The proteasome regulates the UV-induced activation of the AP-1-like transcription factor Gcn4

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The proteasome is well known for its regulation of the cell cycle and degradation of mis-folded proteins, yet many of its functions are still unknown. We show that *RPN11*, a gene encoding a subunit of the regulatory cap of the proteasome, is required for UV-stimulated activation of Gcn4p target genes, but is dispensable for their activation by the general control pathway. We provide evidence that *RPN11* functions downstream of *RAS2*, and show that mutation of two additional proteasome subunits results in identical phenotypes. Our analysis defines a novel function of the proteasome: regulation of the RAS- and AP-1 transcription factor-dependent UV resistance pathway.

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Ultraviolet (UV) radiation and other environmental stresses continuously damage DNA and other cellular components. Eukaryotes have evolved highly regulated systems to combat these assaults, and an essential component of all known resistance pathways is the ability to alter the patterns of gene expression in response to stress. At least two independent pathways exist in eukaryotes to resist damage caused by UV radiation. The best characterized of the two is the DNA damage–cell cycle checkpoint pathway that responds to DNA lesions, resulting in cell cycle arrest and the increased expression of DNA repair genes (Elledge 1996; Weinert 1998). The second, less characterized, pathway responds to the nongenomic effects of UV irradiation, and its signals are generated by cytoplasmic determinants (Devary et al. 1993; Radler-Pohl et al. 1993). In mammalian cells, this second response is dependent upon the membrane-bound Ha-Ras molecule, which in turn activates c-Jun N-terminal kinase (JNK), a component of a mitogen-activated protein (MAP) kinase cascade (Davis 1994; Dérijard et al. 1994; Karin 1998). Phosphorylation of c-Jun and ATF-2 increases their ability to activate transcription of various stress resistance genes (for review, see Davis 1994; Karin 1998).

As in higher eukaryotes, UV irradiation of the budding yeast *Saccharomyces cerevisiae* activates the AP-1-like transcription factor Gcn4p through a *RAS2*-dependent pathway (Engelberg et al. 1994). UV-mediated activation of Gcn4p occurs in the absence of *GCN2* function and in cells expressing a constitutive version of *GCN4* that escapes translational control (Engelberg et al. 1994); thus it is separate from the well characterized amino acid starvation response (Hinnebusch 1988; Dever et al. 1992). The only genes that are known to be required for this response are *RAS2* and *GCN4*. Unfortunately, genes functioning downstream of *RAS2* and upstream of *GCN4* have not yet been identified.

A connection between protein degradation and the AP-1 transcription factor pathway has emerged with the identification of genes required for the function of AP-1 transcription factors in mediating stress responses. The *Schizosaccharomyces pombe* gene *pad1*$^+$ (*pap*-dependent transcriptional activator 1) and its human homolog *POH1* confer multidrug and UV resistance when overexpressed in their native organisms (Shimanuki et al. 1995; Spataro et al. 1997). *Pad1+* is a positive regulator of *pap1*$^+$ (pombe AP-1)-dependent transcription, and although its mechanism is unknown, it clearly does not do so by regulating the levels of Pap1 or its DNA-binding activity (Shimanuki et al. 1995). Biochemical analyses of Pad1+ and Poh1 indicate that they are regulatory subunits of the 26S proteasome (Spataro et al. 1997; Penney et al. 1998). Both *pad1+* and *POH1* are related to the *S. cerevisiae* gene *RPN11/MPR1*. Rpn11p is likewise a bona-fide subunit of the 26S proteasome (Glickman et al. 1998), and its gene was initially cloned for its role in maintaining mitochondrial function and cell cycle progression through the G_2/M checkpoint (Rinaldi et al. 1998).

Here, we describe the characterization of *RPN11*, the gene of a regulatory subunit of the proteasome. Using mutant alleles of *RPN11* and other proteasome subunit genes, we show that the proteasome is required for the UV- and methyl methanesulfonate (MMS)-induced activation of Gcn4p target genes, but not for their activation through the amino acid starvation pathway. We provide evidence that *RPN11* acts downstream of *RAS2* and upstream of *GCN4*. Our analysis has identified a novel function for the proteasome: the regulation of AP-1-like transcription factors in stress-mediated pathways.

