Pheromone-regulated Sumoylation of Transcription Factors That Mediate the Invasive to Mating Developmental Switch in Yeast*

Yuqi Wang and Henrik G. Dohlman 1

From the Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, North Carolina 27599-7260

A fundamental question in biology is how different signaling pathways use common signaling proteins to attain different developmental outcomes. The yeast transcription factor Ste12 is required in at least two distinct signaling processes, each regulated by many of the same protein kinases. Whereas Ste12-Ste12 homoimers promote transcription of genes required for mating, Ste12-Tec1 heterodimers activate genes required for invasive growth. We report that Ste12 and Tec1 undergo covalent modification by the ubiquitin-related modifier SUMO. Stimulation by mating pheromone promotes sumoylation of Ste12 and diminishes the sumoylation of Tec1. In the absence of sumoylation Tec1 is more rapidly degraded. We propose that pheromone-regulated sumoylation of Ste12 and Tec1 promotes a developmental switch from the invasive to the mating differentiation program.

The budding yeast Saccharomyces cerevisiae can initiate distinct developmental programs depending on the presence or absence of specific external cues. Mating is initiated when α and α haploid cell types secrete and respond to cell type-specific pheromones acting through G protein-coupled receptors; once activated, the α and α cells fuse to form an α/α diploid cell. Invasive or filamentous growth occurs in nutrient-poor conditions and is manifested by altered budding and formation of long branching filaments, as well as increased adhesion and invasion of the substratum. Both developmental outcomes require activation of a protein kinase cascade comprised of Ste20, Ste11, Ste7, and Fus3 or Kss1 (1, 2).

Fus3 and Kss1 are mitogen-activated protein (MAP)2 kinases that phosphorylate substrates required for signaling in both the mating and invasive growth pathways. Phosphorylation of Ste12 promotes binding to a specific DNA sequence called the pheromone-response element (PRE) (3–6), where it initiates transcription of genes required for efficient mating (7–9). Ste12 can also assemble with another transcription factor, Tec1 (10–13). Ste12-Tec1 heterodimers bind cooperatively to a distinct DNA sequence called the filamentation- and invasive-response element (FRE) (10, 11) present in the promoter region of genes that regulate invasive or pseudohyphal growth (9, 14). The kinase activity of Kss1 increases filamentation, whereas the kinase activity of Fus3 suppresses filamentation (1, 8, 10, 15, 16). Both pathways are negatively regulated by Ste12-binding proteins Dig1/Rst1 and Dig2/Rst2 (7, 17–19) as well as by Ste12 binding to the unphosphorylated and inactive form of Kss1 (20).

Although the mechanisms of MAP kinase and transcription factor activation are well established, less understood is how signaling pathways that share the same components attain different developmental fates (21). Signal identity has been ascribed to differences in signal magnitude, duration, and frequency (22), as well as to the scaffolded association of protein kinase components (23). Even where such differences have been documented, the kinase signals must still be interpreted by nuclear transcription factors to initiate expression of a distinct set of genes (9). Here we have shown that Ste12 and Tec1 are covalently modified by the small ubiquitin-related protein SUMO (Smt3 in yeast). Although stimulation by mating pheromone promotes sumoylation of Ste12, the same treatment inhibits sumoylation of Tec1 and the protein is instead degraded. These findings suggest that pheromone-regulated sumoylation of transcription factors underlies the developmental switch from the invasive to the mating differentiation program.

EXPERIMENTAL PROCEDURES

Strains and Plasmids—Standard methods for the growth, maintenance, and transformation of bacteria and for the manipulation of DNA were used throughout. Details of plasmid construction are available from the authors. Except where stated otherwise, TEC1 and STE12 were expressed from single copy plasmids using the native promoter.

