with Hsc70 class chaperones; it also may have a lower affinity for the substrates that CSPα unfolds.

These studies highlight a dual role for α-synuclein in neurodegeneration: mutant α-synuclein or the abnormal accumulation of wild-type α-synuclein induces neurodegeneration, whereas the normal functions of α-synuclein help to enhance synaptic activity and integrity. A key question is whether and how these two conflicting roles of α-synuclein are connected. For example, given that mutant α-synuclein plays a key role in the development of PD and other neurodegenerative diseases, it would be interesting to know whether upregulation of β-synuclein—which does not form pathological cellular inclusions—would also be able to rescue the function of CSPα. Does sequestration of α-synuclein in pathological inclusions lead to its depletion at nerve terminals, thereby reducing chaperone activity that is critical for nerve terminal integrity? Does altered CSPα function contribute to PD? Does upregulation of CSPα hold therapeutic promise for treating neurodegenerative diseases as has been suggested for the chaperone Hsp70 (Auluck et al., 2002)? Although these ideas are speculative, the study by Chandra, Südhof, and colleagues provides the first compelling evidence for the physiological activity of normal α-synuclein in vivo. Their findings present a framework for understanding the normal functions of α-synuclein and how aberrant activity of this protein leads to neurodegeneration in PD and other synucleinopathies.

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The Proteasome: Not Just Degrading Anymore

The proteasome is a large multiprotein complex that has a critical role in the degradation of ubiquitylated proteins. A fascinating paper in this issue of *Cell* (Lee et al., 2005) now reveals that the proteasome recruits the SAGA histone acetyltransferase complex to a target promoter during gene activation. This finding adds to the growing body of evidence indicating that the proteasome has nonproteolytic functions.

Gene expression in eukaryotes is regulated by transcriptional activators that recruit chromatin-modifying and -remodeling enzymes to promoters. These enzymes relieve the inhibition of transcription caused by nucleosomes, which are the fundamental repeating unit of chromatin consisting of histone proteins wrapped in DNA. The posttranslational modification of histones regulates a number of cellular processes, such as gene silencing, DNA repair, and transcription. Among these modifications, acetylation and methylation of lysine residues have been extensively studied for their effects on gene expression. Recently, it has been shown that the 19S regulatory particle (19S RP) of the 26S proteasome, once thought to function exclusively in ubiquitin-mediated protein degradation, also plays a distinct nonproteolytic role in regulating transcription through an interaction with the transcriptional activator protein Gal4 (Muratani and Tansey, 2003). In this issue of *Cell*, Workman, Tansey, and colleagues (Lee et al., 2005) show that the 19S RP promotes the targeting of the Spt-Ada-Gcn5-acetyltransferase (SAGA) coactivator complex to the *GAL1-GAL10* promoter. This targeting—facilitated by the interaction between 19S RP and Gal4—results in increased histone H3 acetylation and *GAL1* transcription (Figure 1).

Protein degradation is the most well-established role of the proteasome (reviewed in Kinyamu et al. [2005]). In its classic representation, the proteasome consists of two main subcomplexes, 19S RP and 20S, which have very distinct functions. Together, the 19S RP and 20S complexes function as the 26S holoenzyme, in which the 20S core is sandwiched between two 19S RPs (Figure 1). The 19S RP recognizes proteins that have been polyubuquitylated by ubiquitin ligase enzymes and directs them to the barrel-like 20S catalytic core, where they are cleaved into small peptides through a number of proteolytic mechanisms. The tight regulation of this pathway modulates a number of cellular processes of which cell cycle progress is the most highly studied.