Results and Discussion

RPN11/TSG6/MPR1 was isolated as a gene-dosage suppressor of a TATA box binding protein associated factor (TAF) mutant, *taf68-9* (Reese et al. 2000; J. Reese and M. Green, in prep). Recently, Rpn11p was verified to be a non-ATPase subunit of the 19S regulatory "cap" of the proteasome (Glickman et al. 1998) and has been implicated in controlling mitochondrial function and G_2/M

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progression (Rinaldi et al. 1998). From this point on, we will refer to *RPN11/TSG6/MPR1* as *RPN11*. In an attempt to characterize the potential transcriptional regulatory functions of *RPN11*, a screen for temperature-sensitive (ts-) mutants was performed and four mutants were chosen for characterization. Figure 1A shows the growth phenotypes of these mutants at 23°C, 30°C, and 37°C. Three of the four mutants (*rpn11-8*, *rpn1-14*, and *rpn11-25*) displayed growth defects at permissive temperatures and arrested growth at 37°C, whereas the *rpn1-22* allele grew well at 23°C and 30°C and displayed a weaker ts- phenotype at 37°C.

Because a previously identified mutation in *RPN11* caused cell cycle arrest in G_2/M at the restrictive temperature (*mpr1-1*, described in Rinaldi et al. 1998), the cell cycle phenotype of the mutants described here was examined by flow cytometry. Even at the permissive temperature (30°C), all four mutants displayed a significant accumulation of cells with a 2N DNA content, suggesting a delay in G_2/M (data not shown). Consistent with a delay in G_2/M, microscopic examination of the cells revealed that most contained a single large bud (not shown).

Overexpression of *POH1* resulted in a measurable increase in the resistance of tissue culture cells to UV radiation (Spataro et al. 1997), indicating that it may regulate UV resistance pathways in mammals. We therefore examined the sensitivity of the *RPN11* mutants to UV radiation. The results presented in Figure 1B show that all four mutants were significantly more sensitive to UV radiation compared to the wild-type strain. Moreover, the sensitivity of the mutants to UV radiation correlated with the severity of their growth defects (Fig. 1, cf. A and B). The two mutants that are truncated within the C terminus, *rpn11–8* and *rpn11–14*, were approximately 100- and 1000-fold more sensitive than the wild type to UV, respectively. In contrast, the *rpn11-22* and *rpn11-25* alleles were less sensitive, displaying a roughly 10-fold increase in sensitivity. We next tested the sensitivity of the mutants to MMS, a DNA alkylating agent. Figure 1B shows that all strains grew at 30°C on plates containing 0.01% MMS, and that the *rpn11-8* and *rpn11-14* alleles failed to grow on medium containing 0.03% MMS. Neither the *rpn11-22* nor the *rpn11-25* allele was more sensitive than the wild-type strain, even at the maximal permissive concentration of MMS (0.05%, not shown). Moreover, despite showing some sensitivity to MMS, the *rpn11-8* and *rpn11-14* mutants were only threefold more sensitive to MMS; thus, they are significantly less sensitive to MMS compared to UV radiation.

To ascertain the functions of *RPN11* in mediating the resistance to UV radiation, we examined the integrity of the two known UV resistance pathways in yeast. The first pathway is dependent upon the DNA damage cell cycle checkpoint genes and regulates the expression of DNA repair genes (Elledge et al. 1993; Kiser and Weinert 1996; Basrai et al. 1999). We examined the integrity of the DNA damage checkpoint pathway in the *RPN11* mutants by monitoring the induction of two prototypical target genes whose expression is dependent upon checkpoint function, namely *RNR3* and *HUG1* (Elledge et al. 1993; Kiser and Weinert 1996; Basrai et al. 1999). The Northern blot presented in Figure 1C shows that the induction of *RNR3* and *HUG1* mRNA was not affected by mutations in *RPN11*, indicating that the DNA damage checkpoint pathways are not dependent upon *RPN11*.

The second resistance pathway does not require the DNA damage checkpoint genes in yeast (J. Reese, unpubl.) or nuclear function in mammals (Devary et al. 1993; Radler-Pohl et al. 1993) and is mediated by *RAS2* and the AP-1-like transcription factor Gcn4p (Engelberg et al. 1994). Given the genetic evidence implicating *RPN11* in the regulation of AP-1-like transcription factors (Shiminuki et al. 1995), we examined the UV-induced expression of Gcn4p target genes in the *RPN11* mutants. As reported in a previous study (Engelberg et al. 1994), the treatment of wild-type cells with UV radiation increased the expression of two well-characterized Gcn4p target genes, *HIS3* and *HIS4* (Fig. 2A). In contrast, UV-induced activation of both genes was abolished in all four *RPN11* mutants even when the cells were main-

