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Growth arrest in yeast with aberrant [kinetochore] is rescued by the loss of [Gcn5]

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Abstract

The kinetochore provides the end-on attachment of central core complexes, such as Ndc80, to the spindle microtubules (MTs) for a balanced chromosome segregation. The K-acetyltransferase Gcn5, involved in acetylation dependent processes, is also engaged in the control of the cell cycle progression. Here we show that in budding yeast the deletion of the KAT GCN5 gene leads to a complete growth recovery of a strain missing the central kinetochore component Ndc80. Accordingly, we obtained full deletion of the essential, highly conserved, Ndc80 KT subunit in the gcn5Δ strain. We also demonstrated that the deletion of Gim3, a subunit of the tubulin chaperone, abrogates the recovery of cell growth in the *ndc80-1-gcn5*Δ strain suggesting an involvement of the kinetochore-MT attachment in this process. Our observations suggest the notion that KAT Gcn5 exerts a regulatory role in the interaction of the central kinetochore Ndc80 complex to the spindle microtubules exhorting us to dig more into the mechanistic of this process.

Introduction

Mitotic stability requires a successful attachment of the kinetochore (KT) to the mitotic spindle [1] [2] achieved also through the activity of the central KMN network [3] [4]. In *S.cerevisiae,* mutations in KT and KMN subunits are lethal due to kinetochore aberrations preventing the accomplishment of mitosis. The central KT-hub is the Ndc80 complex composed by Ndc80, Spc24, Spc25 and Nuf2 proteins, conserved from fungi to humans and implicated in several essential functions like binding to microtubules and, recruitment of the mitotic checkpoint [5] [6]. Depletion of Ndc80 and Nuf2 results in mislocalization of several kinetochore subunits [7]. Ndc80c is a 4 unit dumbbell shaped coiled coil complex with 2 positively charged head domains (Ndc80/Nuf2) on one end, Ndc80 binds the negative microtubule surface [8], on the opposite site, C-terminal domains of Spc24/Spc25 bind to the kinetochore [9]. It has been proposed that Nuf2 is not directly involved in the binding with microtubules suggesting a substantiallly different contribution compared to Ndc80 [10]. Ndc80 also shows an internal loop that mediates the recruitment of Dam1 [11], a component of the ring embracing the mitotic spindle [12] and a toe CH domain that mediates the microtubule binding at high affinity [13]. Finally, Ndc80 is essential to recruit the SAC proteins Mad1 and Mad2 to unattached kinetochores while KMN-Spc105 recruits Bub1/Bub3 and Mad1/Mad2 necessary to delay cell division [14].

Objective

Here we assay the role of K-acetyltransferase Gcn5 on kinetochore function and its interaction with mitotic spindle. Unexpected results highlighted the relevance of Gcn5 in this phenomenon and show that in absence of Gcn5 cells can override mitotic arrest due to an aberrant kinetochore and recover growth.

