doi:10.1016/j.jmb.2006.08.073 J. Mol. Biol. (2006) 363, 660–672

JMB

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blm3-1 Is an Allele of *UBP3*, a Ubiquitin Protease that Appears to Act During Transcription of Damaged DNA

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Yeast Blm10 and mammalian PA200 proteins share significant sequence similarity and both cap the ends of 20 S proteasomes and enhance degradation of some peptide substrates. Blm10 was identified as a suppressor of the yeast *blm3-1* mutation, and initially was thought to be the Blm3 protein. Both the *blm3-1* and *blm10*-Δ mutations were reported to cause sensitivity to bleomycin and other forms of DNA damage, suggesting a role for Blm10/PA200–proteasome complexes in DNA repair. We have been unable to observe significant DNA damage sensitivity in *blm10*-Δ mutants in several genetic backgrounds, and we have therefore further investigated the relationship between *BLM10* and *blm3-1*. We find that *blm3-1* is a nonsense mutation in the ubiquitin protease gene *UBP3*. Deleting *UBP3* causes phenotypes similar to those caused by *blm3-1*, but neither causes a general defect in DNA repair. Ubp3 has several known functions, and genetic interaction data presented here suggest an additional role in transcriptional elongation. The phenotypes caused by *blm3-1* and *ubp3*-Δ mutations are not suppressed by over-expression of *BLM10*, nor are they affected by deletion of *BLM10*. These results remove key components of the previously reported connection between Blm10/PA200–proteasome complexes and DNA repair, and they suggest a novel way to interpret sensitivity to bleomycin as resulting from defects in transcription elongation.

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Keywords: Blm3; Blm10/PA200; Ubp3; transcription; bleomycin

Introduction

Bleomycin is a toxin that binds to DNA and to iron, and can cause DNA damage by producing reactive oxygen species near genomic DNA.[1] Bleomycin has been used successfully as a chemotherapeutic agent in humans.[1] As part of an effort to understand how bleomycin damages cells and how cells respond to this damage, the yeast *Saccharomyces cerevisiae* was used to identify mutations that cause sensitivity to bleomycin.[2] A plasmid carrying a portion of the gene now known as *BLM10* was reported to suppress the bleomycin sensitivity of one of these mutants, *blm3-1*, and deletion of the *BLM10* gene was reported to cause hypersensitivity to killing by the related toxin phleomycin.[3] It was therefore concluded that *BLM10* was the gene affected by the *blm3-1* mutation.[3]

PA200 is an approximately 200 kDa proteasome activator protein that was purified from mammalian cells by assaying the ability to increase the rate of digestion of some peptide substrates by the 20 S proteasome.[4] The gene encoding human PA200 was identified, and the protein sequence was found to share significant similarity with yeast Blm10.[4] Several lines of evidence indicate that the physiological role of PA200/Blm10 protein family members involves regulation of 20 S proteasome activity. Both mammalian PA200 and yeast Blm10 bind to the ends of proteasome barrels and activate their ability to digest some peptide substrates.[4–7] High-throughput screens for interacting proteins indicated that Blm10 protein is physically associated with proteasome subunits in yeast cell extracts.[8,9] It has been reported that Blm10 is a component of nascent proteasomes, and that deletion of *BLM10* causes a delay in proteasome assembly.[10] However, the binding of PA200 and Blm10 to intact proteasomes indicates that if Blm10

Abbreviations used: MMS, methylmethane sulfonate; ORF, open reading frame; HU, hydroxyurea; 6-AU, 6-azauracil.

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acts during proteasome biogenesis it also has a function that involves the mature complexes. The ties between PA200/Blm10 proteins and proteasomes, together with the reported defects in repair associated with yeast *blm10* mutants, suggested that this conserved family of proteins regulates proteasome function in a way that is important to allow repair of damage induced by agents like bleomycin.[4]

