Biochemical and Biophysical Research Communications 438 (2013) 746–752

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The functional role of SUMO E3 ligase Mms21p in the maintenance of subtelomeric silencing in budding yeast

Yakun Wan [a,*,1], Xiao Zuo [a,1], Ya Zhuo [a], MIn Zhu [a], Samuel A. Danziger [b], Zikai Zhou [a]

[a] The Key Laboratory of Developmental Genes and Human Disease, Ministry of Education, Institute of Life Sciences, Southeast University, Nanjing 210096, China
[b] Institute for Systems Biology, Seattle, WA 98109, United States

ARTICLE INFO

Article history:
Received 19 July 2013
Available online 31 July 2013

Keywords:
Telomere silencing
E3 ligase
H3K4 tri-methylation
SIR complex

ABSTRACT

In *Saccharomyces cerevisiae*, subtelomeric silencing is involved in the propagation of Silent Information Regulator (SIR) proteins toward euchromatin. Numerous mechanisms are involved in antagonizing the local spread of Sir-dependent silent chromatin into neighboring euchromatin. Here, we identified a novel role for sumoylation E3 ligase Mms21 in the maintenance of subtelomeric silencing. We found that disruption of E3 ligase activity of Mms21 results in the de-repression of subtelomeric silencing. Deletion of E3 ligase domain of Mms21 led to decreased binding of Sir2p, Sir3p and Sir4 at subtelomeric chromatins and increased H3K4 tri-methylation at telomere-distal euchromatin regions, correlating with increased gene expression in two subtelomeric reporter genes. In addition, a *mms21Δsl* mutant caused a severe growth defect in combination with *htz1Δ* deletion and showed an enhanced association of Htz1 with telomere proximal regions. Taken together, our findings suggest an important role of Mms21p; it contributes to subtelomeric silencing during the formation of a heterochromatin boundary.

1. Introduction

Eukaryotic genomes are packaged into either lightly packed, transcriptionally active euchromatin or denser, transcriptionally inactive heterochromatin [1,2]. This separation into two types of chromatin provides a unique mechanism where gene expression can be regulated by chromatin organization and both chromatin types have significant roles in gene expression and genomic stability [3]. Heterochromatin is important not only for chromosome integrity but also is involved in various biological processes, for example cell proliferation and differentiation, as well as development and aging [4]. The molecular components that appear to regulate the spreading of heterochromatin involve histone modifications, DNA methylation and nucleosome remodeling [5,6]; however, the underlying mechanisms are still under active investigation.

The yeast *Saccharomyces cerevisiae* is a highly tractable model organism for investigating the molecular mechanisms underlying heterochromatin establishment and maintenance. Establishment of heterochromatin-like chromatin in yeast is mediated by direct recruitment of the Sir complex (silent information regulators) to DNA elements termed "silencers". Sir2 NAD^+-dependent histone deacetylase activity located at these silencers eliminate acetyl groups from adjoining histone H4 tails, which in turn facilitates the recruitment of additional Sir complexes. Iterative cycles of histone deacetylation and Sir complex binding then expands heterochromatin over several kilobases of DNA [7,8].

The combined work of numerous studies has begun to uncover the molecular mechanisms that regulate how the propagation of heterochromatin into euchromatin is antagonized. The yeast SAS (Something About Silencing) complex and the histone variant H2A.Z have been linked to an antisilencing function at subtelomeric regions [9]. A recent study has also established a functional role for histone H4K12 acetylation at telomeric heterochromatin [10]. Set1p-mediated H3K4 methylation and Dot1p-mediated H3K79 methylation have been identified as specific epigenetic marks for the organization and maintenance of telomeric heterochromatin [11,12]. Our previous studies also demonstrated that histone chaperone Chz1 is involved in subtelomeric silencing by regulating H2B ubiquitination [13,14]. Although significant progress has been made in the identification of regulators of heterochromatin maintenance, the mechanisms underlying the subtelomeric silencing still remain largely unknown.

The covalent protein modification SUMO (small ubiquitin-like modifier) has been shown to be a key regulatory post translational modification in various biological processes [15,16]. In budding yeast, sumoylation of several telomeric proteins has been found to regulate telomere length. PIAS-like SUMO E3 ligase Siz2 can sumoylate both Yku70/80 and Sir4, facilitating telomere attaching to the nuclear periphery [17]. In higher organisms, the Mms21 SUMO ligase can sumoylate multiple telomere-binding proteins, which is a key mechanism in the maintenance of telomere length

* Corresponding author. Fax: +86 25 83790970.
E-mail address: ywansystemsbiology@gmail.com (Y. Wan).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.07.096

[18]. While intriguing, it remains unclear if there is a functional link between subtelomeric silencing and sumoylation.

Considering that sumoylation is involved multiple telomere processes, we sought to test if sumoylation is also involved in the regulation of heterochromatin spreading. The *S. cerevisiae* genome encodes four known E3 ligases: Siz1, Siz2, Mms21 and Zip3. Of these, global SUMO modification levels in budding yeast are primarily controlled by Siz1, Siz2 and Mms21 [17]. Therefore, to determine which SUMO E3s are involved in subtelomeric silencing, we examined mutations to the non-essential Siz1 and Siz2 and the essential Mms21. In this study, we have uncovered a previously uncharacterized role for the E3 ligase Mms21p in the maintenance of subtelomeric silencing. Defects in subtelomeric silencing seen in *mms21* mutants appear to be linked to increased levels of histone H3K4 tri-methylation at subtelomeric regions, which results in the redistribution of SIR proteins. Moreover, we present evidence that Mms21 mediates enhanced association of Htz1 with subtelomeric regions. Overall, this study reveals a novel role of the E3 ligase Mms21p in heterochromatin formation and maintenance.