Unless otherwise indicated, the S. cerevisiae strains used in this study were BY4741 (MATa leu2Δ met15Δ his3Δ ura3Δ) and BY4741-derived mutants lacking SIZ1, SIZ2, FUS3, KSS1, or TEC1 (Research Genetics) or containing the Schizosaccharomyces pombe his4+ gene and a tandem affinity purification (TAP) tag at STE12 or TEC1 (24). Analysis of UBC9 was carried out in strains MHY500 (MATα his3ΔΔ leu2-3,112 ura3-52 lys2-801 trpl-1 gal2Δ) and MHY1620 (MHY500 LEU2::ubc9-1 ubc9Δ::TRP1) (from M. Hochstrasser) maintained at the semipermissive temperature of 24 °C. Protein turnover experiments were conducted in strains Z1256 (MATα ade2-1 trpl-1 can1-100 leu2-3,112 his3-11,15 ura3) (from R. Young), Z1783-fus3 (Z1783 fus3Δ::G418), and Z1783-kss1 (Z1783 kss1Δ::G418) (this study). The invasive growth and FRE reporter transcription assays were performed in the Δ1278-based invasive strain MLY218a (MATα leu2Δ ura3Δ, from J. Heitman) and MLY218a-tec1Δ::URA3 (this study).

Growth, Transformation, and Degradation Bioassays—The pheromone-dependent growth inhibition (halo) and reporter-transcription assays were conducted as described previously (25). Unless indicated otherwise, the concentration of α-factor was 3 μM, which is 10-fold above the EC50 for transcriptional induction. To monitor the loss of Tec1 over time, mid-log cell cultures were treated with α-factor for 60 min, followed by cycloheximide addition as described previously (26). Immunoprecipitations, 6.5% gel electrophoresis, and immunoblot analysis
were carried out as described previously (26) using antibodies against SUMO (from C. Hoege and S. Jentsch), Protein A (Sigma), FLAG (Sigma), and ubiquitin (Sigma).

RESULTS

A long-standing question in cell regulation is how signaling pathways that share component proteins can attain different developmental fates. To address this question we investigated whether Ste12 or Tec1 is covalently modified by the small ubiquitin-related protein SUMO (Smt3 in yeast) and whether pheromone-regulated modification of these signaling proteins could underlie the switch from the invasive growth to mating differentiation transcription program. We focused initially on sumoylation because it is a reversible modification known to regulate the activity of nuclear transcription factors, co-activators, and co-repressors (27, 28). In addition, mass spectrometry sequencing identified Ste12 as one of several hundred proteins that co-purify with Smt3, making it a likely substrate for sumoylation (29).

Ste12, fused to a TAP tag (24), was immunopurified, resolved by gel electrophoresis, and detected by immunoblotting. The antibodies strongly recognized the native form of the protein (Fig. 1, A and B), as well as additional species ~12 and 24 kDa larger than the native protein, a difference corresponding to the molecular mass of one or two copies of mature SUMO, respectively. The presence of multiple high molecular mass species suggested that Ste12 undergoes sumoylation at two or more sites. The larger forms of the protein were more abundant in cells treated with α-factor pheromone, were less abundant in a mutant strain lacking the E3 SUMO ligase SIZ1 (Fig. 1A), and were completely absent in a ubc9–1 mutant strain deficient in SUMO-conjugating enzyme activity (Fig. 1B) (30). Ste12 also migrated more slowly in the ubc9–1 mutant strain; however, this mobility shift did not appear to be pheromone regulated. Moreover, the shift is too small to arise from addition of ubiquitin or a ubiquitin-like modifier and may instead result from another known modification such as phosphorylation (3–6, 31–33).

To confirm that Ste12 is sumoylated we immunopurified Ste12 tagged with the FLAG epitope. The higher molecular mass form of Ste12 was recognized by SUMO antibodies and was again enriched by
pheromone stimulation (Fig. 1C). The increase in sumoylation was proportionately greater than the induction of Ste12 expression that is typically observed in pheromone-treated cells. Moreover, deletion of either *FUS3* or *KSS1* dampened the effect of pheromone on Ste12 sumoylation, consistent with the partially overlapping function of the two kinases in this pathway (1, 16, 34). Kss1 and Fus3 phosphorylate many of the same proteins but with distinct substrate specificities, and this presumably accounts for the differences in Ste12 sumoylation in the two mutants (35). We conclude from these data that Ste12 is sumoylated and that sumoylation is enhanced in response to pheromone stimulation.

