The Role of Small Ubiquitin-like Modifiers and SUMO-Interacting Motifs in Replication Stress

Jack Hedberg, Yee Mon Thu, Tianji Zhang, LeeAnn Higgins, Sue Van Riper, and Anja-Katrin Bielinsky

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, 55455; Center for Mass Spectrometry and Proteomics, University of Minnesota, Saint Paul, MN, 55108; Massachusetts General Hospital Cancer Center, Harvard Medical School, Charlestown, MA 02129.

Abstract

Accurate DNA replication is essential for genome stability. The successful creation of new, healthy cells relies on proper function of replication protein complexes, which must have effective mechanisms for both recognizing and responding to replication stress. Replication stress arises from many different sources, but can be broadly defined as the slowing or halting of replication forks1. One way replication proteins chemically communicate is through post-translational modifications (PTMs) in which their functions are altered by the attachment and removal of chemical groups or small peptide chains. Attachment of Small Ubiquitin-like Modifiers (SUMOs) to target proteins is one of the most characterized PTMs, with SUMOylation occurring commonly in multiple organisms including yeast and mammalian cells. Delineation of the functional significance of SUMOylation in these species is ongoing, including discovery of SUMO-interacting motifs (SIMs) in SUMO conjugates and their roles in the interaction of SUMO-modified proteins with SUMOylation targets. Here we review the current status of SUMOylation in replication stress and direct our attention to the functional significance of SUMOylation in replication stress signaling, using studies conducted in the Bielinsky Laboratory and elsewhere as examples. The most notable question is how SUMOylation can facilitate cells progressing through replication stress, and future testing will explore this mechanism in more detail.

Introduction

DNA replication requires prolonged timing and coordination of many protein complexes2. At the origin of replication, enzymes separate double-stranded DNA, stabilize the single-stranded DNA, and proteins downstream synthesize new complementary strands for each single strand. When the structure of a DNA or replication protein is aberrantly altered in a way that compromises the quality of DNA replication, replication stress is present. A variety of cellular processes, including cell cycle, gene expression, and metabolism, are affected. For example, replication stress caused by chemical agents (e.g., ionizing radiation, ultraviolet light) or infection (e.g., HIV) results in DNA damage that can be repaired by DNA repair pathways to respond to replication fork stalling and stress9, they do have their limitations, and unintended changes in the DNA can still transpire through all subsequent cell divisions. If these genetic changes occur within pro-oncogenes, uncontrolled cell growth and eventually cancer may result10-13.

Here replication stress was continuously present in yeast cells due to a mutation in the subunit mcm10-1, which contains mutations that denature the protein and render it nonfunctional at 37°C. The role of replication stress was continually present in yeast cells due to a mutation in the subunit mcm10-1, which contains mutations that denature the protein and render it nonfunctional at 37°C. The strain used throughout the Bielinsky Laboratory containing mutations in mcm10-1 was amplified through PCR. Gel electrophoresis confirms which samples have integrated the strain containing mutations in mcm10-1. The four SIMs of N4 were already known, and N4F was used as a positive control.

Differential SUMOylation of proteins in wild-type vs. mcm10-1 mutants indicates SUMOylation protects genome integrity

The second step recommended in the bioinformatical SIM detection publication17 was testing the conservation of potential SIMs throughout various species. The rationale was that highly conserved SIMs are more likely to be in evolutionarily conserved stages of protein subunits of chromosome passenger complex. Creating opportunity for further laboratory testing, understanding the functional significance of SUMOylation in CPC subunits can provide insights into how SUMO regulates cell cycle progression and diverse cellular processes.

Figure 3. (A) West: CPC is composed of the subunits Brf1, Npl4, Sim1, and Brr1. Human analogues of each subunit are written in red. It is hypothesized that yeast CPC complex is a pathway which decreases SUMOylation of Brf1 and Sim1, thus preventing formation of CPC complexes.

Figure 4. (A) Mass spectrometry work conducted by the Bielinsky Laboratory indicates that many proteins are differentially SUMOylated between mutant and wild-type strains used throughout the Bielinsky Laboratory containing mutations in mcm10-1. The four SIMs of N4 were already known, and N4F was used as a positive control.

Future Directions

• Further laboratory testing of potential SIMs of CPC subunits in both Saccharomyces cerevisiae and Homo sapiens can be conducted.

Bioinformatics predicts several potential SIMs in Saccharomyces cerevisiae CPC subunits

Some SIMs in human CPC subunits contain conserved cysteine residues, which are rendered nonfunctional when denatured at 37°C. Potential SIMs were used to design a primer in PCR to amplify potential SIMs in human genomic DNA. Gel electrophoresis confirms which samples have integrated the primer into human genomic DNA. The primer used to integrate potential SIMs into human genomic DNA was amplified through PCR. Gel electrophoresis confirms which samples have integrated the primer into human genomic DNA. Integrated potential SIMs were noted.

Bioinformatical SIM detection

• Further laboratory testing of potential SIMs of CPC subunits in both Saccharomyces cerevisiae and Homo sapiens can be performed on other differentially SUMOylated proteins in mcm10-1 mutants.

References


Figure 5. (A) A flowchart illustrating how bioinformatical SIM detection can be conducted. The first step recommended in the bioinformatical SIM detection publication17 was testing the conservation of potential SIMs throughout various species. The rationale was that highly conserved SIMs are more likely to be in evolutionarily conserved stages of protein subunits of chromosome passenger complex. Creating opportunity for further laboratory testing, understanding the functional significance of SUMOylation in CPC subunits can provide insights into how SUMO regulates cell cycle progression and diverse cellular processes.

Bioinformatics predicts several potential SIMs in Saccharomyces cerevisiae CPC subunits

• Further laboratory testing of potential SIMs of CPC subunits in both Saccharomyces cerevisiae and Homo sapiens can be conducted.

Bioinformatical SIM detection

• Further laboratory testing of potential SIMs of CPC subunits in both Saccharomyces cerevisiae and Homo sapiens can be performed on other differentially SUMOylated proteins in mcm10-1 mutants.

References


Figure 5. (A) A flowchart illustrating how bioinformatical SIM detection can be conducted. The first step recommended in the bioinformatical SIM detection publication17 was testing the conservation of potential SIMs throughout various species. The rationale was that highly conserved SIMs are more likely to be in evolutionarily conserved stages of protein subunits of chromosome passenger complex.