2. Materials and methods

2.1. Yeast strains and growth conditions

The yeast strains used in this study are listed in Table S1. Yeast strains were genetically manipulated according to the one-step PCR-mediated recombination as previously described [13,14]. Yeast transformations were performed using a lithium acetate method [19]. The *mms21*Δ*sl* mutant allele was created by integrative yeast transformation with a fragment from plasmid pRR71 cutting by PvuII and XbaI.

2.2. Growth assay

To assay the growth of different yeast strains, cells were grown in YPD or synthetic complete medium lacking the appropriate amino acids to OD_{600}∼1. 8 μl of 5-fold serial dilutions of each cell suspension was spotted on the appropriate plates. The plates were incubated at 25 °C, 30 °C, 33 °C and 37 °C for 2–5 days and photographed.

2.3. Quantitative reverse transcription PCR (qRT-PCR)

Total RNA was prepared from exponentially growing yeast cells in appropriate mediums by using the Trizol RNA isolation protocol. mRNAs of individual genes were analyzed using the Applied Biosystems 7300 Fast systems and Fast start universal SYBR Green Master (Roche). A list of primers used for the qRT-PCR is in Table S2.

2.4. Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed as described previously with some modification [20]. ChIP samples were then analyzed by quantitative real-time PCR using the Applied Biosystems 7300 Fast systems. The sequences of primers used in ChIP experiments are shown in Table S2. The occupancy level of each protein at target DNAs was determined by dividing the relative abundance at telomeric DNA by the relative abundance of an internal control. All ChIP experiments were performed in triplicate. Error bars represent standard deviation.

2.5. Histone H3K4 tri-methylation analysis

Yeast whole cell extracts were prepared as previously described with minor modifications [13,14]. Briefly, about 5×10^8 cells from log-phase cultures were harvested by centrifugation and then lysed in 300 μl of lysis buffer (50 mM HEPES, pH7.9, 2 mM EDTA, 0.25 M NaCl, 0.1% SDS, 0.1% sodium deoxycholate, 1% Triton X-100 and 1× Protease Inhibitor Cocktail (PIC)) by vortexing with acid-washed glass beads. Equal amount of proteins were immunoblotted with the H3K4 tri-methylation antibody.

2.6. FACS analysis

Cells were grown in YPD to a cell density of $\sim 1 \times 10^7$ cells/ml. A FACS assay was performed as described previously [21]. For each sample, fluorescence intensities of 10,000 cells were measured using a FACS Caliber flow cytometer. Data were analyzed with WinMDI 2.8 (at http://FACS.scripps.edu/).

3. Results

3.1. The *mms21Δsl* mutant exhibits de-repression of subtelomeric silencing

Several studies have demonstrated that heterochromatin-proximal genes can switch between active and inactive states in a heritable manner [22]. To test if E3 ligases are important for the spreading of heterochromatin, quantitative RT-PCR was employed to quantitate mRNA levels of two genes that have previously been shown to be susceptible to heterochromatic position effects. We examined mutants of the nonessential E3 ligases Siz1 and Siz2 and a mutant *mms21Δsl* allele, which lacks the RING domain at the C-terminus of the essential E3 ligase Mms21p. Compared to wild-type cells, *siz2*Δ mutant cells have no detectable effect on the expression levels of both telomere-proximal and mating type-adjacent reporter genes. YIR042c expression increased by 2.5-fold in the *siz1*Δ mutant and further increased in the siz1Δ siz2Δ double mutant. Interestingly, YIR042c expression was substantially higher in the *mms21Δsl* mutant than in the *siz1*Δ *siz2*Δ double mutant. Compared to *YIR042c*, there is no significant difference in the expression of mating type proximal reporter *GIT1* in both *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants (Fig. 1A, B). Similar to *YIR042c*, we also observed the de-repressing effect in the *mms21*Δ*sl* mutant on another reporter gene *YFR057w*, which is located in the vicinity of the right telomeres of chromosome VI (Fig. 1C). To better characterize the effect of *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants on subtelomeric silencing, we analyzed the expression of a reporter gene in these strains, which has integrated with a gene encoding Ura3-GFP located in a subtelomeric region adjacent to chromosome end III-R [23]. FACS analysis suggested that both *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants can lead to the enhanced fluorescence intensity compared with a wild-type strain (Fig. 1D). Together, these data collectively indicate that sumoylation E3 ligases are involved in the regulation of transcription with telomere proximal genes.

3.2. Genetic interaction between E3 ligase and SIR genes

Genetic interaction analysis has been showed to be an important approach for assessing gene function *in vivo*. We focused on the *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants, which show significant induction of *YIR042c* and *YFR057w* compared to wild-type. The *mms21*Δ*sl* mutant, which lacks the E3 ligase domain, is slow growing when compared with wild-type cells at 30 °C. When shifted to 37 °C, growth of the*mms21*Δ*sl* mutant is severely reduced (Fig. 1E). Interestingly, Sir3 and Sir4 deletions in *siz1*Δ *siz2*Δ mutants result in no significant change compared to the *siz1*Δ *siz2*Δ mutant itself at 30 °C. We also tested the effect of overexpression of SIR2, SIR3 and SIR4 on growth defects in the *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants. As shown in Fig. 1F, while overexpression of Sir2, Sir3 and Sir4 does not affect growth of wild- type cells, increased