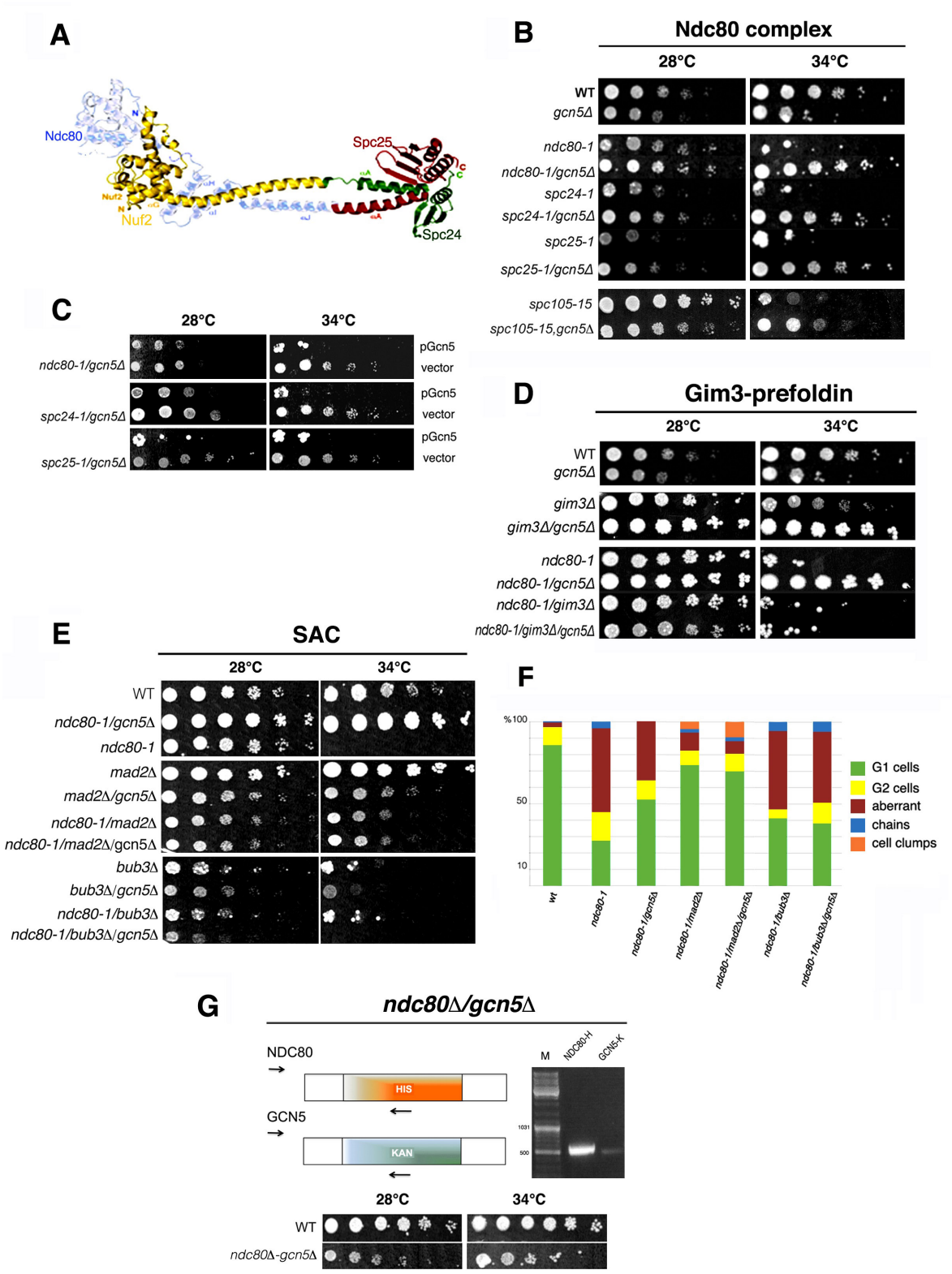

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Figure Legend

Figure 1.

(A) Schematic representation of Ndc8o complex.

(B) Temperature sensitivity analysis of *S.cerevisiae* KT mutants. 5-fold serial dilutions of indicated strains spotted on solid YPD medium and incubated at permissive (28℃) and non permissive (34℃) temperatures.
(C) Strains *ndc80-1/gcn5*Δ (ySM2), *spc24-1/gcn5*Δ (yCC5) and *spc25-1/gcn5*Δ (yCC4) carrying wild type Gcn5 expressed at physiological levels in a centromeric vector (pGcn5) were tested respect to empty plasmid pRS316 (vector).
(D) Growth spot assay of indicated strains deleted in the Gim3 subunit of the tubulin chaperone GIM complex.
(E) Spot assay of *ndc80-1* and *gcn5*Δ strains disrupted respectively in SAC checkpoint proteins Mad2 and Bub3.
(F) Percentage of yeast cell morphologies found in the indicated strains: round G1 (green) and G2 cells (yellow), aberrant and elongated cells (purple), undivided cell chains (blue) and cell clumps aggregates (orange).
(G) Strain *ndc80*Δ-*gcn5*Δ (yRB027) carrying the disruption of the central kinetochore subunit NDC80. Schemes with integration cassettes used (NDC80::HIS3 and GCN5::KAN), colony PCR amplifications of NDC80 and GCN5 insertions in yRB027 and its growth recovery.