Ste12 binds to PReS present within the promoters of several genes involved in mating. The promoter of the *FUS1* gene contains multiple PReS and has been widely used to monitor pheromone-stimulated transcription activity (25). Thus, a reporter consisting of the PRE promoter fused to lacZ (β-galactosidase) was used to determine how sumoylation affects Ste12 function. Initially we attempted to block sumoylation in Ste12 by substituting Arg for Lys-174 and Lys-409, the two residues that most closely match the preferred sequence for sumoylation (Ψ-K-X-D/E, where Ψ is a hydrophobic residue and X is any residue) (36). However, neither mutation, tested alone or in combination, blocked the pheromone-stimulated mobility shift, suggesting that one or more of the other 42 Lys residues in Ste12 is modified (data not shown). We also investigated how the pheromone response is altered in the *ubc9* mutant strain. In this mutant PRE-lacZ induction was substantially reduced relative to the wild-type, suggesting that sumoylation is required for full Ste12 activity (data not shown). However, even though the PRE reporter is highly specific for Ste12 activity, the *ubc9* mutant could have multiple effects that indirectly affect reporter transcription activity. As an alternative approach we examined PRE-lacZ activity in cells expressing a SUMO-Ste12 fusion protein. Similar SUMO substrate fusions have been used previously to show that sumoylation can diminish transcription factor function (37–39). In the absence of pheromone, cells expressing SUMO-Ste12 exhibited no change in basal lacZ activity, suggesting that sumoylation of Ste12 is not sufficient to initiate new gene transcription. In the presence of pheromone, however, cells expressing SUMO-Ste12 exhibited a >2-fold increase in maximum transcription activity (Fig. 1D). The SUMO-Ste12 fusion also increased the pheromone-mediated growth arrest response, as indicated by a larger zone of growth inhibition surrounding a source of α-factor (the response was also more transient, as indicated by resumption of colony growth and the formation of a turbid halo) (Fig. 1E). Finally, activation of the mating response by Ste12-SUMO resulted in a concomitant decrease in the invasive growth response (Fig. 1E). Expression levels of the SUMO-Ste12 fusion were not elevated compared with Ste12 (Fig. 1F). However, whereas native Ste12 is only partially sumoylated after pheromone stimulation, the SUMO-Ste12 fusion resembles a protein that is fully sumoylated all the time, and this presumably accounts for the higher transcription and growth arrest response compared with the native partially sumoylated protein. Thus, sumoylation of Ste12 appears to confer a gain-of-function phenotype, but transcription activity is still contingent on stimulation by upstream components in the pathway.

We then considered whether Tec1 is also regulated by sumoylation. As before, a FLAG-Tec1 fusion was immunopurified and probed by immunoblotting with antibodies to FLAG as well as SUMO. Once again we could detect multiple bands with the FLAG antibody, one corresponding to native Tec1 and a second corresponding to Tec1 potentially modified by sumoylation. The higher molecular mass species was also detected using anti-SUMO-antibodies, and both bands were absent in cells containing only the parent vector (Fig. 2A). Pheromone treatment resulted in diminished expression and substantially diminished sumoylation of Tec1 (Fig. 2B). The pheromone-dependent decrease in Tec1 sumoylation is in marked contrast to the pheromone-dependent increase observed for Ste12.

**FIGURE 2.** Pheromone regulates sumoylation and ubiquitination of Tec1. A, whole cell lysates from tec1Δ cells transformed with FLAG-tagged Tec1 or the parent vector were immunoprecipitated with M2 anti-FLAG resin and subjected to immunoblotting using anti-FLAG or anti-SUMO antibodies. B, the same cells were transformed with triple-FLAG-tagged Tec1, a Lys-54-Arg Tec1 mutant (Tec1K54R), or the parent vector and then treated with α-factor, lysed, immunoprecipitated with M2 anti-FLAG resin, and subjected to immunoblotting using anti-FLAG or anti-SUMO antibodies. Numbers under each lane refer to the difference in band intensity of Tec1 and SUMO-Tec1 (where visible) relative to lane 1, as determined by scanning densitometry. ND, not determined. C, the same cells as in panel B were spotted onto solid YPD medium and after 2 days rubbed vigorously under a stream of water to detect invasive growth. D, transcription activity was measured in the same cells transformed with a plasmid containing an invasive-specific PRE reporter (TEC1 promoter, lacZ reporter) or the mating-specific PRE reporter described in Fig. 1.
mutant exhibited slightly elevated FRE-lacZ and unmodified form of Tec1. Myc-tagged Tec1 was used for these experiments. Accumulation of the unsumoylated Tec1 mutant was ubiquitinated at a reduced level relative to the wild-type protein (data not shown); the residual ubiquitination was most likely due to modification of an alternate site when the primary site (Lys-54 in this case) becomes unavailable. Moreover, Tec1 K54R accumulates a prominent species with reduced mobility but that is not recognized by the anti-SUMO antibodies (Fig. 2B); we presume that this band is due to an accumulation of phosphorylated but poorly ubiquitinated protein.