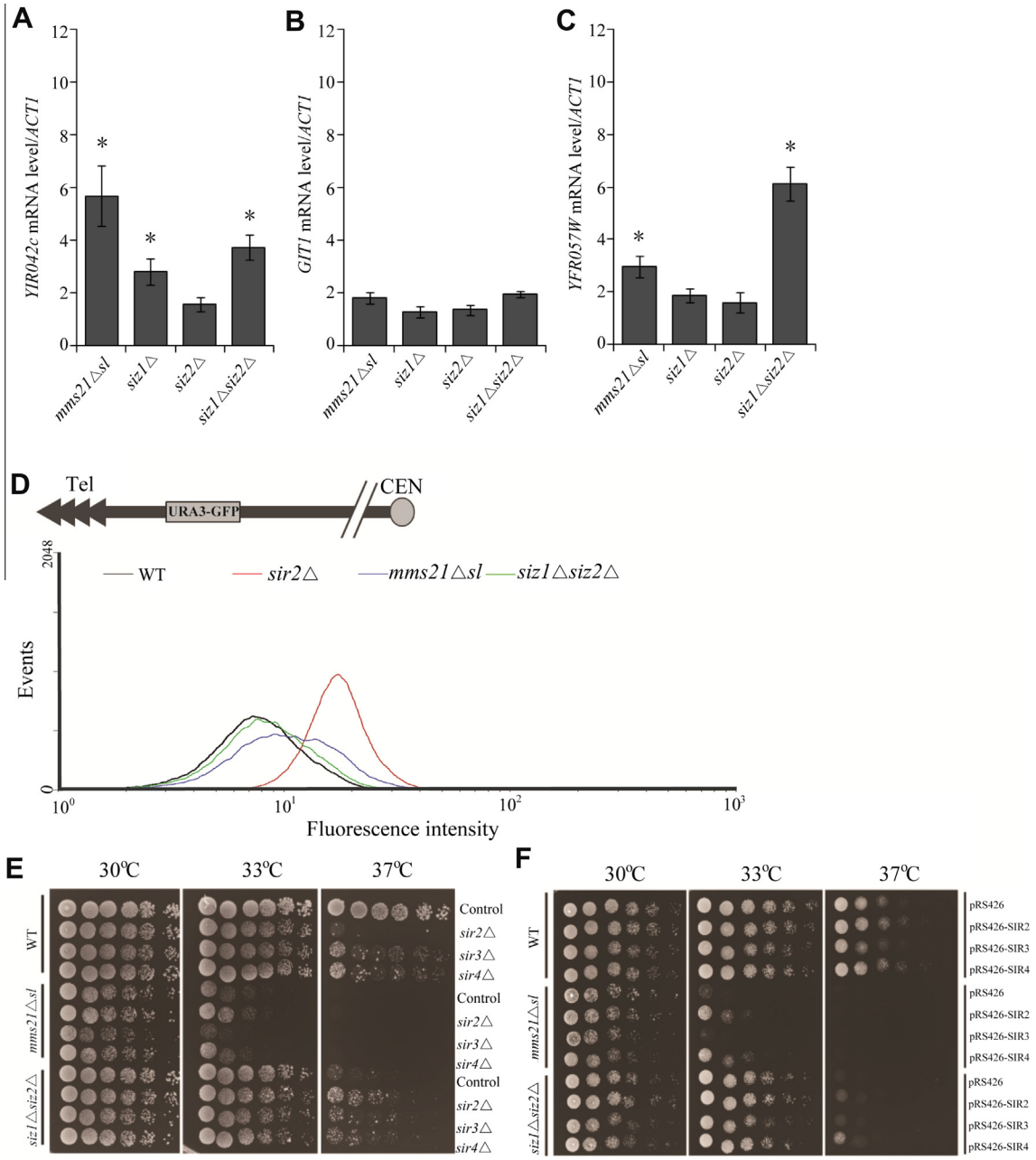

Fig. 1. Mms21 E3 ligase activity is required for subtelomeric silencing. mRNA levels of three reporter genes, *YIR042c* (A), *GIT6* (B) and *YFR057w* (C), were determined by qRT-PCR. mRNA level of *ACT1* was used as a loading control for normalization and mRNA level/*ACT1* in the different mutants has been normalized with the wild-type strain. Errors bars represent standard deviations from the means of three independent experiments replications. Strains integrated with a reporter Ura3–GFP, which is located to the subtelomeric loci, were used to test the effects of individual mutant on subtelomeric silencing by FACS analysis (D). Fivefold serial dilutions of each yeast strains were spotted on either YPD plates (E) or SC-his plates (F) at 30 °C, 33 °C and 37 °C and photographed. Asterisks mark statistically significant results (p-value $\leqslant 0.05$).

dosage of Sir2 and Sir4 partially rescues growth defects observed in *mms21Δsl* at 33 °C and in *siz1Δ siz2Δ* mutants at 37 °C. Together, these results clearly indicate that E3 ligases can genetically interact with SIR complex components.

3.3. Abolishment of SUMO E3 ligase alters the distribution of SIR complex at subtelomeric regions

To investigate whether Mms21 regulates subtelomeric silencing through interactions with subtelomeric chromatin, we immunoprecipicated Mms21p and examined its association with subtelomeric DNA; However, our ChIP RT-PCR failed to detect stable association of Mms21p with subtelomeric chromatin (Figure S2). We then hypothesized that induction of *YIR042c* and *YFR057w* in *mms21Δsl* or *siz1Δ siz2Δ* mutants may be involved in the redistribution of the SIR complex. To test this hypothesis, we measured the association of Rap1, Sir2, Sir3 and Sir4 to 32 subtelomeric loci, which cover 16 chromosomes and 32 subtelomeric regions. Rap1p, Sir2p, Sir3p and Sir4p association at 7.7 kb from telomere 6R toward euchromatin appeared to be at background levels, consistent with our previous report [13,14]. In the *siz1Δ siz2Δ* mutant Rap1p binding to 23 telomere proximal regions did not show a significant defect compared with wild-type. In the *mms21Δsl* mutant, 15 out of 32 subtelomeric loci showed the decreased binding of Rap1p (Fig. 2). The association of Sir2p with subtelomeric chromatin in the *mms21Δsl* mutant was dramatically decreased in 31 out of

32 subtelomeric loci (Fig. 2). The severe defect of association of Sir3p and Sir4p across 32 subtelomeric chromatin was also observed in the *mms21Δsl* mutant (Fig. 2). The impaired binding of Sir2p and Sir3p with subtelomeric chromatin in the *mms21Δsl* mutant does not appear to be a result of transcriptional decrease in *SIR* genes as we did not observe altered transcript levels of *SIR2*, *SIR3*, *SIR4* and *RAP1* (Figure S1). All together, these results demonstrate that *mms21Δsl* and *siz1Δ siz2Δ* mutants have different mechanisms for disturbing the binding of Rap1p, Sir2p, Sir3p and Sir4p to subtelomeric chromatin.