Results & Discussion

Ndc80 central kinetochore component is a tetrameric elongated complex composed of Ndc80, Nuf2, Spc24 and Spc25 proteins (Fig. 1A) that connects the kinetochore to the spindle microtubules. Almost all the KT subunits are essential for viability in budding yeast, indeed the corresponding TS mutants [15] [16] are lethal at restrictive temperature (34℃). The involvement of Gcn5 in mitotic progression [17] is due to its many roles, among others the acetylation of regulatory proteins [18] and the expressions of several cell cycle-related genes [19] was demonstrated. In an attempt to analyze additional effects of KAT Gcn5 on the kinetochore functions, we carried out a screening of *S.cerevisiae* TS mutants in kinetochore subunits where we deleted K-acetyltransferase Gcn5.

Loss of Gcn5 induces cell growth in Ndc80 complex conditionally lethal-mutants

Here we show that TS mutants *ndc80-1, spc24-1 spc25-1* and *spc105-15* of central kinetochore, involved in the interaction with spindle microtubules, grow normally at 28℃ and lose viability at non permissive temperatures (34℃). After deletion of KAT-Gcn5 we surprisingly found that the double mutants *ndc80-1-gcn5*Δ, *spc24-1-gcn5*Δ and *spc25-1-gcn5*Δ and *spc105-15-gcn5*Δ strains were able to rescue the growth arrest even at 34℃ (Fig. 1B). To confirm the direct role of Gcn5 in this observation, we re-induced the expression of GCN5 in those double mutants by introducing the centromeric plasmid pRS316 containing a functional copy of Gcn5 (pGcn5) and empty vector pRS316 (vector) was used as control. According to our previous findings, the re-introduction of a functional wild type Gcn5p abolished the growth recovery also at 34℃, while the empty vector control did not (Fig. 1C). These results provide a direct evidence that Gcn5 acts as a strict regulator of the cell cycle during the kinetochore-MT interaction.

Role of the tubulin chaperon subunit Gim3

The chaperon complex Gim, composed by five subunits (Gim1-5) in yeast is involved in the formation of functional α and γ-tubulins, needed for a correct assembly of MTs [20]. In order to understand if the dynamics and the stability of spindle MTs can play a role in the cell growth recovery of defective kinetochore mutants in absence of Gcn5 we disrupted the Gim3 prefoldin, component of the tubulin chaperon Gim complex. Loss of Gim3 abolished the growth recovery in *ndc80-1-gcn5*Δ mutant, as shown by the growth assay of the triple mutant *ndc80-1-gim3*Δ-*gcn5*Δ (Fig. 1D). This result demonstrates that the recovery of aberrant Ndc80 kinetochore in absence of Gcn5 is related to kinetochore-MT interaction and the relevance of acetylation on mitotic spindle with stabilising effects caused by Gcn5 dependent ipoacetylation.

Role of SAC mitotic checkpoint

Next, we wanted to test the component of SAC mitotic checkpont [21] devoted to control the recovery of aberrant or unattached kinetochores by deleting the checkpoint proteins Mad2 and Bub3 in the *ndc80-1* mutant. Spot growth assay (Fig. 1E) shows that

the absence of Mad2 does not abrogate the growth recovery observed in *ndc80-1-gcn5*Δ mutant, while the deletion of Bub3 subunit is unable to skip the cell cycle arrest under restrictive temperature conditions. These results suggest that the recovery of growth in absence of Gcn5 in the *ndc80-1* mutant is dependent on Mad2 and not Bub3 in the cell. Next, we analyzed cell morphology of the different mutants by optical microscopy, in figure 1F shows the distribution of the different cell types observed: normal G1 and G2 cells, aberrant elongated cells, undivided cell chains and unhealthy cell clumps. Results show that Mad2 deletion increased the number of dividing G1 cells in *ndc80-1*, while in absence of Bub3 there was a drastic decrease of dividing G1 cells and an enhancement of inviable aberrant or undivided chains indicating defects in cytokinesis. Collectively, the number and type of cell morphologies observed were in full agreement with the growth assay shown above. The recovery obtained both in *ndc80-1-mad2*Δ and in *ndc80-1-gcn5*Δ is similar to the triple *ndc80-1-mad2*Δ-*gcn5*Δ strain suggesting that, in absence of Gcn5, cells may overcome the Mad2 dependent cell cycle arrest possibly for improved kinetochore-MT interactions.