Consistent with a role in DNA repair, PA200 was found to localize to nuclear foci in response to DNA damage in human cells,[4] and transcription of *BLM10* in yeast was found to increase in response to exposure to the alkylating agent methylmethane sulfonate (MMS) or to any of several oxidizing agents that can damage DNA (summarized in the *Saccharomyces* Genome Database)†. Further, the *blm3-1* mutation that was reported to be suppressed by over-expression of *BLM10* was itself reported to cause sensitivity to MMS, ionizing radiation, and hydrogen peroxide.[2,3] However, in our tests with several genetic backgrounds, deleting *BLM10* did not cause significant sensitivity to any of several DNA-damaging agents, including MMS, hydrogen peroxide and bleomycin.[5] Further, the level of sensitivity that others have documented for a *blm10*-Δ deletion strain is small compared with the sensitivity displayed by the original *blm3-1* strain.[7] Finally, *BLM10* has not been identified in any of the other numerous screens for DNA repair factors in yeast.[11–13] These results suggest that the function of the conserved PA200/Blm10 family may not be related to a general DNA repair mechanism.[14] Consistent with this interpretation, mice lacking PA200 were found to have normal capacity to repair double-strand breaks.[15]

In the absence of a direct phenotype linking *BLM10* to a significant DNA repair defect, the principal remaining evidence suggesting a role for Blm10–proteasome complexes in DNA repair is the reported suppression of *blm3-1* by over-expression of Blm10 protein.[3] We have therefore investigated the nature of the *blm3-1* mutation and its interaction with *BLM10* as part of our analysis of the physiological role of Blm10. Here, we report that *blm3-1* is an allele of the ubiquitin protease gene *UBP3*. Ubp3 has been implicated in several processes, including protein degradation, vesicular sorting, transcriptional silencing, and transcriptional initiation.[16–23] Data presented here support an additional function for Ubp3 in transcriptional elongation. These results suggest that the *blm3-1* mutation may not cause sensitivity to bleomycin because of a defect in a standard DNA repair pathway, but because of a defect in transcription, possibly in the process that allows recovery from stalling of RNA polymerase II at sites of bleomycin-induced damage. Further, we are unable to observe suppression of the phenotypes caused by either *blm3-1* or *ubp3*-Δ mutations by over-expression of *BLM10*. The function of Blm10/PA200 therefore remains unclear, but this factor does not appear to be required for a general DNA damage repair pathway.

† http://www.yeastgenome.org/

Results

BLM10 encodes a 2143 residue protein

A database search revealed similarity of the N-terminal region of human PA200 to the *Saccharomyces cerevisiae* gene *YFL007w*, and similarity of the C-terminal region of human PA200 to *YFL006w*.[4] As *YFL007w* and *YFL006w* are adjacent to one another, this pattern of similarity suggested that these two open reading frames (ORFs) might be separated by a sequencing error. To investigate this, we amplified the region between the two putative ORFs by PCR and checked the genomic DNA sequence. We and others found that a single base deletion error was responsible for a frameshift (Figure 1).[4,24,25] When corrected, the *YFL007w-YFL006w* region encodes a 2143 residue protein with homology to the full-length human PA200 protein (Figure 1).[4,24,25] *YFL007w* had been called *BLM3*,[3] but this region was subsequently found to have the wild-type sequence in a *blm3-1* mutant.[24] The fusion of *YFL007w* and *YFL006w* was therefore renamed *BLM10*.[24]

blm3-1 is not associated with *BLM10*

In contrast to the initial report,[3] we found that deleting *BLM10* did not cause DNA damage sensitivity in three strain backgrounds we tested,[5] and others found that it caused only about a fivefold decrease in viability on medium containing bleomycin.[7] This level of sensitivity is much lower than initially reported for a *blm3-1* strain.[2] We therefore obtained the *blm3-1* mutant CM1469-5C described previously for comparison.[3] As reported, the *blm3-1* strain was very sensitive to phleomycin (Figures 1 and 2) as well as to bleomycin (see Figure 4 below).

To examine the relationship between *blm3-1* and *BLM10*, we first performed genetic crosses to determine whether the genes are linked. CM1469-5C (*blm3-1*) was mated to a strain from the A364a genetic background carrying a deletion of *BLM10* marked with the *TRP1* gene, and the haploid segregants were tested for various phenotypes after sporulation and dissection. Sensitivity to bleomycin and phleomycin was variable among the haploids obtained, suggesting segregation of multiple mutations affecting this phenotype. However, tighter sensitivity to both agents co-segregated in two of the spores from each tetrad. As sensitivity to bleomycin was the primary reason for naming the mutation *blm3-1*, we followed sensitivity to bleomycin and phleomycin in subsequent crosses as an indicator of the *blm3-1* mutation. If *blm3-1* was an allele of *BLM10*, bleomycin sensitivity should have segregated away from the *blm10*-Δ(::*TRP1*) marker in all cases. Instead, both the tight phleomycin