3.4. H3K4. tri-methylation is increased in *mms21Δsl* mutants

Sas2-mediated H4K16, Set1-mediated H3K4 methylation, and Dot1-mediated H3K79 are required for the organization and maintenance of specialized chromatin regions like subtelomeric DNA [11,12]. Disruption of these histone modifications changes the distribution of SIR proteins and impairs subtelomeric silencing. We examined these three major histone modifications in two subtelomeric regions, which contained two reporter genes, to illuminate any specific defects imparted by the *mms21Δsl* or *siz1Δ*

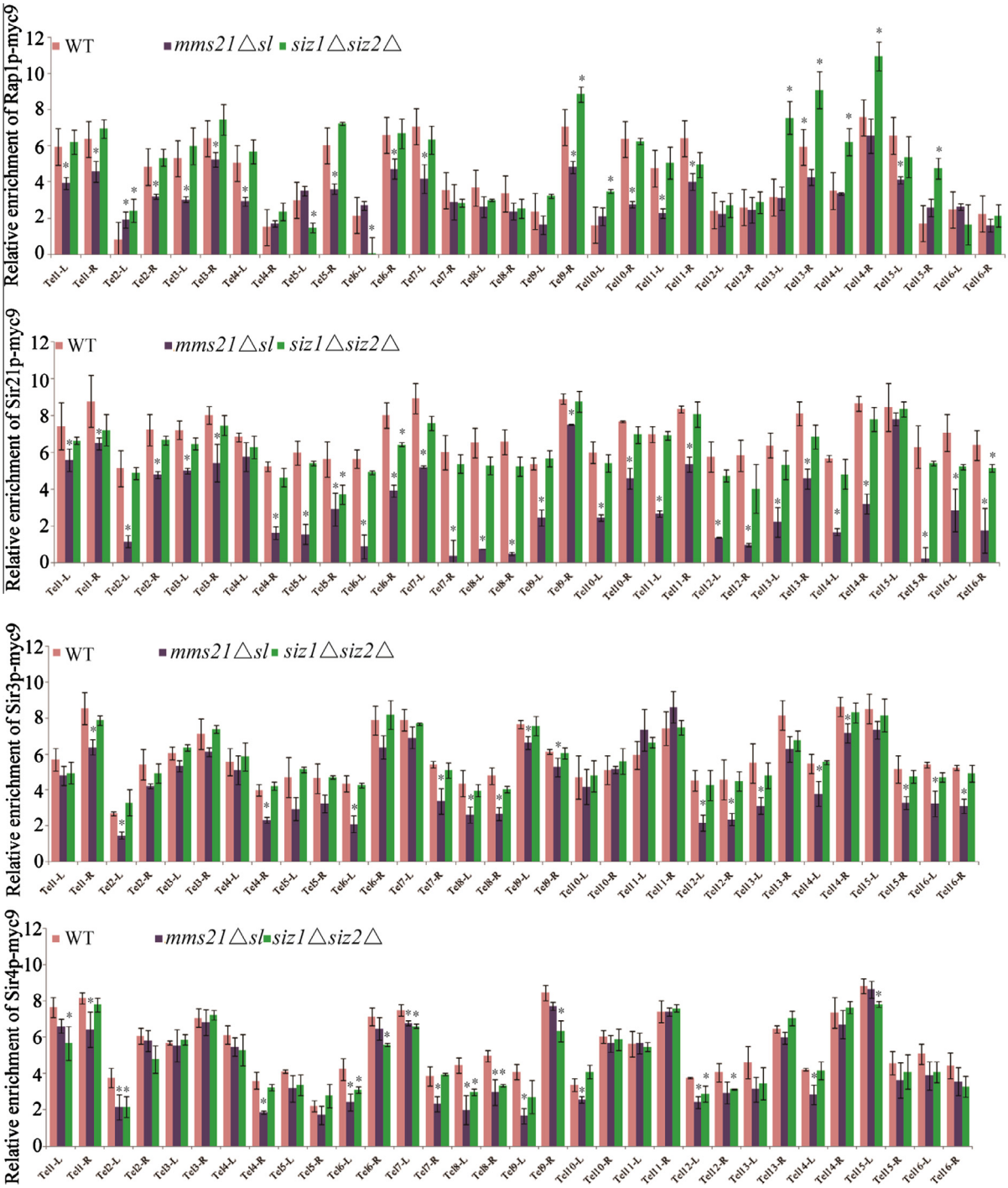

Fig. 2. Sumoylation E3 ligase can affect the distribution of the SIR complex. The association of Rap1p (A), Sir2p (B), Sir3p (C) and Sir4p (D) with 32 subtelomeric chromatins was determined by ChIP using anti-myc antibody, followed by qPCR. The values of the y axis indicate the ratio of normalized signals of enrichment relative to the internal control. Errors bar represents the standard deviation from three independent biological replications. Asterisks mark statistically significant results (p-value $\leqslant 0.05$).

