Recruitment of a 19S Proteasome Subcomplex to an Activated Promoter

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The 19S proteasome regulatory particle plays a critical role in cellular proteolysis. However, recent reports have demonstrated that 19S proteins play a nonproteolytic role in nucleotide excision repair and transcription elongation. We show by chromatin immunoprecipitation assays that proteins comprising the 19S complex are recruited to the GAL1-10 promoter by the Gal4 transactivator upon induction with galactose. This recruited complex does not contain proteins from the 20S proteolytic particle and includes a subset of the 19S proteins. This subset is also specifically retained from an extract by the Gal4 activation domain. These data indicate that in vivo, the base of the 19S complex functions independently of the larger complex and plays a direct, nonproteolytic role in RNA polymerase II transcription.

The 26S proteasome is composed of a 20S proteolytic barrel and a 19S regulatory cap (1). The latter contains at least 18 proteins (2), including six highly related adenosine triphosphatases (ATPases) of the ATPases associated with various cellular activities (AAA) family (3). Five of these ATPases have been linked to transcription either biochemically or genetically (4–7). However, these observations have been presumed to reflect the indirect effects of proteasome-mediated proteolysis of transcription factors (8, 9), and the 19S complex is generally thought solely to stimulate and regulate 20S-mediated proteolysis. However, recent biochemical studies have provided evidence that the 19S complex plays a nonproteolytic role in transcription elongation (7). This view was supported by the observation that certain mutations in SUG1/RPT6 render yeast highly sensitive to 6-azauracil (7), a hallmark of a defect in elongation (10), but more direct in vivo evidence for a role of the 19S in transcription has been lacking. Here we provide evidence that a subset of the 19S complex is involved in transcription in vivo.

If the 19S complex is involved directly in transcription, it should be physically associated with the promoter region of a gene. To test this hypothesis, we examined the GAL1-10 promoter of yeast by chromatin immunoprecipitation (ChIP) assays (11). As a positive control, we precipitated the promoter with antibodies against the regulator of the GAL1-10 promoter, Gal4 (12). It is known that Gal4 protein is present on the promoter under both noninducing (raffinose) and inducing (galactose) conditions (13). A portion of the promoter encompassing the Gal4 binding sites was immunoprecipitated by antibodies to Gal4 under both conditions (Fig. 1A). Preimmune serum or antibodies raised against cyclophilin did not precipitate the promoter (Fig. 1A) (14), nor was the promoter precipitated with the same anti-Gal4 antibodies against an extract from a strain deleted for GAL4 (Fig. 1B). Amplification of an irrelevant portion of the yeast genome from chromosome VII showed no signal above background (15).

The SUG1/RPT6 gene was originally identified genetically on the basis of mutations that suppressed defects in the COOH-terminal activation domain of Gal4 (4). Sug1/Rpt6 is one of the six highly conserved ATPases of the AAA class in the 19S. Anti-Sug1/Rpt6 antibodies precipitated little, if any, of the promoter region in raffinose medium (Fig. 1A). However, within 10 min of inducing the GAL1-10 genes, Sug1/Rpt6 was associated with the promoter. The same pattern of precipitation was also seen with antibodies raised against Sug2/Rpt4 (14) and Yta1/Rpt5 (Fig. 1A), two other ATPases of the 19S complex. These results indicate that the Sug1/Rpt6, Sug2/Rpt4, and Yta1/Rpt5 proteins are recruited to the GAL1-10 promoter region rapidly upon induction of transcription. This association with the promoter is Gal4 dependent because little or no promoter was precipitated by anti-Sug1 antibodies from a Gal4-deletion strain (Fig. 1B).

In contrast, antibodies generated against the 20S complex did not precipitate the promoter in raffinose- or galactose-grown cells (Fig. 1A). Antibodies raised against Rpn9, a non-ATPase 19S subunit, also did not precipitate the promoter after induction with galactose (Fig. 1A). Control experiments demonstrated the ability of the anti-20S and anti-Rpn9 antibodies to immunoprecipitate each of the corresponding proteins from an extract (15). Rpn9 is part of the so-called “lid” subcomplex of the 19S particle, which can be separated biochemically in high-salt buffers from the base (which includes the six ATPases, Rpn1, and Rpn2) (16, 17). Both the 20S and lid components are present on the GAL1 gene at later times after induction (14). These data therefore indicate that the 19S base is recruited to the promoter independently of the 20S and lid subcomponents.

To determine whether recruitment of the 19S ATPases was restricted to the promoter, we repeated the ChIP assays using polymerase chain reaction (PCR) primers targeted to regions around the GAL1 gene. Antibodies raised against Gal4 precipitated predominantly the

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promoter region of the \textit{GAL1} gene (Fig. 2). In contrast, using anti-Sug1/Rpt6 antibodies, we found that Sug1/Rpt6 protein was present throughout the length of the \textit{GAL1} gene (Fig. 2, bottom panel). This is consistent with our previous report that the 19S ATPases are important for efficient RNA polymerase II elongation (7).

The Gal4 activation domain (AD) was previously shown to associate with Sug1/Rpt6 and other 19S ATPases when added to a crude extract (5) or an immunopurified 19S complex (6). This suggests that Gal4 directly recruits a fragment of the proteasome that includes the 19S ATPases and perhaps other proteins, but not the 20S proteolytic or 19S lid subunits.

