Understanding the role of sumoylation in mitotic progression

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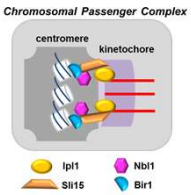
Abstract

Ensuring the integrity of genome across multiple generations is vital for the success of all organisms. Cells have evolved diverse molecular mechanisms that resist deleterious changes at the nucleotide level, such as point mutations, as well as at the structural level, such as chromosome loss or translocation. One such molecular mechanism is protein sumoylation, a process in which concerted action of E1, E2 and E3 ligases facilitates the covalent linkage of small ubiquitin-like modifiers (SUMO) peptide to target proteins. In Saccharomyces cerevisiae, Siz1, Siz2 and Mms21 are the only three E3 SUMO ligases known to date. Functional deficiency of each ligase renders yeast cells sensitive to DNA damaging agents or prone to chromosome instability, implicating the general role of sumoylation in intracellular communication when genome integrity is compromised. Yet, how sumoylation regulates the function of specific proteins remains less understood. Our study dissects the effect of Mms21-dependent sumoylation on mitosis. In S. cerevisiae, Bir1 and Sli15, subunits of chromosomal passenger complex (CPC) have been reported to be potential targets of Mms21. During mitosis, CPC activates the spindle assembly checkpoint (SAC) until bi-orientation is achieved. Proper amount of tension between two sister chromatids serves as a molecular indicator of successful bi-orientation. Lack of tension may result from incorrect attachment of mitotic spindles to the chromosome or unresolved replication intermediates resulting from DNA damage accumulation. Bir1 is thought to sense tension, or the lack thereof, subsequently regulating the SAC. Intriguingly, a decrease in sumoylation of Bir1 or Sli15 coincides with SAC deactivation when replication stress ensues. The existing evidence led us to hypothesize that Mms21-dependent sumoylation of Bir1 or Sli15 regulates SAC activation when the genome integrity is challenged. To test this hypothesis, we generated SUMO mutants of *BIR1* in which Bir1 is constitutively sumoylated or cannot be sumoylated (named *BIR1-SuOn* or *BIR1-SuOff*, respectively). Our preliminary data suggests that, when treated with a DNA damaging agent, methyl methane sulfonate (MMS), these mutants do not exhibit any sensitivity. Similarly, these mutants do not show any sensitivity to microtubule disrupting agents, such as benomyl or nocodazole. Intriguingly, this lack of sensitivity is in agreement with the lack of sensitivity of *bir1* temperature sensitive mutants to the same agents. Our long-term plan is to determine if sumoylation of CPC complex has a role in regulation of mitosis.

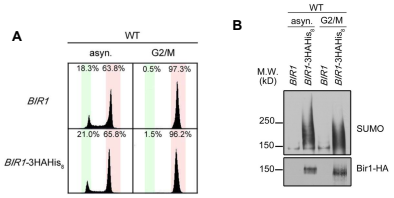
Bir1 is a subunit of Chromosomal Passenger Complex (CPC) that has been shown to be sumoylated



Modified from Carmena M, *et al.*, *Nat. Rev. Mol. Cell Bio.* 2012.

Figure 1. Chromosomal passenger complex, composed of Bir1, Sli15, Ipl1 and Nbl1, regulates kinetochore attachment at centromeres during mitosis in *Saccharomyces cerevisiae*. Bir1 and Sli15 have been shown to be sumoylated in *S. cerevisiae* (Montpetit *et al.*, 2006; Albuquerque *et al.*, 2013; Thu *et al.*, 2016).

Bir1 is sumoylated in G2/M phase



Thu *et. al.*, Cell Reports., 2016.

Figure 2. Bir1 is sumoylated in G/2M phase. **(A)** Wild-type cells with 3HAHis8 epitope tag at the endogenous locus of *BIR1* were treated with α-factor (150ng/ml) for 3 hrs to synchronize them in G1 phase. After 3 hrs, cells were grown in YPD at 35°C for 75 mins. Up to this time point, the majority of the population move synchronously to G2/M. **(B)** Cells from either asynchronous or G2/M populations were collected and subjected to purification of Bir1 via the His epitope tag, followed by immunoblotting with SUMO antibody or HA antibody. (Figures taken from Thu *et. al.*, 2016.)

Sumoylated Bir1 is reduced in the presence of replication stress

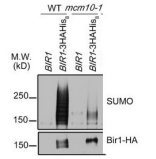


Figure 3. Sumoylation of Bir1 in a replication mutant *mcm10-1*. Bir1 tagged with His and HA were purified from indicated strains grown at 33°C. Eluates from these purifications were immunoblotted with anti-SUMO or HA antibody. (Modified from Thu *et al.*, 2016)

Modified from Thu *et. al.*, Cell Reports., 2016.