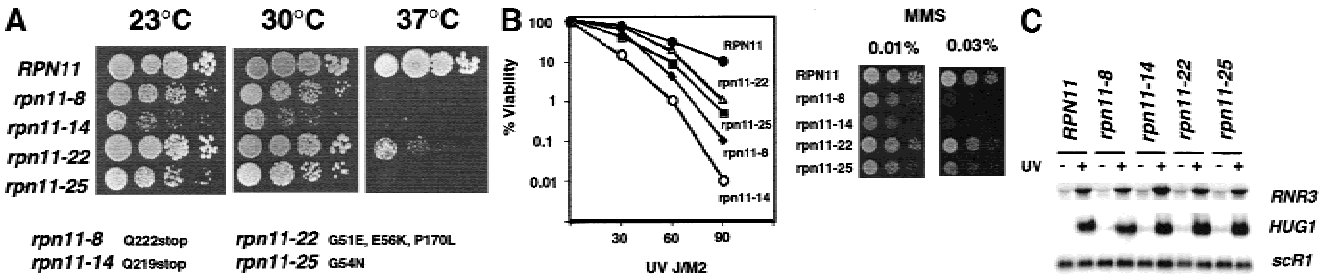

Figure 1. *RPN11* is required for normal resistance to UV radiation (*A*). Isolation of temperature-sensitive mutants. Tenfold serial dilutions of cultures of YJR124-0 (*RPN11*), YJR124-8 (*rpn11-8*), YJR124-14 (*rpn11-14*), YJR124-22 (*rpn11-22*), YJR124-25 (*rpn11-25*) were spotted onto YPAD plates and incubated at 23°C, 30°C, or 37°C for 3 d. The amino acid substitutions in each allele are indicated below. (*B*) UV (left) and MMS (right) sensitivity of *RPN11* mutants. For UV sensitivity studies, cells (YJR124) were grown in YPAD at 30°C until mid-log, and then appropriate dilutions were spread onto pre-warmed YPAD plates in triplicate. The plates were treated with the doses of UV radiation indicated in the figure and then incubated at 30°C in the dark. Sensitivity to MMS was measured on plates containing 0.01% and 0.03% MMS incubated at 30°C for 2 and 4 d, respectively. (*C*) Expression of *RNR3* and *HUG1* in *RPN11* mutants. Cells were treated as described in Fig. 2A. *RNR3* and *HUG1* mRNA was detected by Northern blotting. *ScR1* served as a loading control.

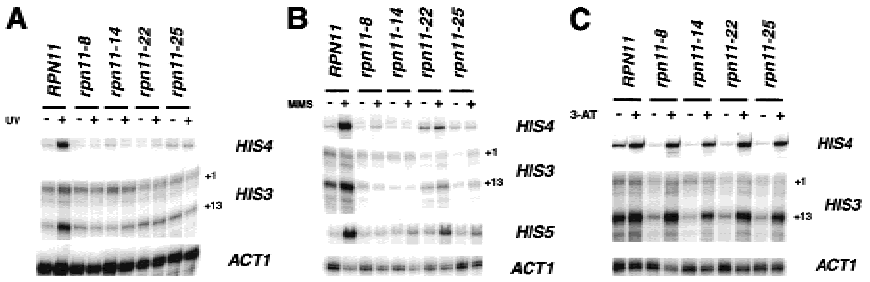

Figure 2. *RPN11* is required for the UV- and MMS-induced expression of Gcn4p target genes. (*A*) UV-induced expression of *HIS3* and *HIS4*. Wild-type and mutant cells (JR493) were treated on plates with 70 J/m^2 UV and returned to prewarmed YPAD for 75 min. *HIS3* and *ACT1* mRNAs were detected by S1 nuclease protection, and *HIS4* mRNA by primer extension. +1 and +13 indicate the two major transcription start sites of *HIS3*. (*B*) MMS-induced induction of *HIS3*, *HIS4*, and *HIS5*. Cells were treated with (+) or without (–) 0.1% MMS for 1 h at 30°C. *HIS5* mRNA was detected by Northern blotting. (*C*) Examination of *HIS3* and *HIS4* expression under amino acid starvation. Cells were grown at 30°C in liquid SC media (–) or in media lacking histidine and treated with 15 mM 3-AT for 60 min (+). RNA was analyzed as described in *A*.

tained at a permissive temperature for growth, 30°C (Fig. 2A, lanes 4,6,8,10). Moreover, mutation of *RPN11* resulted in the selective reduction in the transcript initiating at +13 (Fig. 2A), which is preferentially utilized by Gcn4p (Collart and Struhl 1993).