The findings of Lee et al. (2005) add to the rapidly accumulating body of evidence that the proteasome has extensive functions beyond the ubiquitin-degradation pathway. From their work and others, it is becoming clear that the proteasome plays an active role in multiple aspects of gene expression, including the recruitment of coactivators to promoters, the initiation of transcription, and elongation (Figure 1). Lee et al. (2005) find that the proteasome recruits the SAGA coactivator

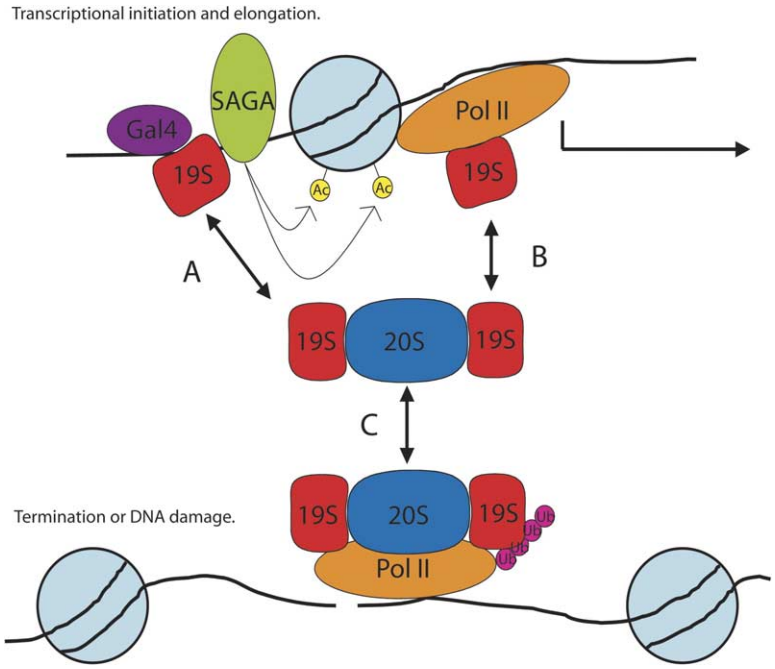

Figure 1. The Proteasome Functions in Multiple Facets of Transcription

The proteasome 19S RP assists in the Gal4-dependent recruitment of the SAGA acetyltransferase complex to target promoters, leading to enhanced acetylation (Ac) of histone H3 (A) and is required for efficient transcription elongation (B). Furthermore, the 26S holoenzyme (20S and two 19S RP subunits) can proteolytically remove stalled, polyubiquitylated RNA polymerase II at termination sites or sites of DNA damage (C).

to gene promoters. SAGA is a 1.8 MDa complex that contains a number of chromatin-modifying and/or binding proteins, including the histone acetyltransferase Gcn5. The SAGA complex acetylates histone lysines and coordinates other histone modifications in the establishment of transcriptionally competent chromatin at the gene promoters to which it is recruited. Lysine acetylation is believed to generate a more "open" chromatin structure and creates a binding surface for effector proteins that facilitates access of general transcription factors, such as TBP, to the DNA (Torok and Grant, 2004).

The proteasome may also function in transcription elongation. Recent coimmunoprecipitation studies have shown that the proteasome interacts with both RNA polymerase II and the transcription elongation factor Cdc68 (Ferdous et al., 2001; Gillette et al., 2004). Mutation of the 19S RP subunit Sug1 results in a buildup of shortened transcripts, indicating a direct role in elongation. Additionally, genetic data indicate that other mutations in 19S RP cause defects in elongation (Muratani and Tansey, 2003).

Although degradation-independent roles for 19S RP have been clearly described in gene expression, there is also evidence indicating that there may be a transcriptional function for proteolysis by the 26S holoenzyme (Figure 1). Inhibition of the proteolytic activity of 26S leads to read-through of the transcription termination site by RNA polymerase II. Additionally, chromatin immunoprecipitation studies have shown that the 26S proteasome localizes at sites of stalled polymerase II, specifically, termination sequences and sites of DNA damage (Gillette et al., 2004). These data imply that although the 19S RP may function on its own to facilitate transcription elongation, the 26S proteasome in its entirety may engage in turnover of stalled RNA polymerase (Figure 1). It has also been shown that ubiquitin-mediated degradation of the Gal4 activator protein is required for the appropriate phosphorylation of the C-terminal domain of RNA polymerase II and subsequent RNA processing (Muratani et al., 2005). Thus, it appears that the proteasome functions not only to stimulate gene expression but also to tightly regulate transcription factor turnover at promoters.