*siz2*Δ mutants. Consistent with previous studies, H4K16 acetylation, H3K4 methylation and H3K79 methylation are more abundant distal to the telomere end [13,14] (Fig. 3). No significant changes in the distribution of H4K16 acetylation and H3K79methylation are observed in the *mms21*Δ*sl* or *siz1*Δ *siz2*Δ mutants (B-F). Interestingly, tri-methylation of H3K4 is significantly increased in the *mms21*Δ*sl* mutant throughout the two subtelomeric regions examined. While both *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants show a de-repression of subtelomeric silencing, the *siz1*Δ *siz2*Δ mutant did not show any detectable change of H3K4 tri-methylation, suggesting that Mms21p plays an important role in regulating specific histone modifications.

It is possible that the increased levels of chromatin associated H3K4 tri-methylation observed in the *mms21*Δ*sl* mutant are the result of a global increase in levels of H3K4 tri-methylation. Total cellular levels of H3K4 tri-methylation in the *mms21*Δ*sl* mutant were compared to those in the wild-type strain and surprisingly, a noticeable decrease of global levels of tri-methylation H3K4 was detected in *mms21*Δ*sl* mutant (Fig. 4A). It will be interesting to study how global levels of H3K4 tri-methylation were regulated in *mms21*Δ*sl*. We further investigated the relationship between Mms21 and H3K4 tri-methylation by performing a growth analysis of gene deletions to understand the genetic interactions between *mms21*Δ*sl* and *SET1*, which mediates H3K4 tri-methylation (Fig. 4B). *mms21*Δ*sl* and *set1*Δ double mutant cells do not survive at 37 °C and are severely affected at 30 °C and 33 °C. All together, these data indicate that *mms21*Δ*sl* and *siz1*Δ *siz2*Δ mutants act through different mechanisms to regulate subtelomeric silencing in yeast. However, it could be possible that some uncharacterized histone modifications located at subtelomeric regions may be affected in the *siz1*Δ *siz2*Δ mutant.

3.5. Association of Htz1p is increased in the mms21 mutant

The histone variant Htz1p has been shown to bind preferentially near telomeres and it is an intrinsic component

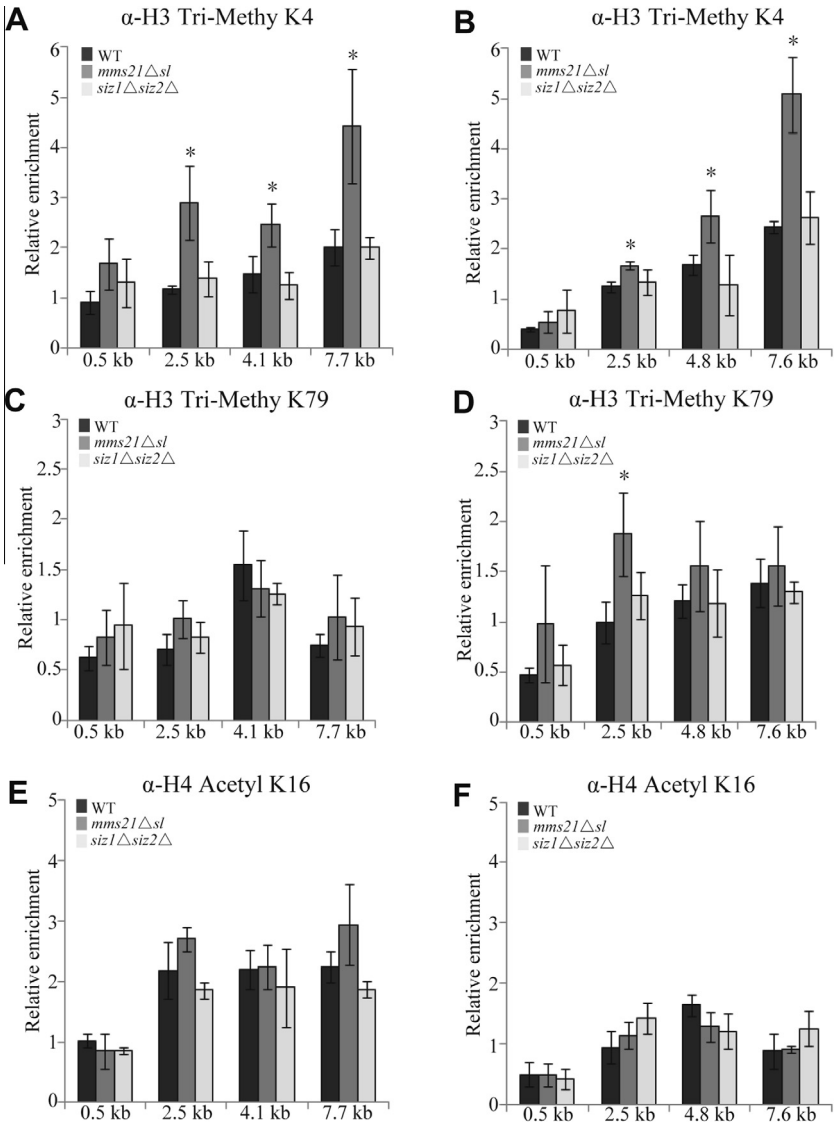

Fig. 3. Sumoylation E3 ligase activity of Mms21 is required for normal H3K4 tri-methylation in subtelomeric chromatin. Relative abundance of H3K4 tri-methylation (A), H3K79 tri-methylation (C) and H4K16 acetylation (E) at chromosome 6R subtelomeric chromatin was studied on wild-type, *mms21*Δ*sl* and *siz1*Δ*siz2*Δ mutant strains. Relative abundance of H3K4 tri-methylation (B), H3K79 tri-methylation (D) and H4K16 acetylation (F) at chromosome 9R subtelomeric chromatin was studied on wild-type, *mms21*Δ*sl* and *siz1*Δ*siz2*Δ mutant strains. Non-promoter IGRi YMR325W was used as an internal control to normalize signals of enrichment for individual histone modification. Asterisks mark statistically significant results (p-value $\leqslant 0.05$).