A genome-wide analysis of transcription revealed that most Gcn4p-dependent genes are induced by MMS treatment (Jelinsky and Samson 1999). Therefore, we examined the induction of three representative genes using MMS as an inducing agent. Similar to what was observed upon UV irradiation, treatment of wild-type cells with 0.1% MMS caused a strong induction of *HIS3* (+13) and *HIS4* mRNA (Fig. 2B). Also consistent with the results shown in Figure 2A, MMS-induced expression of these genes was eliminated in all four mutants (Fig. 2B). In this experiment, the *RPN11* mutants displayed reduced levels of the +13 transcript of *HIS3*, even in the absence of MMS treatment. This difference is likely caused by residual Gcn4p activity in the untreated wild-type cells, which is reduced in the mutants.

GCN4 was initially identified by its regulation of the amino acid starvation response, and most studies carried out to date have focused on this function (for review, see Hinnebusch 1988; Grant et al. 1998). Gcn4p is also activated by UV irradiation through a *RAS2*-dependent, but *GCN2*- and amino acid starvation–independent pathway (Engelberg et al. 1994). To determine whether *RPN11* specifically regulates the UV response pathway, and whether it broadly affects *GCN4* function, we examined the activation of *HIS3* and *HIS4* by inducing amino acid starvation conditions using 3-amino-1H-1,2,4-triazole (3-AT), a competitive inhibitor of the *HIS3* gene product (Kanazawa et al. 1988). The data shown in Figure 2C show that amino acid starvation strongly induced *HIS3* and *HIS4* expression in the mutant strains; thus, the amino acid starvation pathway is intact in *RPN11* mutants. These results clearly demonstrate that *RPN11* is specifically required for the *GCN4*-dependent UV resistance pathway, and that it does not generally control *GCN4* function.

The activation of Gcn4p by UV irradiation requires *RAS2* (Engelberg et al. 1994). To rule out the possibility that *RPN11* simply regulates RAS activity, rather than specifically affecting *GCN4* function, we examined the expression of three *RAS2*-dependent genes. Overexpression of *RAS2* suppresses the transcription of *CTT1, DDR48, TRX2* and heat shock genes, and conversely, mutation of *RAS2* causes their constitutive expression (Stanhill et al. 1999). Therefore, if *RPN11* controls RAS activity, changes in the expression of these genes would be evident in the mutants. The results shown in Figure 3 A and B demonstrate that the uninduced and stress-induced levels of transcription of *TRX2, DDR48*, and *CTT1* were not affected in the *RPN11* mutants. In addition to the data presented here, we also verified that the induction of *CUP1* by copper sulfate and that of *SSA4* by heat shock were unaffected in all four mutants (data not shown). These results indicate that *RPN11* is not generally required for stress-induced transcription, and that it does not affect the activity of *RAS2*.

Expression of an activated form of Ras2p, Ras2^{val19}p,

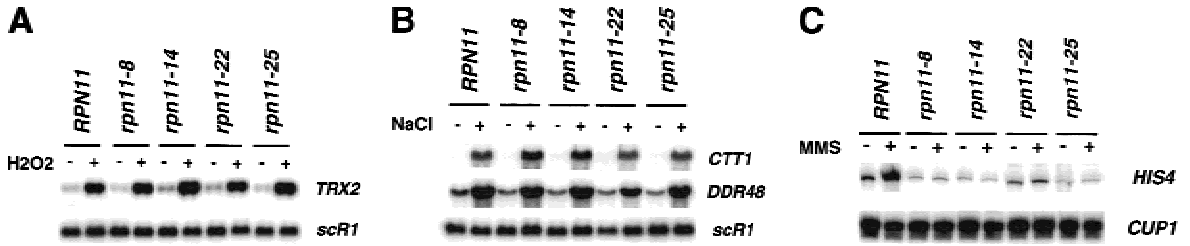

Figure 3. *RPN11* functions downstream of RAS2. (*A,B*) The expression of stress-induced genes is not affected by *RPN11* mutations. Wild-type (JR124-0) and mutant strains were grown in YPAD at 30°C to an OD of 0.4 and then treated with 1 mM hydrogen peroxide (H2O2, *A*) or 1 M NaCl (*B*) for 90 min. RNA levels were measured by Northern blotting. (*C*) MMS-induced expression of *HIS4* in strains overexpressing Ras2^{val19}. Wild-type and mutant strains containing a high-copy number plasmid expressing the activated form of *RAS2* (Whistler and Rine 1997) were grown in selective media and treated with 0.1% MMS for 1 h (+). RNA was analyzed as described in Fig. 2B.