In addition to its roles in transcription, the proteasome has been shown to interact with the ubiquitin-like domain of the nucleotide excision repair (NER) protein Rad23. This interaction was initially thought to function in proteasomal turnover of NER complexes. More recently, it has been shown that inhibition of proteolysis has no effect on NER efficiency, but optimal NER activity still requires this interaction (Russell et al., 1999), indicating that proteasomal involvement in NER is degradation independent. It is possible that the binding of ubiquitin or a similar molecule mediates the localization of the proteasome in its various cell functions outside of protein degradation.

The discovery by Lee et al., (2005) may also shed light on the connection between transcription and several histone modifications, including ubiquitylation. Previous work has clearly established a role for ubiquitylation in transcription. Monoubiquitylation of histone H2B lysine 123 by Rad6 is associated with active transcription, as it is required for methylation of histone H3 lysine 4. Recently, these modifications have been linked by the proteasome (Ezhkova and Tansey, 2004). H2B ubiquitylation was shown to recruit 19S RP to promoter sequences, and a proteasomal mutation caused defects in H3 methylation irrespective of the ubiquitin modification. In this model, 19S RP interacts with ubiquitin moieties and subsequently facilitates H3 methylation coincident with activation of transcription. Chd1, a SAGA subunit containing tandem chromodomains,

binds specifically to methylated H3 lysine 4. This interaction precedes H3 acetylation by Gcn5 and the subsequent gene activation (Pray-Grant et al., 2005). Thus, the ultimate function of 19S RP in transcription initiation may be to coordinate Chd1 binding by targeting SAGA to the same promoters at which H3 lysine 4 is methylated, which are those that have been monoubiquitylated at H2B lysine 123 by Rad6. Another component of the SAGA complex, the ubiquitin protease Ubp8, has been shown to deubiquitylate histone H2B lysine 123 (Daniel et al., 2004). This modification is also important for gene induction, and hence it is thought that a transient sequence of ubiquitylation followed by deubiquitylation precedes transcription (Henry et al., 2003). Given this evidence, it is tempting to speculate that deubiquitylation of H2B releases 19S RP from the promoter region, which allows association with RNA polymerase II during elongation.

An important consideration to point out is that most of the studies referenced thus far were performed on the *Saccharomyces cerevisiae GAL1-GAL10* gene loci. It remains to be determined whether the proteasome functions similarly in multiple steps of transcription activation in mammalian cells, although it is clear that the proteasome plays a role in regulation of nuclear receptors, including those for estrogen, progesterone, glucocorticoid, and others. In general, nuclear receptors are bound by activating ligands, and following ligand binding they directly interact with chromatin at promoter elements. This interaction is thought to recruit activators and basal transcriptional machinery to the promoter, which induces gene expression. Recent evidence shows that the proteasome tightly controls the exchange rate of ligand bound nuclear receptors at promoters, thereby directly influencing transcriptional output (Kinyamu et al., 2005).

Another important question for future studies is to determine how the proteasomal ATPase activity specifically functions in the targeting of SAGA to the gene promoter. Because the targeting effect was observed even on naked DNA, the work of Lee et al. (2005) indicates that the activity of an ATPase subunit of the proteasome, Sug1, is directed toward SAGA rather than chromatin. Mutations in the ATPase domain of Sug1 decrease the recruitment of the acetyltransferase subunit of SAGA, Gcn5, to the *GAL1-GAL10* promoter. Because of this ATP dependence, the authors postulate that 19S RP loads SAGA onto the promoter in a manner similar to the loading of the Mcm2-7 DNA helicase complex onto replication origins by ORC (origin recognition complex). Clarifying the mechanisms for this process may provide insight into how the proteasome functions in other DNA-related events.

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