A Conserved RING Finger Protein Required for Histone H2B Monoubiquitination and Cell Size Control

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Summary

Monoubiquitination of histone H2B is required for methylation of histone H3 on lysine 4 (K4), a modification associated with active chromatin. The identity of the cognate ubiquitin ligase is unknown. We identify Bre1 as an evolutionarily conserved RING finger protein required in vivo for both H2B ubiquitination and H3 K4 methylation. The RING domain of Bre1 is essential for both of these modifications as is Lge1 (Large 1), a protein required for cell size control that copurifies with Bre1. In cells lacking the euchromatin-associated histone variant H2A.Z, BRE1, RAD6, and LGE1 are each essential for cell viability, supporting redundant functions for H2B ubiquitination and H2A substitution in the formation of active chromatin. Notably, analysis of mutants demonstrates a function for Bre1/Lge1-dependent H2B monoubiquitination in the control of cell size.

Introduction

Covalent modifications of histones play crucial roles in several aspects of chromosome behavior, especially transcription (Jenuwein and Allis, 2001). In several instances, a modification at one position of a histone can influence subsequent modification at a second site. For example, in fission yeast and metazoans, methylation of histone H3 K4, a modification associated with active chromatin, is mutually exclusive with the heterochromatin-associated modification of H3 K9 (Jenuwein and Allis, 2001). Moreover, it has recently been shown in the budding yeast, S. cerevisiae, that monoubiquitination of K123 on histone H2B is required for methylation of H3 K4, demonstrating that modification of one histone subunit can regulate the subsequent modification of a different subunit (Dover et al., 2002; Sun and Allis, 2002). Despite increasing recognition of the importance of histone ubiquitination and the fact that histones were the first proteins found to be subject to covalent modification by ubiquitin (Hunt and Dayhoff, 1977; Olson et al., 1976), our understanding of the machinery responsible for this modification, its regulation, and its functions remains incomplete.

Ubiquitination is catalyzed by a system of three enzymes that includes a generic ubiquitin-activating enzyme or E1, a ubiquitin-conjugating enzyme or E2, and a ubiquitin ligase or E3 (Glickman and Ciechanover, 2002). Cells have many more ubiquitin ligases than ubiquitin-conjugating enzymes; the ligases are the substrate recognition components of the system, and they are generally the target of biological regulation. In S. cerevisiae, the Rad6 ubiquitin-conjugating enzyme has been shown to be required for histone H2B monoubiquitination on K123 and consequently methylation of H3 on K4 (Robzyk et al., 2000). Rad6 is known to function in conjunction with one of three RING finger ubiquitin ligase homologs: Ubr1, Rad18, or Rad5 (Bailly et al., 1994; Dohmen et al., 1991; Johnson et al., 1992). Ubr1 functions in the N-end rule protein degradation pathway (Bartel et al., 1990), whereas Rad18 and Rad5 function in DNA repair, where PCNA is a key substrate (Hoege et al., 2002). The ubiquitin ligase for histone monoubiquitination has not previously been reported.

Here we identify a conserved RING finger domain protein, encoded by BRE1 in S. cerevisiae, that is required for histone H2B monoubiquitination in vivo. A BRE1-associated protein, LGE1, is also required for H2B mono-ubiquitination. As predicted from recent studies, both Bre1 and Lge1 are required for histone H3 K4 methylation as well. In contrast, the Rad5, Rad18, and Ubr1 RING proteins shown previously to function with Rad6 are dispensable for H2B monoubiquitination and H3 K4 methylation. The lge1Δ (large 1) mutant was originally identified in a screen for cell size mutants. We show here that bre1Δ mutants and strains harboring a single amino acid change in the H2B monoubiquitination site also display a large cell phenotype. Cells lacking components of the H3 K4 methylase display a size distribution that is intermediate between those of wild-type and bre1Δ or lge1Δ cells, demonstrating a role for histone H2B monoubiquitination in cell size determination that is in part distinct from its requirement for histone H3 K4 methylation.