Production of ndc80Δ-gcn5Δ strain

Based on our collected results showing that the depletion of GCN5 in the conditionally-lethal mutant *ndc80-1* is viable, we wanted to push further our assay asking whether we may fully disrupt the essential NDC80 gene in absence of Gcn5 (*gcn5*Δ) and obtain a viable strain. We therefore transformed *gcn5*Δ strain with the integrative cassette NDC80::HIS. HIS3 trasformants obtained were analyzed by colony PCR analysis was performed to confirm the correct chromosomal integration of NDC80::HIS cassette and the concomitant presence of the GCN5::KAN.MX in the strain (Fig. 1G). According to our predictions, we obtained the double deleted mutant ndc80Δ-gcn5Δ viable both at permissive and restrictive temperatures (28℃ and 34℃). This result reinforces the meaning of our findings thus confirming the role of Gcn5 in the control of the central KT Ndc80 complex and its interaction with spindle microtubules during chromosome segregation.

Conclusions

Ndc80 in the complex macrostructure of kinetochore is an essential subunit of central layer devoted to the attachment of the KMN to spindle microtubules which is the prerequisite for faithful chromosome segregation. Despite the relevance of Ndc80 in kinetochore the mechanisms governing KT-MT interactions with microtubules are still unclear. Acetylation was associated to microtubule stabilization and deacetylation to their destabilization [22] [23], however its role is still debated. Here we demonstate that the absence of Gcn5 allows growth of cells with aberrant kinetochores in mutants for Ndc80 complex components. We demonstrated that lack of Gcn5 allows growth of mutants in the central Ndc80 complex at non permissive temperature. We also obtained an unbiased demonstration of the role of Gcn5 in this process by producing the full deletion of the essential Ndc80 subunit in the double deleted strain *ndc80*Δ-*gcn5*Δ. Moreover, we have shown that the recovery of cell growth is linked to the Gim3 subunit of the tubulin chaperone and is regulated by SAC checkpoint Mad2.

Limitations

In *S.cerevisiae* the kinetochore is simplified respect to higher Eukaryotes. It binds to a single microtubule while, in humans, it interacts with a complex bundles of multiple microtubules. However, while yeast shows a simplifyed interaction of kinetochore with a single microtubule it offers the unique opportunity to carry on defined genetic analysis to dissect protein networks and functional interactions. As a proof of concept our results suprisingly outlined the effects induced by Gcn5 on the interaction between the budding yeast kinetochore Ndc80 complex and the mitotic spindle.

Our collected results demonstrate that the absence of Gcn5 allows the interaction between aberrant kinetochores missing an essential central structure and the spindle microtubules. Microtubules are subjected to a number of different post translational modifications determining their stability and dynamics [24]. Despite these studies the mechanism and the role of this regulation is far to be determined. Our observations set the

stage to carry on further characterizations on the mechanisms involved in such process. To this end, the *ndc80Δ-gcn5Δ* strain we have obtained represents an excellent strain for future studies on the mechanisms involved and the identification of additional regulatory components. We believe that our experimental data may lead to additional new experiments to dissect the role of acetylation in the interaction of central kinetochore with the mitotic spindle [12].

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201705000006.

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Ethics Statement

Not Applicable.

Citations

[1] Sue Biggins. "The Composition, Functions, and Regulation of the Budding Yeast Kinetochore". In: *Genetics* 194.4 (Aug. 2013), pp. 817–846. DOI: 10.1534/genetics.112.145276. URL: https://doi.org/10.1534/genetics.112.145276.