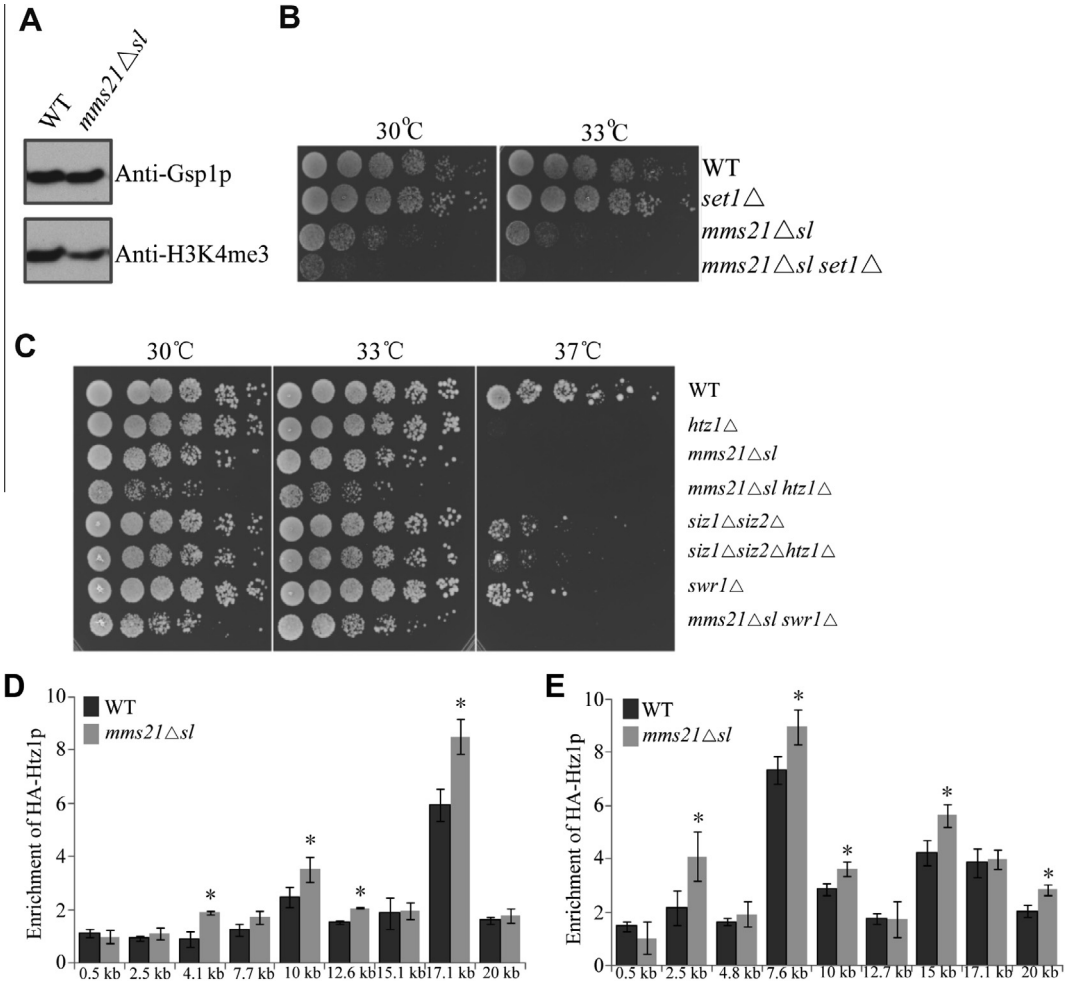

Fig. 4. Mms21 negatively regulates global histone H3K4 tri-methylation. (A) Global steady-state H3K4 tri-methylation levels from whole-cell lysates were determined by using an H3K4 tri-methylation antibody in wild-type and *mms21Δsl* strains. (B) Genetic interaction between *mms21Δsl* and *set1Δ* was assayed by fivefold serial dilutions of each yeast strains on YPD plates at 30 °C and 33 °C for 3 days and photographed. (C) Genetic interaction between *mms21Δsl* and *htz1Δ*. (D) Relative abundance of HA-Htz1 at chromosome 6R subtelomeric chromatin was studied on wild-type and *mms21Δsl* mutant strains. (E) Relative abundance of HA-Htz1 at chromosome 9R subtelomeric chromatin was studied on wild-type and *mms21Δsl* mutant strains. Asterisks mark statistically significant results (p-value $\leqslant 0.05$).

of euchromatin, functioning to antagonize the spread of heterochromatin toward euchromatin [9,24]. Deletions of *HTZ1* result in a growth defect at 37 °C but not at the lower temperatures tested (Fig. 4C). In contrast, severe growth defects are observed when the *HTZ1* deletion is combined with *mms21Δsl* at both 30 °C and 33 °C. The defect was not observed in *htz1Δsiz1Δ siz2Δ* triple mutants. As many of the phenotypes of *htz1Δ* mutant have been shown to be due to aberrant Swr1p activity [25], we decided to investigate whether genetic interaction between *mms21Δsl* and *htz1Δ* mutant was linked to Swr1p. As shown in Fig. 4C, we did not observe a weak growth rate in the *mms21Δsl swr1Δ* double mutant compared to the *mms21Δsl* single mutant. Genetic interactions between *mms21Δsl* and Htz1 may reflect a functional dependency during the regulation of subtelomeric silencing.

We further measured the effect of the *mms21Δsl* mutant background on Htz1p occupancy, testing Htz1 occupancy on the right arm of chromosome 6 and chromosome 9 (Fig. 4D, E). As previously reported, levels of Htz1p increase from the telomere proximal to the telomere distal probes [14]. Interestingly, in the *mms21Δsl* mutant, the association of Htz1 with telomere-proximal regions was dramatically increased at two loci. These results indicate that the E3 SUMO ligase Mms21 may functionally cooperate with Htz1p to maintain subtelomeric silencing in yeast.

