# 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

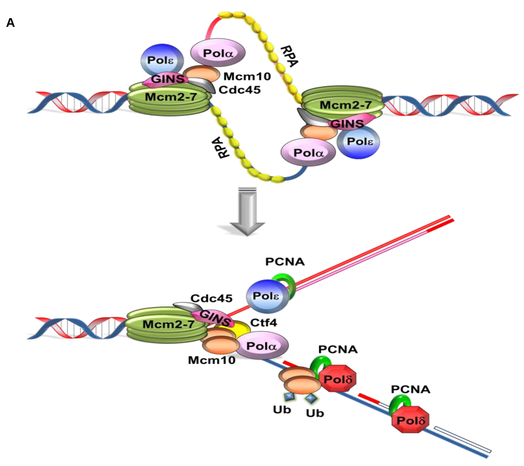
Accurate DNA replication is essential for genome stability. The successful creation of new, healthy cells relies on proper function of replication protein complexes, which must have effective methods for both recognizing and responding to replication stress. Replication stress arises from many different sources, but can be broadly defined as the slowing or stalling of replication forks[1]. 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 a type of PTM. SUMO chains are "read" or recognized by SUMO-Interacting Motifs, and SUMO-SIM interaction can promote the formation of stable protein complexes[2]. Previous work of Yee-Mon Thu, Ph.D., and other members of the Bielinsky Laboratory has shown that SUMOylation is required for the survival of *Saccharomyces cerevisiae* cells containing mutations of their Mcm10 replication proteins, which creates a steady source of replication stress within the cells[3]. Of particular interest was the finding that protein subunits of a complex called chromosome passenger complex (CPC) had different levels of SUMOylation between *mcm10-1* mutant cells and wild type cells. CPC is responsible for regulating mitosis[4], and we hypothesized that CPC subunits contain SIMs and SUMO consensus sequences. To test this hypothesis, bioinformatics software was used to find potential SIMs on protein subunits of yeast CPC based on primary structures, and mutants of one particular CPC subunit called Bir1 are currently being studied. Additionally, we used bioinformatics to predict several potential SIMs in *Homo sapiens* CPC, creating opportunity for further laboratory testing. Understanding the functional significance of SIMs in CPC subunits can provide insights into how SUMO regulates cell cycle progression and diverse cellular processes.

## Introduction

DNA replication requires profound timing and coordination of many protein complexes[5-8]. At the origin of replication, enzymes separate double-stranded DNA, stabilize the single-stranded DNA, and then proteins downstream synthesize new complementary strands for each single strand. When the structure of DNA or replication proteins is abnormally altered in a way that compromises the quality of DNA replication, replication stress is present. A wide variety of both internal and external agents are capable of inducing replication stress, including free radicals, ionizing radiation, and mutations to DNA. One example is ultraviolet radiation penetrating through biological tissue and creating abnormal bonds between adjacent thymine bases. These structures cannot fit into the catalytic pockets of replication proteins, and thus cause replication fork stalling. While there are several highly evolved DNA repair pathways to respond to replication fork stalling and stress[9], they do have their limits, and unintended changes in the DNA can still transferred through all subsequent cell divisions. If these genetic changes occur within proto-oncogenes, uncontrolled cell growth and eventually cancer may result[10-13].

Here replication stress was continually present in yeast cells due to a mutation in the replication protein Mcm10. Mcm10 is required for replication initiation and elongation of DNA strands[14-16], and the *mcm10-1* strain used throughout the Bielinsky Laboratory contains mutations that denature the protein and render it nonfunctional at 37°C.

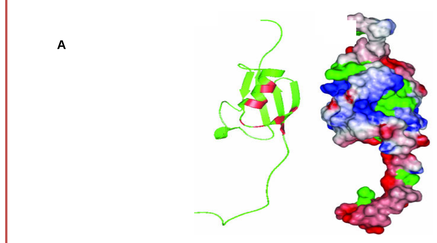
## Mcm10 facilitates DNA replication initiation and elongation



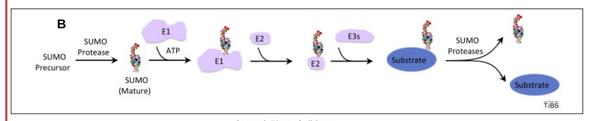

Thu and Bielinsky, *Semin Cell Dev Biol.* 2014

Figure 1. **(A)** DNA replication requires the coordination of many proteins. At the origin of replication, double-stranded DNA is split into single strands. On the leading strand, DNA complementary to the single parent strand is synthesized by polymerase epsilon, and on the lagging strand it is synthesized by polymerase delta. Mcm10 can be seen regulating the unwinding of DNA, and it is thought to orchestrate DNA synthesis as well. *mcm10-1* mutants are not able to regulate DNA unwinding or adequate timing of polymerase recruitment, and it follows that this is a source of replication stress.