[2] Andrea Musacchio and Arshad Desai and. "A Molecular View of Kinetochore Assembly and Function". In: *Biology* 6.1 (Jan. 2017), p. 5. DOI: 10.3390/biology6010005. URL: https://doi.org/10.3390/biology6010005.

[3] Dileep Varma and E. D. Salmon. "The KMN protein network - chief conductors of the kinetochore orchestra". In: *Journal of Cell Science* 125.24 (Dec. 2012), pp. 5927–5936. DOI: 10.1242/jcs.093724. URL: https://doi.org/10.1242/jcs.093724.

[4] Iain M. Cheeseman et al. "The Conserved KMN Network Constitutes the Core Microtubule-Binding Site of the Kinetochore". In: *Cell* 127.5 (Dec. 2006), pp. 983–997. DOI: 10.1016/j.cell.2006.09.039. URL: https://doi.org/10.1016/j.cell.2006.09.039.

[5] Claudio Ciferri, Andrea Musacchio, and Arsen Petrovic and. "The Ndc80 complex: Hub of kinetochore activity". In: *FEBS Letters* 581.15 (May 2007), pp. 2862–2869. DOI: 10.1016/j.febslet.2007.05.012. URL: https://doi.org/10.1016/j.febslet.2007.05.012.

[6] Yanchang Wang et al. "The current view for the silencing of the spindle assembly checkpoint". In: *Cell Cycle* 13.11 (Apr. 2014), pp. 1694–1701. DOI: 10.4161/cc.29027. URL: https://doi.org/10.4161/cc.29027.

[7] Mark L. McCleland et al. "The highly conserved Ndc80 complex is required for kinetochore assembly, chromosome congression, and spindle checkpoint activity". In: *Genes and Development* 17.1 (Jan. 2003), pp. 101–114. DOI: 10.1101/gad.1040903. URL: https://doi.org/10.1101/gad.1040903.

[8] Neil T. Umbreit et al. "The Ndc80 kinetochore complex directly modulates microtubule dynamics". In: *Proceedings of the National Academy of Sciences* 109.40 (Aug. 2012), pp. 16113–16118. DOI: 10.1073/pnas.1209615109. URL: https://doi.org/10.1073/pnas.1209615109.

[9] Arsen Petrovic et al. "Modular Assembly of RWD Domains on the Mis12 Complex Underlies Outer Kinetochore Organization". In: *Molecular Cell* 53.4 (Feb. 2014), pp. 591–605. DOI: 10.1016/j.molcel.2014.01.019. URL: https://doi.org/10.1016/j.molcel.2014.01.019.

[10] Lynsie J.R. Sundin, Geoffrey J. Guimaraes, and Jennifer G. DeLuca. "The NDC80 complex proteins Nuf2 and Hec1 make distinct contributions to kinetochore-microtubule attachment in mitosis". In: *Molecular Biology of the Cell* 22.6 (Jan. 2011), pp. 759–768. DOI: 10.1091/mbc.e10-08-0671. URL: https://doi.org/10.1091/mbc.e10-08-0671.

[11] Jean-François Maure et al. "The Ndc80 Loop Region Facilitates Formation of Kinetochore Attachment to the Dynamic Microtubule Plus End". In: *Current Biology* 21.3 (Feb. 2011), pp. 207–213. DOI: 10.1016/j.cub.2010.12.050. URL: https://doi.org/10.1016/j.cub.2010.12.050.

[12] JJ L Miranda et al. "The yeast DASH complex forms closed rings on microtubules". In: *Nature Structural and Molecular Biology* 12.2 (Jan. 2005), pp. 138–143. DOI: 10.1038/nsmb896. URL: https://doi.org/10.1038/nsmb896.

[13] Kuo-Shun Hsu and Takashi Toda and. "Ndc80 Internal Loop Interacts with Dis1/TOG to Ensure Proper Kinetochore-Spindle Attachment in Fission Yeast". In: *Current Biology* 21.3 (Feb. 2011), pp. 214–220. DOI: 10.1016/j.cub.2010.12.048. URL: https://doi.org/10.1016/j.cub.2010.12.048.

