Conformational exchange at a C_2H_2 zinc-binding site facilitates redox sensing by the PML protein

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INTRODUCTION

The promyelocytic leukemia protein (PML) plays a vital role in response to various types of cellular stress, including oxidative stress. Within the nucleus, PML is enriched within spherical structures called PML nuclear bodies (PML-NBs) (Fig. 1A). These PML-NBs recruit numerous, diverse partner proteins and modulate their activity through various means. In response to oxidation, PML multimerizes and forms mesh-like structures that serve as the outer shell of PML nuclear bodies. Multimerization occurs through intermolecular disulfide bonds formed between the side chains of cysteine residues, which are highly enriched within three zinc-binding domains of PML (Fig. 1B). PML multimerization is the key event in formation of NBs; however, its molecular mechanism remains poorly understood. Here we identify the B-box1 domain of PML as highly redox-sensitive and use NMR studies of molecular dynamics to connect the enhanced oxidation susceptibility of this domain to conformational exchange within its C_2H_2-type zinc-coordinating site. Our results suggest a role for B-box1 in redox sensing by the PML protein.

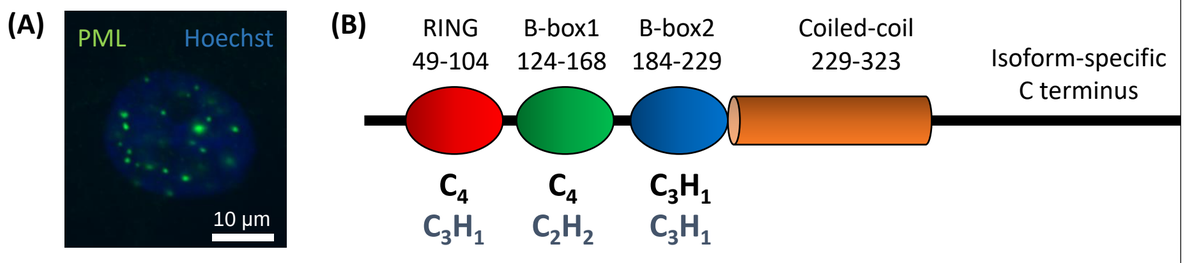

Fig. 1: (A) Immunofluorescence image of PML-NBs within the nucleus. (B) An illustration of the domain architecture of PML. The RING, B-box1, and B-box2 domains each coordinate two zinc ions through a set of four cysteine and histidine residues per zinc. Numbers above each domain indicate boundaries. Text below the zinc-binding domains lists the composition of the residues that coordinate each zinc ion.

RESULTS

B-box1 is more oxidation-prone than RING

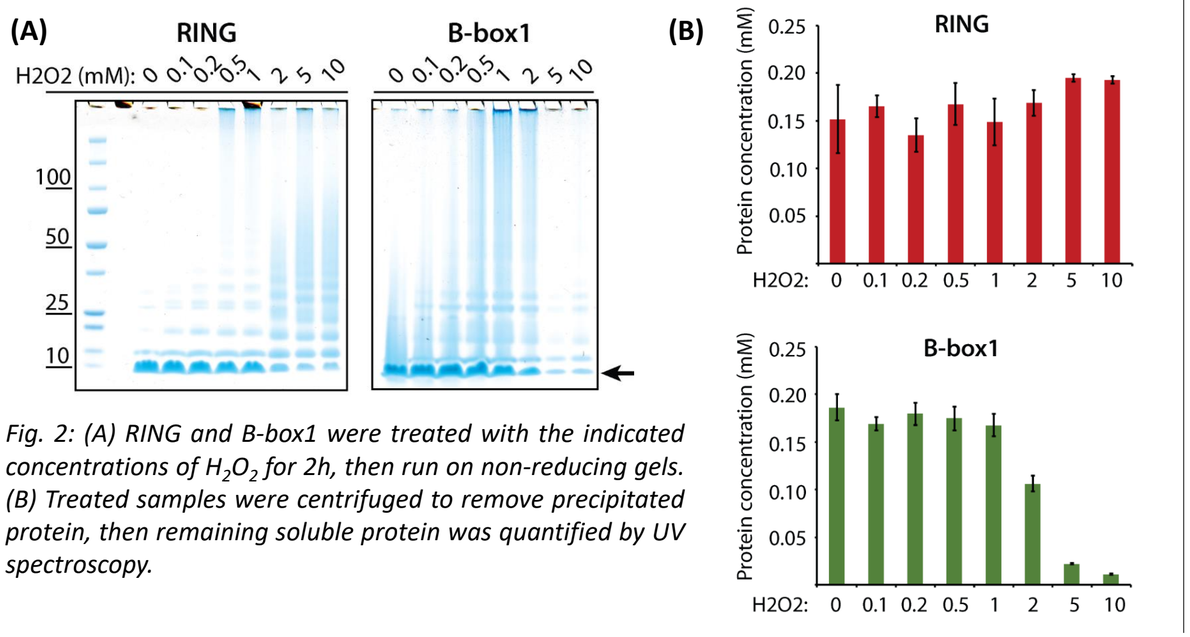

Fig. 2: (A) RING and B-box1 were treated with the indicated concentrations of H_2O_2 for 2h, then run on non-reducing gels. (B) Treated samples were centrifuged to remove precipitated protein, then remaining soluble protein was quantified by UV spectroscopy.