Tec1 ubiquitination is regulated by the MAP kinases Fus3 and Kss1 (42–44). These MAP kinases phosphorylate many common substrates and have partially overlapping function in vivo. Expression of either kinase is sufficient to partially sustain the mating response (Fig. 1C) (1, 16, 34). However, significant differences have been reported for the invasive pathway. Deletion of KSS1 is sufficient to block invasive growth, whereas deletion of FLIS3 promotes invasive behavior. Moreover, deletion of FLIS3 (but not KSS1) results in an increase in FRE binding activity (9) and transcription of FRE-containing genes (8, 10, 16). To determine whether sumoylation might underlie these differences we compared Tec1 expression in fus3Δ and kss1Δ mutant strains. Deletion of FLIS3 (but not KSS1) resulted in elevated expression of Tec1 and also of the sumoylated form of Tec1 (Fig. 3A). Moreover, pheromone treatment of the fus3Δ mutant strain no longer diminished, and even increased slightly, the expression of Tec1. These differences in expression could be ascribed to differences in protein stability because Tec1 was degraded more slowly in the absence of Fus3 (but not Kss1) and also of the sumoylated form of Tec1 (Fig. 3C).

Because increased sumoylation promotes Ste12 activity we anticipated that decreased sumoylation might result in diminished Tec1 activity. To test this model we replaced a consensus site Lys at position 54 and found that it successfully eliminated detectable sumoylation concomitant with higher overall expression levels (Fig. 2B). We then measured invasive growth and transcription induction using a FRE promoter fused to lacZ. Rather than decrease activity, the sumoylation-deficient Tec1 K54R mutant exhibited slightly elevated FRE-lacZ and invasive growth activities (Fig. 2, C and D) (8, 40, 41). Together these data reveal that sumoylation is not required for full Tec1 transcription activity or proper regulation of invasive growth behavior.

The higher abundance of Tec1 K54R could result from a loss of ubiquitination. Ubiquitin attachment typically leads to rapid capture and degradation of the substrate by the proteasome protease complex (27), and it was recently documented that Tec1 undergoes phosphorylation-dependent ubiquitination and accelerated degradation in pheromone-stimulated cells (42–44). Moreover, modification by SUMO and ubiquitin are often antagonistic and can sometimes occur on the same Lys residue (45, 46). In agreement with this model, the unsumoylated Tec1 K54R mutant was ubiquitinated at a reduced level relative to the wild-type protein (data not shown); the residual ubiquitination was most likely due to modification of an alternate site when the primary site (Lys-54 in this case) becomes unavailable. Moreover, Tec1 K54R accumulates a prominent species with reduced mobility but that is not recognized by the anti-SUMO antibodies (Fig. 2B); we presume that this band is due to an accumulation of phosphorylated but poorly ubiquitinated protein.

FIGURE 3. Regulation of Tec1 expression by Fus3. A, wild-type or isogenic fus3Δ or kss1Δ mutant cells containing integrated triple-Myc-tagged Tec1 were treated with α-factor and subjected to immunoblotting of whole cell lysates using anti-Myc antibodies as indicated. Numbers under each lane refer to the difference in band intensity of Tec1 and SUMO-Tec1 (where visible) relative to lane 3, as determined by scanning densitometry. ND, not determined. B, the same cells as in panel A were treated with α-factor pheromone as indicated, treated with cycloheximide for the indicated times to block new protein translation, and subjected to immunoblotting of whole cell lysates using anti-Myc antibodies. 

Because increased sumoylation promotes Ste12 activity we anticipated that decreased sumoylation might result in diminished Tec1 activity. To test this model we replaced a consensus site Lys at position 54 and found that it successfully eliminated detectable sumoylation concomitant with higher overall expression levels (Fig. 2B). We then measured invasive growth and transcription induction using a FRE promoter fused to lacZ. Rather than decrease activity, the sumoylation-deficient Tec1 K54R mutant exhibited slightly elevated FRE-lacZ and invasive growth activities (Fig. 2, C and D) (8, 40, 41). Together these data reveal that sumoylation is not required for full Tec1 transcription activity or proper regulation of invasive growth behavior.