4. Discussion

Covalent protein modification by SUMO has been shown to be involved in a wide range of biological processes including transcriptional regulation, DNA replication and repair, and signal transduction [26]. A requirement for sumoylation in telomere length control has also been previously documented, wherein sumoylation acts as a negative regulator of telomere length. In this study, we report that impairment of the sumoylation E3 ligase activity of Mms21 causes no changes in mating type silencing, despite its overwhelming requirement in subtelomeric silencing, which adds an additional layer of complexity onto the already complex mechanisms of heterochromatin silencing.

Previous work has shown that the SUMO ligase activity of Mms21 is required for maintenance of chromosome integrity in budding yeast [27]. Sumoylation has also been shown to be involved in the subnuclear positioning of telomeres in budding yeast. While the PIAS homologue Siz2-mediated sumoylation of both Yku70/80 and Sir4 was found to promote telomere positioning to the nuclear periphery [17], in this study, we did not detect a significant defect in subtelomeric silencing in *siz2Δ* mutant cells. Mutation of *MMS21* shows a significant perturbation to subtelomeric silencing, although it showed the normal telomere length compared to wild-type [28]. Our results are strongly indicative of a functional relationship between Mms21p and subtelomeric silencing.

But how does Mms21p contribute to subtelomeric silencing? Our data suggest that the mms21 mutant shows a strong genetic interaction with SIR complex components. In particular, the growth defect observed for*mms21*Δsl and *siz1*Δ*siz2*Δ can, at least in part, be rescued by overexpression of Sir2p and Sir4p. We hypothesize that overexpression of Sir2p and Sir4p may facilitate restoration of more normal subtelomeric silencing and thus partially rescue the growth phenotype. However, it is unclear why overexpression of Sir3p does not rescue the growth defects observed for the E3 ligase deletions. This underscores the functional differences between components of the SIR complex. In vitro data has shown that Sir3p has an altered binding affinity for H4K16 modified histones and Sir3p clearly preferentially associates with unmodified chromatin [7,29]. It is possible that impaired sumoylation in the *mms21*Δsl and *siz1*Δ*siz2*Δ mutants impedes the incorporation of Sir3p into chromatin.

The histone variant H2A.Z and Set1-mediated H3K4 methylation can antagonize the local spread of Sir-dependent silencing chromatin into adjacent euchromatic regions, with Set1 and H2A.Z playing redundant roles in antagonizing Sir-dependent subtelomeric silencing [29,30]. In our study, we have shown that *MMS21* can genetically interact with both *SET1* and *HTZ1*. We also show that impairment of sumoylation activity of Mms21p results in an increase in the abundance of chromatin associated with H3K4 tri-methylation at subtelomeric DNA, which is not a result of increased cellular levels of H3K4 tri-methylation. In subtelomeric regions, increased H3K4 tri-methylation can lead to the de-repression of subtelomeric silencing. Although loss of SUMO ligation function in both the *mms21*Δ*sl* and *siz1*Δ*siz2*Δ mutants can perturb subtelomeric silencing, the molecular mechanisms by which this occurs are not well understood and are most likely different. In the *mms21*Δ*sl* mutant background, the association of Sir2p and Sir3p with subtelometric DNA is significantly decreased. This suggests that Mms21-mediated sumoylation targets may affect the binding affinity of SIR to heterochromatin. Given that Siz1/Siz2 mediated sumoylation does not appear to regulate the association of Sir2p and Sir3p with subtelomeric DNA, it is possible that Mms21 and Siz1/Siz2 function in distinct physiological pathways to regulate subtelomeric silencing.

In summary, in this study we have clearly demonstrated functional roles of Mms21 and Siz1/Siz2-mediated sumoylation in the regulation of subtelomeric silencing. Further investigation into the distinct molecular mechanisms mediated by Mms21 and Siz1/Siz2 will be required for fully understanding the functional roles of each SUMO E3 ligase in chromatin regulation.

Acknowledgments

This work was supported by grants from The National Natural Science Foundation of China (31071146, 31271365), Natural Science Foundation of Jiangsu Province (BK2011599) and the Excellent Young Teachers Program of Southeast University (3231001201) to Yakun Wan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.07.096.