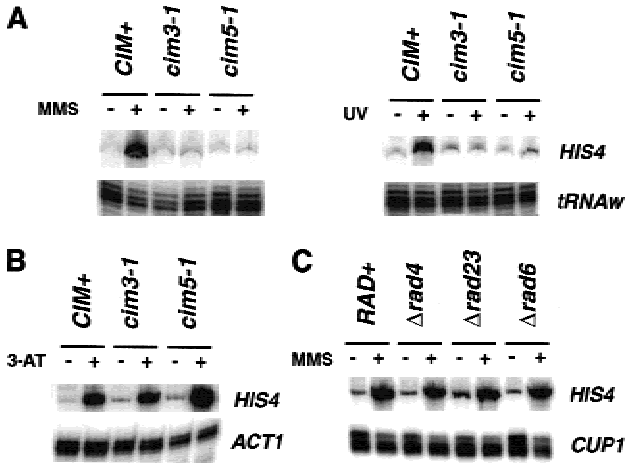

Figure 4. The regulatory cap of the proteasome regulates Gcn4p activity independent of its nucleotide excision repair functions. (*A*). UV- and MMS-induced expression of *HIS4*. Wild-type (CIM+), *cim3-1*, and *cim5-1* strains were treated with 0.1% MMS (left) or with 70 J/m^2 UV (right) at 30°C and then processed. *HIS4* mRNA was analyzed as described in Figure 2. (*B*) Starvation-induced expression of *HIS4*. Cells were grown to mid-log phase growth in SC medium at 30°C, collected by centrifugation and resuspended in SC (–) or SC minus histidine plus 15 mM 3-AT (+). After 60 min at 30°C the cultures were harvested. (*C*). Expression of *HIS4* in *RAD4, RAD6*, and *RAD23* mutants. Cells were treated as described in Fig. 2B.

increased the expression of Gcn4-target genes (Engelberg et al. 1994). We used this observation to more definitively place *RPN11* downstream of *RAS2* by examining the expression of *HIS4* in cells transformed with a high copy plasmid containing *Ras2^{val19}*. Enhanced RAS activity did not suppress the ts- growth defects or UV sensitivity of the mutant strains (not shown). In addition, the data shown in Figure 3C clearly indicate that overexpression of this hyperactive form of *RAS2* did not suppress the defects in *HIS4* expression observed in the mutants. Collectively, the results presented in Figure 3 indicate that *RPN11* functions downstream of *RAS2*, and that it is not generally required for stress-induced transcription.

Next, we investigated whether *RPN11* specifically regulates the RAS/AP-1-dependent UV resistance pathway, and whether the regulatory cap of the proteasome is required for this function. The 19S regulatory cap of the proteasome is composed of at least 11 subunits, each separated into one of two categories: ATPase and non-ATPase subunits (Glickman et al. 1998). *RPN11* is a representative of the latter category. We examined the requirement of two ATPase domain-containing subunits, Cim3/Sug1/Rpt6 and Cim5/Rpt1 (Glickman et al. 1998) for the UV-mediated activation of Gcn4p. The *cim3-1* and *cim5-1* mutants are moderately sensitive to UV radiation (Schauber et al. 1998; data not shown) and accumulate in the G_2/M phase of the cell cycle (Ghislain et al. 1993); thus, they are good candidates to analyze. As the results in Figure 4A show activation of *HIS4* by either MMS or UV treatment is severely compromised in both the *cim3-1* and *cim5-1* strains. Moreover, similar to the *RPN11* mutants, the *cim3-1* and *cim5-1* mutations had no effect on the starvation-induced activation of *HIS4* expression (Fig. 4B). In fact, we reproducibly observed a slight increase in the accumulation of *HIS4* mRNA in 3-AT-treated *cim5-1* cells (see below). These results indicate that the regulatory cap of the proteasome, and not *RPN11* specifically, is required for this function.