The isolated B-box1 domain is highly rigid, with flexibility limited to the termini

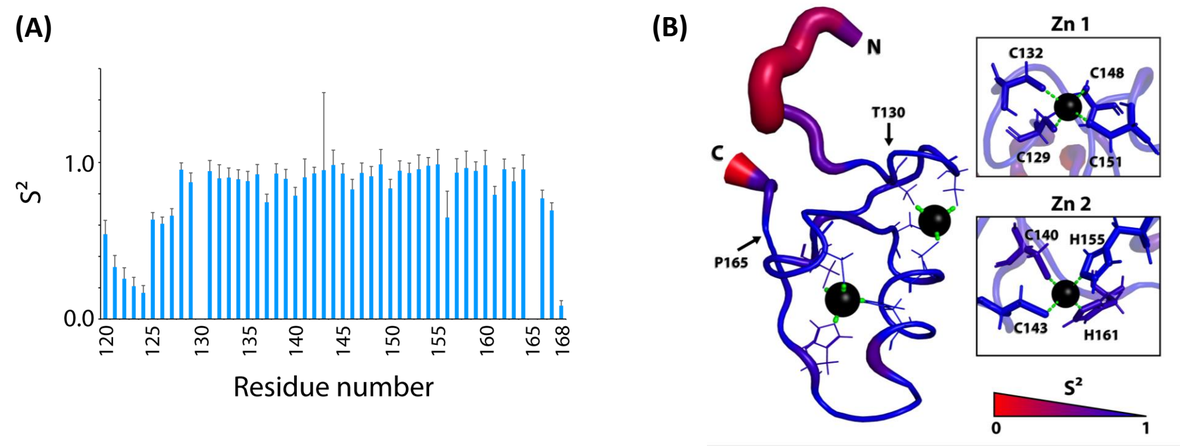

Fig. 3: (A) Per-residue order parameters (S^2) determined from R_1 and R_2 relaxation rates and heteronuclear NOE experiments. (B) Structural depiction of flexible residues within B-box1. Insets show side chains of cysteine and histidine residues involved in zinc coordination.

A loop within the C_2H_2 zinc-coordinating site undergoes conformational exchange

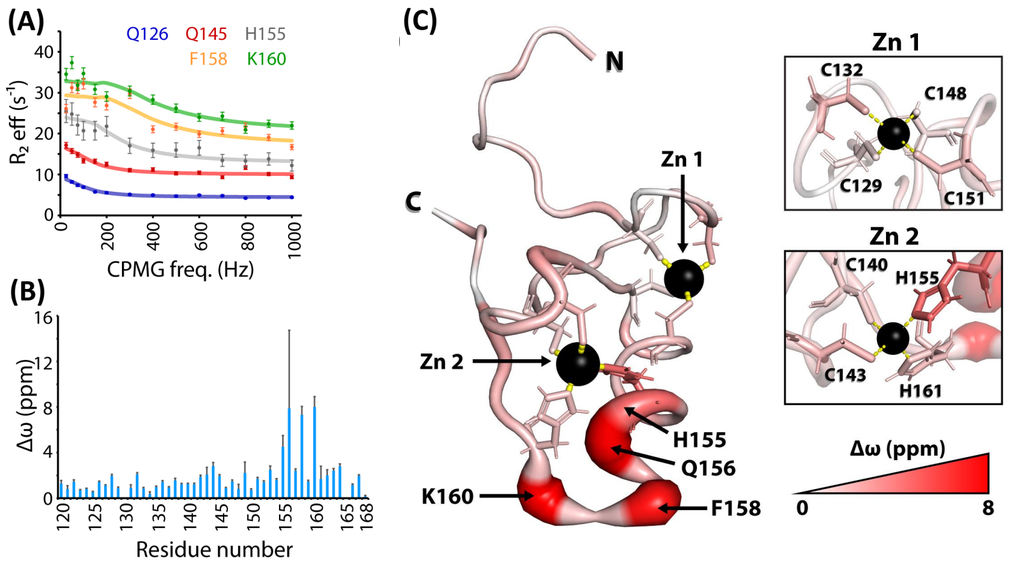

Fig. 4: (A) Dispersion profiles of selected B-box1 residues. (B) Calculated chemical shift differences (Δω) between B-box1 major and minor states by residue. (C) Structural depiction of Δω values highlights a region of conformational exchange around the C_2H_2 site.

CONCLUSIONS AND FUTURE DIRECTIONS

Here we have demonstrated that B-box1 is a highly redox-sensitive domain of PML and used NMR dynamics methods to reveal conformational exchange around its C_2H_2-type zinc-coordinating site. We propose that the observed exchange leads to loss of coordination by H155 and subsequent zinc release, thus exposing the side chains of C140 and C143 for intermolecular disulfide formation. Future studies will examine whether the mode of coordination of zinc impacts the stability and redox susceptibility of this site.

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