because proteasomal protein degradation is a highly ordered and elaborated process, it is obvious that this process also can underlie deregulation as observed in several human diseases that exhibit an imbalance of proliferation and apoptosis as a fundamental pathogenetic feature. Future research will lead to an improved understanding of the modes of pro-

teasomal action with the aim of allowing the development of beneficial therapeutic strategies.

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