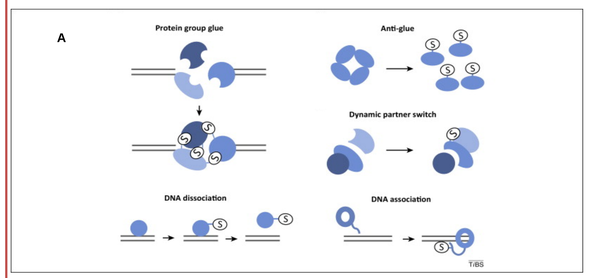
## SUMOylation is required for the survival of *mcm10-1* mutants




Song, Durrin, Wilkinson, Krontiris, Chen, *PNAS.* 2004




Sarangi, Zhao, *Cell Press.* 2015




Sarangi, Zhao, *Cell Press.* 2015

Figure 2. **(A)** Small Ubiquitin-like Modifier proteins are represented in ribbon and molecular diagrams. **(B)** Conjugation of SUMO onto substrate proteins involves a pathway of three enzyme classes called E1, E2, and E3. SUMOs are conjugated onto substrates via the interaction of E2/E3 complexes with consensus sequences on substrates.

Figure 3. **(A)** SIMs are regions on proteins which hydrogen-bond with specific SUMO chain sequences on target proteins. SUMO-SIM interactions mediate a diverse array of protein interactions, and SUMOylation is required for survival in *mcm10-1* mutants.

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

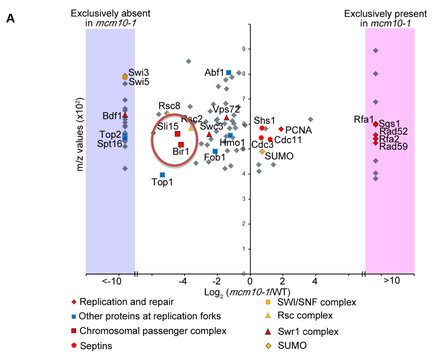



Figure 4. **(A)** Mass spectrometry work conducted by the Bielinsky Laboratory indicates that many proteins are differentially SUMOylated between *mcm10-1* mutants and wild type *Saccharomyces cerevisiae*. Among these proteins are Sli15 and Bir1 (circled in red), two subunits of chromosome passenger complex.

## Decreased SUMOylation of CPC potentially prevents cell cycle progression in mcm10-1

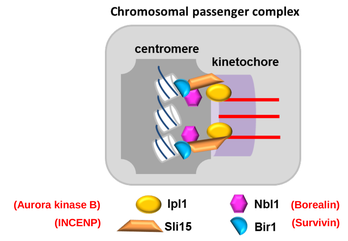



Figure 5. **(A)** Yeast CPC is composed of the subunits Ipl1, Nbl1, Sli15, and Bir1. Human analogues of each subunit are written in red. It is hypothesized that yeast CPC complex formation is mediated by SUMO-SIM interaction, and that replication stress somehow triggers a pathway which decreases SUMOylation of Bir1 and Sli15, thus preventing formation of CPC.

## Bioinformatics predicts several potential SIMs in *Saccharomyces cerevisiae* CPC subunits

A

| Protein Name | Position | Sequence | Score |
|---|---|---|---|
| Bir1 | | | |
| | 149 - 153 | SNPDDEN VINLR KFTFQDN | 53.283 |
| | 308 - 312 | DYNEKTD ISVIQ HNISVLD | 54.142 |
| | 315 - 319 | ISVIQHN ISVLD GAQGENV | 62.665 |
| | 423 - 427 | GDNKDKD LVIDF TSHIIKN | 52.999 |
| | 528 - 532 | GRDSSTN ILIRT QIVDQNL | 55.961 |
| | 723 - 727 | FTNKQET IKILF DVSVKNE | 59.449 |
| | 769 - 773 | EEFSGKE LDIPI DSSTVEI | 53.947 |
| | 780 - 784 | IDSSTVE IKKVI KPEFEPV | 51.131 |
| | 921 - 925 | IDDCDKK LDILR RDYYTAT | 51.262 |
| Sli15 | | | |
| | 400 - 404 | KLTTHKK LAIIA EQKKKSK | 51.94 |
| | 511 - 515 | KNYRLTN LQLLP PAEAERD | 51.294 |
| Ipl1 | | | |
| | 240 - 244 | ILIGFNN VIKLT DFGWSII | 52.366 |
| | 332 - 336 | SQDAQDL ILKLL KYDPKDR | 52.429 |
| Nbl1 | | | |
| | 43 - 47 | KEETLKK LNLLQ QPDATSA | 50.179 |

Figure 6. **(A)** The first step recommended in a publication outlining techniques for bioinformatical detection of SIMs[17] was to run primary protein structures through SIM-detection software[18,19], and this was done with each of the CPC subunits by Yee Mon Thu, Ph.D. Scores of potential SIM sequences were assigned based on similarity to the 151 known SIMs integrated into the software's database, and scores of 50.000 or higher were noted. Each potential SIM sequence is highlighted in blue, and flanking regions are non-highlighted.