The higher abundance of Tec1 K54R could result from a loss of ubiquitination. Ubiquitin attachment typically leads to rapid capture and degradation of the substrate by the proteasome protease complex (27), and it was recently documented that Tec1 undergoes phosphorylation-dependent ubiquitination and accelerated degradation in pheromone-stimulated cells (42–44). Moreover, modification by SUMO and ubiquitin are often antagonistic and can sometimes occur on the same Lys residue (45, 46). In agreement with this model, the unsumoylated Tec1 K54R mutant was ubiquitinated at a reduced level relative to the wild-type protein (data not shown); the residual ubiquitination was most likely due to modification of an alternate site when the primary site (Lys-54 in this case) becomes unavailable. Moreover, Tec1 K54R accumulates a prominent species with reduced mobility but that is not recognized by the anti-SUMO antibodies (Fig. 2B); we presume that this band is due to an accumulation of phosphorylated but poorly ubiquitinated protein.

Tec1 ubiquitination is regulated by the MAP kinases Fus3 and Kss1 (42–44). These MAP kinases phosphorylate many common substrates and have partially overlapping function in vivo. Expression of either kinase is sufficient to partially sustain the mating response (Fig. 1C) (1, 16, 34). However, significant differences have been reported for the invasive pathway. Deletion of KSS1 is sufficient to block invasive growth, whereas deletion of FLIS3 promotes invasive behavior. Moreover, deletion of FLIS3 (but not KSS1) results in an increase in FRE binding activity (9) and transcription of FRE-containing genes (8, 10, 16). To determine whether sumoylation might underlie these differences we compared Tec1 expression in fus3Δ and kss1Δ mutant strains. Deletion of FLIS3 (but not KSS1) resulted in elevated expression of Tec1 and also of the sumoylated form of Tec1 (Fig. 3A). Moreover, pheromone treatment of the fus3Δ mutant strain no longer diminished, and even increased slightly, the expression of Tec1. These differences in expression could be ascribed to differences in protein stability because Tec1 was degraded more slowly in the absence of Fus3 (but not Kss1) and also of the sumoylated form of Tec1 (Fig. 3C).

Because increased sumoylation promotes Ste12 activity we anticipated that decreased sumoylation might result in diminished Tec1 activity. To test this model we replaced a consensus site Lys at position 54 and found that it successfully eliminated detectable sumoylation concomitant with higher overall expression levels (Fig. 2B). We then measured invasive growth and transcription induction using a FRE promoter fused to lacZ. Rather than decrease activity, the sumoylation-deficient Tec1 K54R mutant exhibited slightly elevated FRE-lacZ and invasive growth activities (Fig. 2, C and D) (8, 40, 41). Together these data reveal that sumoylation is not required for full Tec1 transcription activity or proper regulation of invasive growth behavior.

The higher abundance of Tec1 K54R could result from a loss of ubiquitination. Ubiquitin attachment typically leads to rapid capture and degradation of the substrate by the proteasome protease complex (27), and it was recently documented that Tec1 undergoes phosphorylation-dependent ubiquitination and accelerated degradation in pheromone-stimulated cells (42–44). Moreover, modification by SUMO and ubiquitin are often antagonistic and can sometimes occur on the same Lys residue (45, 46). In agreement with this model, the unsumoylated Tec1 K54R mutant was ubiquitinated at a reduced level relative to the wild-type protein (data not shown); the residual ubiquitination was most likely due to modification of an alternate site when the primary site (Lys-54 in this case) becomes unavailable. Moreover, Tec1 K54R accumulates a prominent species with reduced mobility but that is not recognized by the anti-SUMO antibodies (Fig. 2B); we presume that this band is due to an accumulation of phosphorylated but poorly ubiquitinated protein.