Generation of *BIR1* SUMO mutants

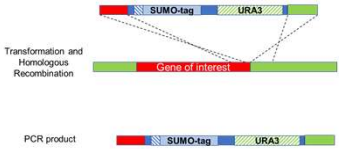


Figure 4. SUMO mutants of *BIR1* were generated by integration of DNA fragment carrying SUMO-tag and a selectable marker *URA3* gene. Depending on whether SUMO-tag will mimic constitutive sumoylation or deficiency in sumoylation of Bir1, they are named SuOn or SuOff, respectively. A DNA fragment carrying a SUMO-tag was generated by using PCR and reagents described in Wei and Zhao, 2016. This strategy was first described in Almedawar *et al.*, 2012.

bir1 and *ipl1* mutants are not sensitive to a DNA damaging agent, MMS

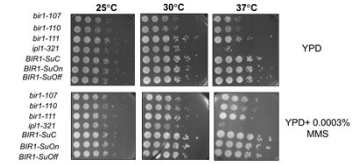


Figure 5. The effect of MMS on *bir1* and *ipl1* mutants. Successive 10-fold serial dilutions of the indicated strains were spotted on YPD **(A)** or YPD+0.0003% MMS **(B)** plates and grown at indicated temperatures for two days.

bir1 and *ipl1* mutants are not sensitive to agents that disrupt microtubule dynamics

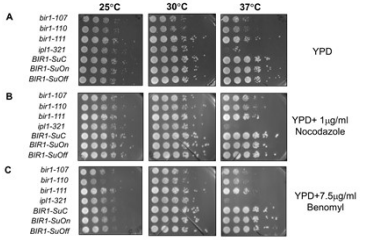


Figure 6. The effect of nocodazole and benomyl on *bir1* and *ipl1* mutants. Successive 10-fold serial dilutions of the indicated strains were spotted on YPD **(A),** YPD+1μg/ml nocodazole **(B)** or YPD+ 7.5μg/ml benomyl plates **(C)** and grown at indicated temperatures for two days.

BIR1-SuOn modification does not rescue *mms21-CH* phenotypes

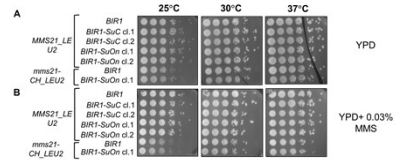


Figure 7. The effect of MMS on *mms21-CH* or *mms21-CH BIR1-SuOn* mutants. Successive 10-fold serial dilutions of the indicated strains were spotted on YPD **(A)** or YPD+0.03% MMS **(B)** plates and grown at indicated temperatures for two days.

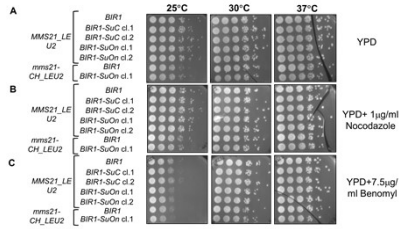


Figure 8. The effect of nocodazole and benomyl on *mms21-CH* or *mms21-CH BIR1-SuOn* mutants. Successive 10-fold serial dilutions of the indicated strains were spotted on YPD **(A),** YPD+1μg/ml nocodazole **(B)** or YPD+ 7.5μg/ml benomyl plates **(C)** and grown at indicated temperatures for two days.

Discussion

It has been reported that Bir1 is subject to sumoylation however, it is unclear how sumoylation affects Bir1's function (Montpetit *et al.*, 2006; Albuquerque *et al.*, 2013; Thu *et al.*, 2016). In order to address this question, we generated SUMO mutants of Bir1 and compared their phenotypes to other well characterized *bir1* mutants and *ilp1* mutant.

Temperature sensitive *bir1* mutants or *ipl1* mutants do not show sensitivity to 0.0003% of MMS or 1ug/ml of nocodazole (Figures 5A and B). Similarly, most of these mutants are not sensitive to benomyl. Only *bir1-110* mutants exhibited slight sensitivity to benomyl (Figure 5B). Both *bir1-107* and *ipl1-321* mutants have defects in chromosome bi-orientation and tension checkpoint (Shimogawa *et al.*, 2009). Although these mutants have defect in mitosis, they exhibit little or no sensitivity to agents that interfere with microtubule dynamics. This implies that optimal function of mitotic checkpoint is not necessary to resist microtubule disrupting agents, especially at low concentrations. SUMO mutants of *BIR1* are similar to *bir1-107* and *ipl1-321* mutants in their drug sensitivity. Due to this similarity, the possibility that SUMO mutants of *BIR1* may have partial defect in mitotic checkpoint regulation still cannot be ruled out. Lack of drug sensitivity in some CPC mutants precludes the possibility of using this phenotypic characteristic as a surrogate marker for mitotic checkpoint defect.

It is interesting to note that *mms21-CH* mutants exhibit moderate growth defect even in the absence of any genotoxic drugs (Figures 7 and 8). These mutants do not show any significant sensitivity to MMS, nocodazole or benomyl at least at the concentrations that were used in this study (Figures 7 and 8). Furthermore, *mms21-CH Bir1-SuOn* mutants phenocopy *mms21-CH* mutants (Figures 7 and 8). This observation suggests two possibilities: 1) Bir1 is not sumoylated continuously in *mms21-CH Bir1-SuOn* mutants as intended by the genetic modification; 2) the growth defect in *mm21-CH* mutants is not due to lack of Bir1 sumoylation. Biochemical studies to determine the sumoylation status of Bir1 in Bir1 SUMO mutants are necessary to distinguish these two possibilities.

References

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