## Bir1's highest score predicted SIM is being tested through mutation and sequencing

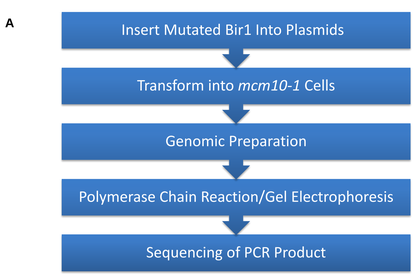



Figure 7. **(A)** Laboratory procedures being used to test a potential SIM on Bir1 positions 315-319. Bir1 with mutations in these positions is inserted into plasmids and transformed into *mcm10-1* cells. The DNA of these cells is isolated, and the endogenous Bir1 region of the cells is amplified through PCR. Gel electrophoresis confirms which samples have integrated the mutated Bir1 into their endogenous regions, and sequencing confirms the presence of the mutation in the SIM.

## Bioinformatics predicts several SIM candidates in *Homo sapiens* CPC subunits

A

| Protein Name | Protein Accession Number | Position | Sequence | Score |
|---|---|---|---|---|
| RNF4 | P78317.1 | | | |
| | | 36 - 40 | ISLEAEP IILVE TAGDEIV | 65.339 |
| | | 46 - 50 | VLETAGDE IVEE CILLEPV | 64.336 |
| | | 57 - 61 | TCESLEP VVVDG THNDSVV | 67.924 |
| | | 67 - 71 | DLTHNDS VVIVD ERRRPRR | 65.335 |
| | | 148 - 152 | EIVQNGR LIVST ECGHVFC | 59.091 |
| | | | | |
| INCENP | Q9NQS7.3 | | | |
| | | 13-Sep | GTTAPGP IILLE LCDQKLM | 54.632 |
| | | 31 - 35 | CNMDNKD LVWLE TIQEEAE | 63.442 |
| | | 168 - 172 | TQCQLVP IVIEQ ISERQNA | 60.001 |
| | | 792 - 796 | GASKALN VVVDV QSPACTS | 58.852 |
| | | | | |
| Aurora Kinase B | Q96GD4.3 | | | |
| | | 39 - 43 | EPVTPSA IVLMS RSNVQPT | 51.583 |
| | | 102 - 106 | RFKKSHP IVALK VLFKSQI | 56.229 |
| | | 137 - 141 | AHLHHPN LRLV NFFYDRR | 55.089 |
| | | 150 - 154 | VFYDRRR IVLE LYAPRGE | 53.55 |
| | | 266 - 270 | RVVLCVP LVVEH PFFESAS | 51.268 |
| | | 286 - 290 | NETYRRI VNVDL KFPASVP | 50.185 |
| | | | | |
| Borealin | NP_001243804.1 | | | |
| | | 32 - 36 | LKDFDRE VEIRI KQIESDR | 59.317 |
| | | 58 - 62 | DNLYNIE IRLP KALREMN | 54.247 |
| | | 227 - 231 | PLADSKE IFLTV PVGGGES | 52.501 |

Figure 8. **(A)** Potential SIM sites were identified on human analogs of yeast CPC subunits (highlighted in blue), and scores of 50.000 or higher were noted. No potential SIMs were predicted on survivin, the human analog of Bir1. The four SIMs of RNF4 were already known, and RNF4 was used as a positive control.

## Multiple sequence alignment shows SIM conservation across several organisms

A

**BOREALIN Predicted SIM #1: Score of 59.317**

| | | | |
|---|---|---|---|
| Human: | LKDFDRE | VEIRI | KQIESDR |
| Frog: | IKDFDSQ | VKLIT | EEMKASV |
| Mouse: | LKDFDRE | VQVRT | KQIESDR |
| Budding Yeast: | LHRMQLE | LETTE | KLIENIK |
| Fly: | LISIDSL | ETKFI | SAVDHQV |
| Fission Yeast: | LENLAFE | LSDKV | RRLRSNA |

**INCENP Predicted SIM #1: Score of 63.442**

| | | | |
|---|---|---|---|
| Human: | CNMDNKD | LVWLE | EIQEEAE |
| Frog: | RTLDSKH | MVWLL | EIEEEAR |
| Mouse: | CNVDNKD | FMWLK | EIEEEAE |
| Budding Yeast: | TTDAHSE | INWLR | NNVYMNT |
| Fly: | RKAHFEE | LD_G | QPEAEAK |
| Fission Yeast: | TNGKMKE | FFWLN | EHMLEVS |