Tec1 ubiquitination is regulated by the MAP kinases Fus3 and Kss1 (42–44). These MAP kinases phosphorylate many common substrates and have partially overlapping function in vivo. Expression of either kinase is sufficient to partially sustain the mating response (Fig. 1C) (1, 16, 34). However, significant differences have been reported for the invasive pathway. Deletion of KSS1 is sufficient to block invasive growth, whereas deletion of FLIS3 promotes invasive behavior. Moreover, deletion of FLIS3 (but not KSS1) results in an increase in FRE binding activity (9) and transcription of FRE-containing genes (8, 10, 16). To determine whether sumoylation might underlie these differences we compared Tec1 expression in fus3Δ and kss1Δ mutant strains. Deletion of FLIS3 (but not KSS1) resulted in elevated expression of Tec1 and also of the sumoylated form of Tec1 (Fig. 3A). Moreover, pheromone treatment of the fus3Δ mutant strain no longer diminished, and even increased slightly, the expression of Tec1. These differences in expression could be ascribed to differences in protein stability because Tec1 was degraded more slowly in the absence of Fus3 (but not Kss1) and also of the sumoylated form of Tec1 (Fig. 3C).

Finally, the relationship between Tec1 sumoylation and abundance sug-
gests that sumoylation might stabilize the protein. A prediction of this model is that sumoylated Tec1 should degrade more slowly than the unmodified protein. To test this, we compared the abundance of sumoylated and non-sumoylated Tec1 over time, following cycloheximide treatment to block new protein synthesis. As predicted, the sumoylated form of the protein persisted much longer than the unmodified species, whether expressed under the control of the native promoter (Fig. 3C) or a heterologous inducible promoter (from GAL1, Fig. 3D).

**REFERENCES**

1. Roberts, R. L., and Fink, G. R. (1994) *Genes Dev.* 8, 2974–2985
2. Liu, H., Styles, C. A., and Fink, G. R. (1993) *Science* 262, 1741–1744
3. Elion, E. A., Satterberg, B., and Kranz, J. E. (1993) *Mol. Biol. Cell* 4, 495–510
4. Dolan, J. W., Kirkman, C., and Fields, S. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 5703–5707
5. Hagen, D. C., McCaffrey, G., and Sprague, G. F., Jr. (1991) *Mol. Cell. Biol.* 11, 2952–2961
6. Sengupta, P., and Cochran, B. H. (1990) *Mol. Cell. Biol.* 10, 6809–6812
7. Breitkreutz, A., Boucher, L., Breitkreutz, B. J., Sultan, M., Jurisica, I., and Tyers, M. (2003) *Genetics* 163, 997–1013
8. Roberts, C. J., Nelson, B., Marton, M. J., Stoughton, R., Meyer, M. R., Bennett, H. A., He, Y. D., Dai, H., Walker, W. L., Hughes, T. R., Tyers, M., Boone, C., and Friend, S. H. (2000) *Science* 287, 873–880
9. Zeitlinger, J., Simon, I., Harbison, C. T., Hannett, N. M., Volkert, T. L., Fink, G. R., and Young, R. A. (2003) *Cell* 113, 395–404
10. Madhani, H. D., and Fink, G. R. (1997) *Science* 275, 1314–1317
11. Baur, M., Esch, R. K., and Errede, B. (1997) *Mol. Cell. Biol.* 17, 4330–4337
12. Laloux, I., Jacobs, E., and Dubois, E. (1994) *Nucleic Acids Res.* 22, 999–1005
13. Gavriès, V., Andrianopoulos, A., Giron, C. J., and Timbers, W. E. (1996) *Mol. Microbiol.* 19, 1255–1263
14. Madhani, H. D., Galtitski, T., Landers, E. S., and Fink, G. R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 12530–12535
15. Cook, J. G., Bardwell, L., and Thorner, J. (1997) *Nature* 390, 85–88
16. Sabbagh, W. Jr., Flatau, L. J., Bardwell, A. J., and Bardwell, L. (2001) *Mol. Cell* 8, 683–691
17. Tedford, K., Kim, S., Sa, D., Stevens, K., and Tyers, M. (1997) *Curr. Biol.* 7, 228–238
18. Cook, J. G., Bardwell, L., Kron, S. J., and Thorner, J. (1996) *Genes Dev.* 10, 2831–2848
19. Bardwell, L., Cook, J. G., Zhu-Shimoni, J. X., Voora, D., and Thorner, J. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 15400–15405
20. Bardwell, L., Cook, J. G., Voora, D., Baggett, D. M., Martinez, A. R., and Thorner, J. (1998) *Genes Dev.* 12, 2887–2898
21. Elion, E. A., Qi, M., and Chen, W. (2005) *Science* 307, 687–688
22. Bhalla, U. S., Ram, P. T., and Iyengar, R. (2002) *Science* 297, 1018–1023
23. Pawson, T., and Scott, J. D. (1997) *Science* 278, 2075–2080
24. Ghaemmaghami, S., Huh, W. K., Bower, K., Howson, R. W., Belle, A., Dephouville, N., O’Shea, E. K., and Weissman, J. S. (2003) *Nature* 425, 737–741
25. Hoffman, G., Garrison, T. R., and Dohlmian, H. G. (2002) *Methods Enzymol.* 344, 617–631
26. Hao, N., Yildirim, N., Wang, Y., Elston, T. C., and Dohlmian, H. G. (2003) *J. Biol. Chem.* 278, 46506–46515
27. Hochstrasser, M. (2000) *Nat. Cell Biol.* 2, E153–E157
28. Seeler, J. S., and Dejean, A. (2003) *Nat. Rev. Mol. Cell. Biol.* 4, 690–699
29. Wohlschlegel, J. A., Johnson, E. S., Reed, S. L., and Yates, J. R., III (2004) *J. Biol. Chem.* 279, 45662–45668
30. Johnson, E. S., and Blobel, G. (1997) *J. Biol. Chem.* 272, 26799–26802
31. Nelson, C., Goto, S., Lund, K., Hung, W., and Sadowski, I. (2003) *Nature* 421, 187–190
32. Hung, W., Olson, K. A., Breitkreutz, A., and Sadowski, I. (1997) *Eur. J. Biochem.* 245, 241–251
33. Song, D., Dolan, J. W., Yuan, Y. L., and Fields, S. (1991) *Genes Dev.* 5, 741–750
34. Gartner, A., Nasmyth, K., and Ammerer, G. (1992) *Genes Dev.* 6, 1280–1292
35. Breitkreutz, A., Boucher, L., and Tyers, M. (2001) *Curr. Biol.* 11, 1266–1271
36. Johnson, E. S., and Blobel, G. (1999) *J. Cell Biol.* 147, 981–994
37. Ross, S., Best, J. L., Zon, L. I., and Gill, G. (2002) *Mol. Cell* 10, 831–842
38. Yang, S. H., Jaffray, E., Hay, R. T., and Sharrocks, A. D. (2003) *Mol. Cell* 12, 63–74
39. Long, J., Wang, G., He, D., and Liu, F. (2004) *Biochem. J.* 379, 23–29
40. Oehlen, L. J., and Cross, F. R. (1998) *Cell Biol. Chem.* 245, 25089–25097
41. Erdman, S., and Snyder, M. (2001) *Genetics* 159, 919–928
42. Rao, M. Z., Schwartz, M. A., Cantin, G. T., Yates, J. R., III, and Madhani, H. D. (2004) *Cell* 119, 991–1000
43. Chou, S., Huang, L., and Lii, H. (2004) *Cell* 119, 981–990
44. Bruckner, S., Kohler, T., Braus, G. H., Heise, B., Bolte, M., and Mosch, H. U. (2004) *Curr. Genet.* 46, 331–342