To more specifically address the physiological relevance of the interaction between the 19S and Gal4 on DNA, we examined whether mutations in \textit{SUG2} that do or do not suppress a defect in the Gal4 AD also differentially affect the association of the 19S with the promoter in vivo. A partial deletion of the Gal4 AD (4D) that yields a galactose-minus phenotype also abolished the association of the Sug1 protein with the promoter (Fig. 4). However, when this gal4D mutation was combined with the sug2-1 mutation that restores the ability to grow on galactose, the association of the Sug1 protein with the promoter was restored (Fig. 4). In contrast, another mutation in \textit{SUG2}, sug2-13, that does not suppress the gal4D defect did not restore Sug1 protein to the promoter (Fig. 4).

This strict correlation of mutations in \textit{Sug1} and \textit{Sug2} with gene expression and recruitment of the Sug proteins to the promoter, especially when considered together with the biochemical associations demonstrated in Fig. 3, imply that the Sug proteins play a functional role in gene expression.

We previously demonstrated a nonproteolytic role for the 19S proteins in elongation in vitro (7). The observation of a rapid, activator-dependent association of at least three of the 19S ATPases with the \textit{GAL1} promoter reported here suggests a functional role in the early events of transcription in vivo as well. The finding that Sug1/Rpt6 protein also associates with the promoter reported here suggests a functional role in the early events of transcription in vivo.

The results reported here demonstrate that the Gal4 AD binds only a subset of proteasomal proteins in vitro and that this subset appears to be the same one that is recruited to the \textit{GAL1} promoter in vivo. The 19S and 20S complexes...
can be separated biochemically, and the former can be further subdivided into base, which contains Rps1-6, Rpn1, and Rpn2 and exhibits chaperonin activity (21, 22), and lid (16, 17). However, there has been no previous evidence that these subspecies represent physiologically relevant complexes. Our results suggest that there is at least one discrete subcomplex of the 19S and that it functions independently of other proteasome subunits in Gal4-mediated transcription. This species, which we call the APIS (AAA proteins independent of 20S) complex, clearly includes the six 19S ATPases (Rpt1 to Rpt6) and perhaps other proteins. The precise composition of the APIS complex, and whether it corresponds to the biochemically defined base, remains to be elucidated.

There has been increasing evidence of a link between ubiquitination and transcription (23–26). Recent work by Tansey and colleagues suggests a mechanism by which they might be linked temporally. They found that for the artificial LexA-VP16 activator, ubiquitination of the activator is required for the activator to function in yeast (26). Importantly, linkage of a single ubiquitin molecule to the activator was shown to lead to activation, but did not signal proteolytic turnover. This suggests that it is ubiquitination per se, and not ubiquitin-linked proteolysis, that is crucial for activator function. Whereas the Gal4 AD alone is capable of binding the APIS complex (Fig. 3), an attached monoubiquitin might enhance this interaction or modulate the activity of the AD-bound complex in a way that is important for transcription to proceed. After induction, the ubiquitin chain on the activator would grow, possibly signaling a switch in activator association from the APIS complex to the full 26S proteasome. The time required for the ubiquitin chain to reach the minimum size needed to signal proteasome-mediated degradation (27) would be used by Gal4 to drive high-level transcription. But after that time, the activator would be subject to degradation, thus placing a “governor” on gene expression of Short Interfering RNAs in Mammalian Cells

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Mammalian genetic approaches to study gene function have been hampered by the lack of tools to generate stable loss-of-function phenotypes efficiently. We report here a new vector system, named pSUPER, which directs the synthesis of small interfering RNAs (siRNAs) in mammalian cells. We show that siRNA expression mediated by this vector causes efficient and specific down-regulation of gene expression, resulting in functional inactivation of the targeted genes. Stable expression of siRNAs using this vector mediates persistent suppression of gene expression, allowing the analysis of loss-of-function phenotypes that develop over longer periods of time. Therefore, the pSUPER vector constitutes a new and powerful system to analyze gene function in a variety of mammalian cell types.

In several organisms, introduction of double-stranded RNA has proven to be a powerful tool to suppress gene expression through a process known as RNA interference (1). However, in most mammalian cells this provokes a strong cytotoxic response (2). This non-specific effect can be circumvented by use of synthetic short [21- to 22-nucleotide(nt) interfering RNAs (siRNAs)], which can mediate strong and specific suppression of gene expression (3). However, this reduction in gene expression is transient, which severely restricts its applications. To overcome this limitation, we designed a mammalian expression vector that directs the synthesis of siRNA-like transcripts (pSUPER, 19 APRIL 2002 VOL 296 SCIENCE www.sciencemag.org

References and Notes
15. Supplementary figures and details of experimental procedures are available on Science Online at www.sciencemag.org/cgi/content/full/296/5567/548/DC1.
19. The RPT genes were amplified by PCR from yeast genomic DNA and cloned into the in vitro transcription vector pTL37N. The resultant RNA was then translated with the TNT rabbit reticulocyte system (Promega).
28. We thank J. Swaffield (North Carolina State University) for antibodies against Rpt1-3 and Rpt-5, and A. Toh-e (University of Tokyo) for antibodies raised against Rpn9 and Rpn12. We also thank E. Webb, L. Zhang, and X. Chen for technical assistance. F.G. was supported in part by an NIH training grant. A.D. was supported in part by the CNRS. This work was supported by unrestricted funds of T.K. and S.A.J.
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