References

[1] H. Tamaru, Confining euchromatin/heterochromatin territory: jumonji crosses the line, Genes Dev. 24 (2010) 1465–1478.
[2] E. Fedorova, D. Zink, Nuclear architecture and gene regulation, Biochim. Biophys. Acta 1783 (2008) 2174–2184.
[3] N. Dillon, Heterochromatin structure and function, Biol. Cell 96 (2004) 631–637.
[4] X. Bi, Functions of chromatin remodeling factors in heterochromatin formation and maintenance, Sci. China Life Sci. 55 (2012) 89–96.
[5] J.L. Hu, B.O. Zhou, R.R. Zhang, K.L. Zhang, J.Q. Zhou, G.L. Xu, The N-terminus of histone H3 is required for de novo DNA methylation in chromatin, Proc. Natl. Acad. Sci. USA 106 (2009) 22187–22192.
[6] I.M. Hall, G.D. Shankaranarayana, K. Noma, N. Ayoub, A. Cohen, S.I. Grewal, Establishment and maintenance of a heterochromatin domain, Science 297 (2002) 2232–2237.
[7] A. Johnson, G. Li, T.W. Sikorski, S. Buratowski, C.L. Woodcock, D. Moazed, Reconstitution of heterochromatin-dependent transcriptional gene silencing, Mol. Cell 35 (2009) 769–781.
[8] F. Xu, Q. Zhang, K. Zhang, W. Xie, M. Grunstein, Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast, Mol. Cell 27 (2007) 890–900.
[9] W.J. Shia, B. Li, J.L. Workman, SAS-mediated acetylation of histone H4 Lys 16 is required for H2A.Z incorporation at subtelomeric regions in*Saccharomyces cerevisiae*, Genes Dev. 20 (2006) 2507–2512.
[10] B.O. Zhou, S.S. Wang, Y. Zhang, X.H. Fu, W. Dang, B.A. Lenzmeier, J.Q. Zhou, Histone H4 lysine 12 acetylation regulates telomeric heterochromatin plasticity in *Saccharomyces cerevisiae*, PLoS Genet. 7 (2011) e1001272.
[11] N.J. Krogan, J. Dover, S. Khorrami, J.F. Greenblatt, J. Schneider, M. Johnston, A. Shilatifard, COMPASS, a histone H3 (Lysine 4) methyltransferase required for telomeric silencing of gene expression, J. Biol. Chem. 277 (2002) 10753–10755.
[12] Y.H. Takahashi, J.M. Schulze, J. Jackson, T. Hentrich, C. Seidel, S.L. Jaspersen, M.S. Kobor, A. Shilatifard, Dot1 and histone H3K79 methylation in natural telomeric and HM silencing, Mol. Cell 42 (2011) 118–126.
[13] Y. Wan, W. Chen, J. Xing, J. Tan, B. Li, H. Chen, Z. Lin, J.H. Chiang, R.A. Saleem, Transcriptome profiling reveals a novel role for trichostatin A in antagonizing histone chaperone Chz1 mediated telomere anti-silencing, FEBS Lett. 585 (2011) 2519–2525.
[14] Y. Wan, J.H. Chiang, C.H. Lin, C.E. Arens, R.A. Saleem, J.J. Smith, J.D. Aitchison, Histone chaperone Chz1p regulates H2B ubiquitination and subtelomeric anti-silencing, Nucleic Acids Res. 38 (2010) 1431–1440.
[15] S.H. Yang, A.D. Sharrocks, PIASxalpha differentially regulates the amplitudes of transcriptional responses following activation of the ERK and p38 MAPK pathways, Mol. Cell 22 (2006) 477–487.
[16] B. Guo, N. Panagiotaki, S. Warwood, A.D. Sharrocks, Dynamic modification of the ETS transcription factor PEA3 by sumoylation and p300-mediated acetylation, Nucleic Acids Res. 39 (2011) 6403–6413.
[17] H.C. Ferreira, B. Luke, H. Schober, V. Kalck, J. Lingner, S.M. Gasser, The PIAS homologue Siz2 regulates perinuclear telomere position and telomerase activity in budding yeast, Nat. Cell Biol. 13 (2011) 867–874.
[18] P.R. Potts, H. Yu, The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins, Nat. Struct. Mol. Biol. 14 (2007) 581–590.
[19] R.D. Gietz, R.A. Woods, Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method, Methods Enzymol. 350 (2002) 87–96.
[20] Y. Wan, R.A. Saleem, A.V. Ratushny, O. Roda, J.J. Smith, C.H. Lin, J.H. Chiang, J.D. Aitchison, Role of the histone variant H2A.Z/Htz1p in TBP recruitment, chromatin dynamics, and regulated expression of oleate-responsive genes, Mol. Cell Biol. 29 (2009) 2346–2358.
[21] J.J. Smith, L.R. Miller, R. Kreisberg, L. Vazquez, Y. Wan, J.D. Aitchison, Environment-responsive transcription factors bind subtelomeric elements and regulate gene silencing, Mol. Syst. Biol. 7 (2011) 455–469.
[22] T. Laroche, S.G. Martin, M. Tsai-Pflugfelder, S.M. Gasser, The dynamics of yeast telomeres and silencing proteins through the cell cycle, J. Struct. Biol. 129 (2000) 159–174.
[23] E.R. Loney, P.W. Inglis, S. Sharp, F.E. Pryde, N.A. Kent, J. Mellor, E.J. Louis, Repressive and non-repressive chromatin at native telomeres in *Saccharomyces cerevisiae*, Epigenet. Chromatin 2 (2009) 18–30.
[24] M.D. Meneghini, M. Wu, H.D. Madhani, Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin, Cell 112 (2003) 725–736.
[25] J.E. Halley, T. Kaplan, A.Y. Wang, M.S. Kobor, J. Rine, Roles for H2A.Z and its acetylation in GAL1 transcription and gene induction, but not GAL1-transcriptional memory, PLoS Biol. 8 (2010) e1000401.
[26] J.R. Gareau, C.D. Lima, The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition, Nat. Rev. Mol. Cell Biol. 11 (2010) 861–871.
[27] R. Rai, S.P. Varma, N. Shinde, S. Ghosh, S.P. Kumaran, G. Skariah, S. Laloraya, Small ubiquitin-related modifier ligase activity of Mms21 is required for maintenance of chromosome integrity during the unperturbed mitotic cell division cycle in *Saccharomyces cerevisiae*, J. Biol. Chem. 286 (2011) 14516–14530.
[28] L.E. Hang, X. Liu, I. Cheung, Y. Yang, X. Zhao, SUMOylation regulates telomere length homeostasis by targeting Cdc13, Nat. Struct. Mol. Biol. 18 (2011) 920–926.
[29] K.J. Armache, J.D. Garlick, D. Canzio, G.J. Narlikar, R.E. Kingston, Structural basis of silencing: Sir3 BAH domain in complex with a nucleosome at 3.0 A resolution, Science 334 (2011) 977–982.
[30] M.B. Chandrasekharan, F. Huang, Y.C. Chen, Z.W. Sun, Histone H2B C-terminal helix mediates trans-histone H3K4 methylation independent of H2B ubiquitination, Mol. Cell Biol. 30 (2010) 3216–3232.