Results and Discussion

We have recently obtained evidence that the universally conserved histone variant, H2A.Z (encoded by HTZ1 in S. cerevisiae), functions to promote the formation of active chromatin (M.D. Meneghini, M. Wu, and H.D.M., submitted). Although htz1Δ deletion mutants are viable, we observed that htz1Δ rad6Δ double mutants are inviable (Figure 1). We systematically screened for other deletion mutants that are synthetically lethal with htz1Δ (our unpublished data) and identified a null mutation in BRE1, which encodes a protein of unknown function identified previously in a screen for mutants that are hypersensitive to the drug brefeldin A (Muren et al., 2001). bre1Δ mutants display synthetic lethality with htz1Δ mutants but, like rad6Δ mutants, are viable in HTZ1+ cells (Figure 1). Database searching and alignment (Figure 2) revealed sequence homologs of Bre1 in Schizosaccharo-
myces pombe (SpBre1A, SpBre1B), Dictyostelium discoideum (DictBre1), Arabadopsis thaliana (AtBre1), Caenorhabditis elegans (CeBre1), Drosophila melanogaster (DmBre1), and humans (HuBre1A, HuBre1B). Multiple domains in Bre1 and its homologs yield strong predictions of helical coiled-coil structure, but the highest sequence conservation is apparent in a canonical RING domain that lies at the extreme C terminus of each family member. Phylogenetic analysis of the similarity between Bre1 homologs from different organisms recapitulates the expected relationships between species, further supporting their assignment as a family of proteins (data not shown). RING domains are characteristic of a large class of ubiquitin ligases and are likely to be diagnostic for this activity (Joazeiro and Weissman, 2000). Since BRE1 shares genetic properties with RAD6 and since the protein has a domain found in ubiquitin ligases, we hypothesized that Bre1 is a component of the ubiquitin ligase that cooperates with Rad6 in the ubiquitination of histone H2B.

As shown in Figure 3A, monoubiquitination of H2B can be readily detected in wild-type cells harboring a functional FLAG-tagged allele of the HTB1 gene (encoding histone H2B) as a slower-migrating form upon SDS-PAGE and immunoblotting of whole-cell extracts with anti-FLAG antibodies. Consistent with previous studies, the ubiquitinated species is absent in cells lacking the E2 Rad6 (Figure 3A). In cells lacking Bre1, monoubiquitination of H2B is similarly...
Conserved RING Protein for Histone Ubiquitination

Figure 3. Analysis of H2B Monoubiquitination and H3 Methylation

(A) Determination of H2B monoubiquitination in selected wild-type and mutant strains. Shown is an immunoblot using anti-FLAG antibodies of extracts from strains of the indicated genotypes harboring FLAG-H2B on a CEN-ARS plasmid.

(B) Bre1 RING domain is essential for H2B monoubiquitination. bre1Δ cells harboring either pADH-BRE1 or pADH-bre1ΔRING were analyzed as in (A).

(C) Determination of H3 K4 methylation in selected wild-type and mutant strains. (Upper panel) Shown is an immunoblot using anti-H4 methyl-K4 antibodies of extracts from strains of the indicated genotypes fractionated by SDS-PAGE. (Lower panel) Reprobing of the blot in the upper panel with antibodies to Pgk1.

(D) Bre1 RING domain is essential for H3 K4 methylation. bre1Δ cells harboring either pADH-BRE1 or pADH-bre1ΔRING were analyzed as in (A).

(E) Determination of H3 K79 methylation in selected wild-type and mutant strains. (Upper panel) Shown is an immunoblot using anti-H4 methyl-K79 antibodies of extracts from strains of the indicated genotypes fractionated by SDS-PAGE. (Lower panel) Reprobing of the blot in the upper panel with antibodies to Pgk1.

eliminated (Figure 3A). In contrast, cells lacking the RING finger proteins Ubr1, Rad5, or Rad18, which have been shown previously to act with Rad6, display no defect in H2B monoubiquitination (Figure 3A). Cells harboring a BRE1 allele containing a truncation of 54 codons corresponding to the C-terminal RING domain (bre1ΔRING) are also defective in ubiquitination, consistent with the known requirement of RING domains for activity of this family of ubiquitin ligases (Figure 3B).

A large-scale protein complex purification study has previously identified two polypeptides associated with Bre1: Lge1 and Yhr149c (Ho et al., 2002). Interestingly, lge1Δ displays synthetic lethality with htz1Δ (Figure 1; our unpublished data). Lge1 has a strongly predicted coiled-coil domain at its C terminus, which may mediate its interaction with Bre1 (our unpublished data). We therefore examined H2B ubiquitination in lge1Δ cells. As shown in Figure 3A, the lge1Δ mutant is defective in H2B ubiquitination, suggesting that Bre1 functions as part of a multiprotein complex.