*Acknowledgments*—We thank Gerald Fink, Richard Young, Mark Hochstrasser, Carsten Hoege, and Stefan Jentsch for providing valuable reagents and Beverly Errede and Jeannine Cooper for helpful comments on the manuscript.
45. Hoege, C., Pfander, B., Moldovan, G. L., Pyrowolakis, G., and Jentsch, S. (2002) Nature 419, 135–141
46. Desterro, J. M., Rodriguez, M. S., and Hay, R. T. (1998) Mol. Cell 2, 231–239
47. Ellison, M. J., and Hochstrasser, M. (1991) J. Biol. Chem. 266, 21150–21157
48. Hicke, L., and Riezman, H. (1996) Cell 84, 277–287
49. Roth, A. F., and Davis, N. G. (1996) J. Cell Biol. 134, 661–674
50. Madura, K., and Varshavsky, A. (1994) Science 265, 1454–1458
51. Marotti, L. A., Jr., Newitt, R., Wang, Y., Aebersold, R., and Dohlman, H. G. (2002) Biochemistry 41, 5067–5074
52. Wang, Y., and Dohlman, H. G. (2002) J. Biol. Chem. 277, 15766–15772
53. Wang, Y., Ge, Q., Houston, D., Thorner, J., Errede, B., and Dohlman, H. G. (2003) J. Biol. Chem. 278, 22284–22289