Rad23p and Rad4p, two components of the nucleotide excision repair system, associate with the regulatory cap of the proteasome (Schauber et al. 1998; Russell et al. 1999). It is possible that the defects in *HIS4* transcription caused by mutations in proteasome subunits result from altered nucleotide excision repair function. To rule out this possibility, we analyzed the expression of *HIS4* in strains containing a deletion of *RAD23* and *RAD4*. The results shown in Figure 4C demonstrate that the stimulation of *HIS4* by MMS is unaffected in these mutants; thus, the proteasome, and not the nucleotide excision repair system, is required for this function. In addition, we examined the induction of *HIS4* transcription in a *RAD6* mutant, a ubiquitin ligase required for normal resistance to UV and for the degradation of Gcn4p (Kornitzer et al. 1994). Because deletion of *RAD6* did not affect *HIS4* expression, the effects we observe in the *RPN11* mutants are unlikely to result from the alteration of Gcn4p half-life. To directly demonstrate that the defects in *HIS4* expression are not the result of altered Gcn4p levels, we immunoblotted for Gcn4p protein in the *RPN11* mutants. Figure 5 shows that Gcn4p levels were not significantly different in the mutants versus the wild type, either prior to or after 60 min of MMS treatment.

RPN11 is specially required for the UV-mediated activation of Gcn4p, but not for general control. These defects are not explained by trivial changes in Gcn4p levels or turnover. We did not find significant differences in the levels of Gcn4p in the mutants compared to a wild-type strain. If protein turnover is the predominant mechanism, we predict that the expression of Gcn4p target genes would increase, and that the starvation response would likewise be affected in a similar fashion (Kornitzer et al. 1994; Meimoun et al. 2000). In fact, the half-life of Gcn4p is extended in a *CIM5/RPT1* mutant (Kornitzer et al. 1994), and consistent with this, we observed a slight increase in *HIS4* transcription in 3-AT-stimulated

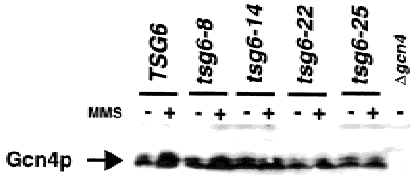

Figure 5. *RPN11* mutations do not affect Gcn4p levels. Cells were grown under nonstarvation conditions in SC complete medium and treated (+) or not treated (–), with 0.1% MMS for 60 min at 30°C. Gcn4p was detected by immunoblotting using anti-Gcn4 antibodies. An extract from a Δgcn4 strain was loaded as a negative control.

cim5-1 cells. In contrast, UV-stimulated expression is completely abolished in this mutant. Secondly, deletion of *RAD6* (Fig. 3C), a gene that regulates Gcn4p half-life (Kornitzer et al. 1994), does not affect the MMS-induced activation of *HIS4*. Thus the defects we observed are not simply the result of changes in Gcn4p levels or turnover rates.

What is the function of the proteasome in this pathway? One possibility is that UV irradiation stimulates the RAS pathway, which in turn triggers the destruction of a negative regulator of Gcn4p through the proteasome. A negative regulator of *GCN4* function has not been discovered; however, most analyses have centered on its translational regulation by *GCN2* (for review, see Hinnebusch 1988). Our data, and those of Engelberg et al. (1994), clearly indicate that two distinct pathways activate Gcn4p. The advantage of an alternate mechanism is that the activation of Gcn4p target genes can occur rapidly to repair damaged cellular compartments prior to the next cell division and can function independently of amino acid availability. The mechanism may be analogous to the regulation of NF-κB in mammalian cells. Like AP-1 transcription factors, NF-κB is stimulated by UV irradiation through the RAS pathway in mammalian cells (Devary et al. 1993). Ubiquitination and proteasome-mediated destruction of IκB, an inhibitor of NF-κB, are required to activate NF-κB-dependent genes (Winston et al. 1999). Alternatively, the proteasome degrades an inhibitor of the MAP kinase pathway that activates Gcn4p under UV stress. Treatment of mammalian cells with proteasome inhibitors prevents the degradation of MAP kinase phosphatase (Brondello et al. 1999), an antagonist of the MAP kinase pathway. Likewise in yeast, the proteasome may degrade a phosphatase (or other type of inhibitor) that antagonizes the actions of the MAP kinase pathway. At this time, the MAP kinase genes that activate Gcn4p in *S. cerevisiae* are not known, but future studies aimed in this direction will help illuminate the functions of the proteasome in the RAS-dependent UV resistance pathway.