As in other species, H3 K4 methylation in S. cerevisiae by the Set1 complex is associated with transcriptionally active chromatin (Bernstein et al., 2002; M. D. Meneghini, M. Wu, and H.D.M., submitted). Since Rad6-dependent monoubiquitination of histone H2B has been demonstrated to be required for H3 K4 methylation (Dover et al., 2002; Sun and Allis, 2002), we predicted that Bre1 and Lge1 should likewise be important for methylation of H3 K4. SDS-PAGE fractionation and immunoblotting of whole-cell extracts using antibodies specific for H3 methyl-K4 revealed a robust band corresponding to methyl-K4 H3 in wild-type cells (Figure 3C). As predicted, bre1Δ mutants, like rad6Δ mutants, were completely defective in H3 K4 methylation. Moreover, the bre1ΔRING allele also abolished H3 K4 methylation (Figure 3D), whereas cells lacking Lge1 showed greatly reduced methylation (Figure 4A). Finally, we examined whether bre1Δ and lge1Δ are required for histone H3 K79 methylation, which has recently been shown to require H2B monoubiquitination (Briggs et al., 2002; Ng
et al., 2002). As with K4 methylation, *bre1*Δ cells lack methylation of K79, whereas *ige1*Δ cells display a partial defect in K79 methylation that is weaker than their defect in K4 methylation (Figure 3E). The residual methylation present in *ige1*Δ cells could be due to a very low amount of residual H2B monoubiquitination (below our detection limit) in the *ige1*Δ mutant, or possibly, Lge1 could play an inhibitory role in methylation that is overcome by ubiquitination.

The *ige1*Δ mutant was originally identified in a large-scale screen for mutants with defective cell size control (Jorgensen et al., 2002). Cells lacking *LGE1* display a large cell phenotype. Inspection of the published genome-wide dataset revealed that *bre1*Δ mutants also display a large cell phenotype. To confirm these data, we examined the population size distributions of wild-type, *bre1*Δ, and *lge1*Δ cells. As controls, we examined mutants displayed a median and mean size that was intermediate between those of wild-type and H2B monoubiquitination-defective mutants (Figure 4A). Thus, the large cell phenotype of *bre1*Δ, *lge1*Δ, and H2B-K123R mutants can only partially be explained by a loss of H3 K4 methylation.

We have identified Bre1 as a RING finger protein required for monoubiquitination of histone H2B on K123 and methylation of histone H3 on K4. We also show that the Bre1-associated protein Lge1 is required for normal levels of H2B ubiquitination and H3 K4 methylation, suggesting that both proteins are components of a multisubunit E3. Although we cannot rule out the formal pos-
sibility that Rad6 and Bre1-Lge1 indirectly activate an unknown ubiquitin ligase that promotes H2B monoubiquitination, their specific requirement in vivo for H2B ubiquitination and the necessity for the Bre1 RING domain for the modification are most simply explained by a direct role. However, demonstration of H2B-specific in vitro ubiquitin ligase activity of the Bre1 complex in a purified system will be necessary to show that it functions as an E3. As noncatalytic subunits of ubiquitin ligase complexes can serve a regulatory function (Carroll and Morgan, 2002), we speculate that Lge1 may function to modulate the activity of the complex in response to regulatory inputs and/or to control substrate selection.

Our analysis of cell size distributions of mutants lacking histone H2B monoubiquitination revealed an unexpected role for this chromatin modification in cell size control. Moreover, our finding that blocking H3 K4 methylation by deletion of genes encoding essential components of the H3 K4 methylase only partially recapitulates the cell size defects of bre1Δ and lge1Δ mutants indicates that H2B monoubiquitination has a role in size control independent of its function in H3 K4 methylation. Methylation of H3 on K79 has been recently shown to require H2B monoubiquitination (Briggs et al., 2002; Ng et al., 2002) and may explain the intermediate cell size phenotype of cells lacking K4 methylation. This requirement for H2B monoubiquitination for size control could reflect an effect on the cell cycle transcriptional program. An intriguing alternative model would be a role for H2B monoubiquitination which is independent of transcription; one possibility is that cells measure the modified histone content of chromatin as part of the still-mysterious biochemical calculation of cell size which is used to time critical transitions in the cell cycle such as START. Such a model would be consistent with the known dependence of cell size on chromosome ploidy that has been established in organisms from yeast to man (Gallitski et al., 1999; Su and O’Farrell, 1998).

Both BRE1 and LGE1 are essential for cell viability in cells lacking the conserved histone variant H2A.Z, and they are required for H3-K4 methylation, a modification associated with active chromatin. Moreover, we have recently shown that H2A.Z promotes the formation of active chromatin (M.D. Meneghini, M. Wu, and H.D.M., submitted). We speculate that Rad6, Bre1, and Lge1 act upstream of H3 K4 methylation in a pathway parallel to that of H2A.Z which also functions to establish and/or maintain the euchromatic state. Our identification of homologs of Bre1 in diverse eukaryotic species suggests that the fundamental elements of the histone-based regulatory circuitry described in this and other recent studies have been conserved during evolution.

Experimental Procedures

Yeast Strains
S. cerevisiae strains used in this study are listed in Supplemental Table S1 at http://www.molecule.org/cgi/content/full/11/1/261/DC1.

Yeast Methods
Standard procedures were used for cultivation and genetic manipulations (Guthrie and Fink, 2002). Strains were of the S288C background. Knockouts were obtained from the Yeast Deletion Consortium collection (Research Genetics), and their genotypes are indicated in the figure legends.
References