**INCENP Predicted SIM #2: Score of 60.001**

| | | | |
|---|---|---|---|
| Human: | TQCQLVP | VVEIQ | ISERQNA |
| Frog: | _____NA | QVKIS | EQERRSA |
| Mouse: | _____A | VVEIS | TSERLSA |
| Budding Yeast: | _____ | _____ | LRESSKT |
| Fly: | _____P | SLNRK | MRRPSSE |
| Fission Yeast: | SFVTLPK | REEI_ | LKRPASL |

Figure 9. **(A)** The second step recommended in the bioinformatical SIM detection publication[17] was testing the conservation of potential SIMs throughout various species. The rationale was that highly conserved SIMs are more likely to exist in nature. A multiple alignment of two Borealin and one INCENP potential SIM was performed using MUSCLE software[20], and the potential SIM sites are highlighted in blue. The third step of bioinformatical SIM detection relies on the fact that functional SIMs tend to lie in disordered regions of proteins and towards the surface, so this step involves using software to determine where potential SIMs lie in terms of a particular protein's 3-dimensional structure. This step is currently being carried out.

## Future Directions

- Further laboratory testing of potential SIMs of CPC subunits in both *Saccharomyces cerevisiae* and *Homo sapiens* can be conducted.
- Bioinformatical detection of SIMs and SUMO consensus sequences can be performed on other differentially SUMOylated proteins in *mcm10-1*.

## References

1) Watts, F. Z. DNA Repair (Amst). 2006, 5, 399–403.
2) Sarangi, P.; Zhao, X. Trends Biochem. Sci. 2015, 40, 233–242.
3) Thu, Y. M.; Bielinsky, A.-K. Semin. Cell Dev. Biol. 2014, 30, 121–130.
4) Ruchaud, S.; Carmena, M.; Earnshaw, W. C. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 798–812.
5) Takisawa, H.; Mimura, S.; Kubota, Y. *Curr. Opin. Cell Biol.* **2000**, *12*, 690–696.
6) Aparicio, O. M.; Weinstein, D. M.; Bell, S. P. Cell 1997, 91, 59–69.
7) Waga, S.; Stillman, B. Annu. Rev. Biochem. 1998, 67, 721–751.
8) Masai, H.; Matsumoto, S.; You, Z.; Yoshizawa-Sugata, N.; Oda, M. *Annu. Rev. Biochem.* **2010**, *79*, 89–130.
9) Liu, G.; Myers, S.; Chen, X.; Bissler, J. J.; Sinden, R. R.; Leffak, M. *J. Biol. Chem.* **2012**, *287*, 33412–33423.
10) Dietlein, F.; Reinhardt, H. C. *Clin. Cancer Res.* **2014**, *20*, 5882–5887.
11) Greenblatt, M. S.; Bennett, W. P.; Hollstein, M.; Harris, C. C. **1994**, 4855–4878.
12) Jackson, A. L.; Loeb, L. A. *Mutat. Res. Mol. Mech. Mutagen.* **2001**, *477*, 7–21.
13) Loeb, L. A.; Loeb, K. R.; Anderson, J. P. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 776–781.
14) Alver, R. C.; Zhang, T.; Josephrajan, A.; Fultz, B. L.; Hendrix, C. J.; Das-Bradoo, S.; Bielinsky, A.-K. *Nucleic Acids Res.* **2014**, *42*, 8389–8404.
15) Chattopadhyay, S.; Bielinsky, A.-K. *Mol. Biol. Cell* **2007**, *18*, 4085–4095.
16) Ricke, R. M.; Bielinsky, A.-K. Mol. Cell 2004, 16, 173–185.
17) Vogt, B.; Hofmann, K. *Ubiquitin Family Modifiers and the Proteasome*; Dohmen, R. J., Scheffner, M., Eds.; Methods in Molecular Biology; Humana Press: Totowa, NJ, 2012; Vol. 832.
18) Ren, J.; Gao, X.; Jin, C.; Zhu, M.; Wang, X.; Shaw, A.; Wen, L.; Yao, X.; Xue, Y. *Proteomics* **2009**, *9*, 3409–3412.
19) Zhao, Q.; Xie, Y.; Zheng, Y.; Jiang, S.; Liu, W.; Mu, W.; Liu, Z.; Zhao, Y.; Xue, Y.; Ren, J. *Nucleic Acids Res.* **2014**, *42*, W325–W330.
20) Edgar, R. C. *Nucleic Acids Res.* **2004**, *32*, 1792–1797.