RPN11 certainly has functions in UV resistance in addition to regulating the RAS-AP-1 response. This is demonstrated by the effect of each allele on the expression of Gcn4p-dependent genes versus their degrees of sensitivity to UV. All four mutants displayed strong effects on the expression of *HIS3* and *HIS4*, and yet the UV sensitivities varied as much as 100-fold among the alleles. Our observations cannot be explained by the reduced expression of Gcn4p-dependent genes not tested, because *gcn4* or *ras2* mutants are only threefold more sensitive to UV radiation compared to wild-type strains (Engelberg et al. 1994), whereas mutation of *RPN11* causes as much as a 1000-fold increase. The proteasome associates with Rad23 and Rad4, two components of the nucleotide excision repair system (Schauber et al. 1998; Russell et al. 1999). Mutation of *RPN11* may affect the association of the Rad23p/Rad4 complex with the proteasome, independent of its function in regulating the UV response. An alternate, but not mutually exclusive, explanation for the more severe phenotype of the mutants is that *RPN11* controls the activity of other transcription factors involved in stress resistance. Genetic evidence suggests that it is another AP-1-like transcription factor (Shimanuki et al. 1995). *S. cerevisiae* have at least 8 bZip transcription factors (YAPs) that have a high degree of sequence homology to Gcn4p (Fernandes et al. 1997), and the functions of these genes have yet to be elucidated. Additional studies such as genome-wide microarray expression experiments and genetic screens using *RPN11* mutants will hopefully identify additional targets of the proteasome that will increase our understanding of its functions in UV and drug resistance.

Materials and methods

Isolation of temperature-sensitive mutants of RPN11

Temperature sensitive mutants of *RPN11* were isolated by the plasmid shuffle technique by introducing a pool of hydroxylamine-treated pRS414-*RPN11* (Guthrie and Fink 1991) into YJR124–0 (*Mat a; ΔRPN11:hisg; ade2-11; his3-11,15; leu2-3,112; ura3-1; trp1-1; can1-100*; [RS426-*RPN11*], a W303 background). Tryptophan prototrophs were replica-plated onto 5-fluoroorotic acid-containing media and placed at 23°C and 37°C, and mutants that supported growth at 23°C, but not 37°C, were isolated for further analysis. Mutants that ceased growth at ≤2 doubling times were sequenced and subjected to additional analysis. The YJR493 background (*HIS3+*) was constructed by transforming the YJR124 strains with *Nsi*I-digested pRS403. The *cim3-1* and *cim5-1* temperature-sensitive mutants (Ghislain et al. 1993) were described in previous publications. The RAD deletion strains were purchased from Research Genetics.

Growth and phenotypic analysis of yeast strains

Cells were typically grown in YPD supplemented with 20 μg/mL adenine sulfate (YPAD) or in the appropriate synthetic medium (Guthrie and Fink 1991) at 30°C unless indicated otherwise. The temperature and drug sensitivity phenotypes of *RPN11* mutants were verified by spotting 10-fold serial dilutions of growing cells onto YPAD agar plates supplemented with the agents indicated in the figure legends. For UV sensitivity studies, 200 μL of the appropriate serial dilutions made from log-phase cultures were spread in triplicate onto YPAD agar plates. Each of the five plates was irradiated with 0 J/m^2, 30 J/m^2, 60 J/m^2, or 90 J/m^2 of ultraviolet radiation, respectively, and immediately placed in the dark at 30°C. Colonies were counted on each plate, and percent viability was calculated by dividing the number of colonies on irradiated plates by the number of colonies on the untreated plates.

RNA isolation and analysis

Strains were typically grown in YPAD at 30°C until an OD of 0.4, treated in culture with the agents indicated in the figures legends, collected by centrifugation and used for RNA isolation as described previously (Apone et al. 1996; Walker et al. 1996). The detection of specific messages was performed by S1 nuclease protection (Cormack and Struhl 1992), primer extension analysis (Engelberg et al. 1994) or Northern blotting (Sambrook et al. 1989). The 3-amino-1H-1,2,4-triazole (3-AT) induction experiments were conducted as described in Natarajan et al. (1998). Cells were treated with UV on solid media as described previously (Kiser and Wienert 1996) and returned to pre-warmed YPAD for 60–90 min prior to collection and RNA isolation.

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