[14] Pavithra Aravamudhan et al. "Dual mechanisms regulate the recruitment of spindle assembly checkpoint proteins to the budding yeast kinetochore". In: *Molecular Biology of the Cell* 27.22 (May 2016), pp. 3405–3417. DOI:

10.1091/mbc.e16-01-0007. URL: https://doi.org/10.1091/mbc.e16-01-0007.

[15] Cinzia Pagliuca et al. "Roles for the Conserved Spc105p/Kre28p Complex in Kinetochore-Microtubule Binding and the Spindle Assembly Checkpoint". In: *PLOS ONE* 4.10 (Oct. 2009), e7640. DOI: 10.1371/journal.pone.0007640. URL: https://doi.org/10.1371/journal.pone.0007640.

[16] Peter De Wulf, Andrew D. McAinsh, and Peter K. Sorger. "Hierarchical assembly of the budding yeast kinetochore from multiple subcomplexes". In: *Genes and Development* 17.23 (Dec. 2003), pp. 2902–2921. DOI: 10.1101/gad.1144403. URL: https://doi.org/10.1101/gad.1144403.

[17] Stefano Vernarecci et al. "Gcn5p Plays an Important Role in Centromere Kinetochore Function in Budding Yeast". In: *Molecular and Cellular Biology* 28.3 (Nov. 2007), pp. 988–996. DOI: 10.1128/mcb.01366-07. URL: https://doi.org/10.1128/mcb.01366-07.

[18] Roberta Paolinelli et al. "Acetylation by GCN5 regulates CDC6 phosphorylation in the S phase of the cell cycle". In: *Nature Structural and Molecular Biology* 16.4 (Apr. 2009), pp. 412–420. DOI: 10.1038/nsmb.1583. URL: https://doi.org/10.1038/nsmb.1583.

[19] Hidehiko Kikuchi, Yasunari Takami, and Tatsuo Nakayama and. "GCN5: a supervisor in all-inclusive control of vertebrate cell cycle progression through transcription regulation of various cell cycle-related genes". In: *Gene* 347.1 (Feb. 2005), pp. 83–97. DOI: 10.1016/j.gene.2004.12.007. URL: https://doi.org/10.1016/j.gene.2004.12.007.

[20] Geissler et al. "A novel protein complex promoting formation of functional α- and γ-tubulin". In: *The EMBO Journal* 17.4 (Feb. 1998), pp. 952–966. DOI: 10.1093/emboj/17.4.952. URL: https://doi.org/10.1093/emboj/17.4.952.

[21] Victor M. Bolanos-Garcia and Tom L. Blundell and. "BUB1 and BUBR1: multifaceted kinases of the cell cycle". In: *Trends in Biochemical Sciences* 36.3 (Mar. 2011), pp. 141–150. DOI: 10.1016/j.tibs.2010.08.004. URL: https://doi.org/10.1016/j.tibs.2010.08.004.

[22] Daniel Perdiz et al. "The ins and outs of tubulin acetylation: More than just a post-translational modification?" In: *Cellular Signalling* 23.5 (May 2011), pp. 763–771. DOI: 10.1016/j.cellsig.2010.10.014. URL: https://doi.org/10.1016/j.cellsig.2010.10.014.

[23] N. Kalebic et al. "Tubulin Acetyltransferase TAT1 Destabilizes Microtubules Independently of Its Acetylation Activity". In: *Molecular and Cellular Biology* 33.6 (Dec. 2012), pp. 1114–1123. DOI: 10.1128/mcb.01044-12. URL: https://doi.org/10.1128/mcb.01044-12.

[24] Yuyu Song and Scott T. Brady and. "Post-translational modifications of tubulin: pathways to functional diversity of microtubules". In: *Trends in Cell Biology* 25.3 (Mar. 2015), pp. 125–136. DOI: 10.1016/j.tcb.2014.10.004. URL: https://doi.org/10.1016/j.tcb.2014.10.004.

[25] Carsten Janke et al. "A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes". In: *Yeast* 21.11 (Aug. 2004), pp. 947–962. DOI: 10.1002/yea.1142. URL: https://doi.org/10.1002/yea.1142.