

SPOTLIGHT

A transcription factor primes the condensin pump

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Chromosome condensation is regulated by the condensin complex but whether this process is subject to transcriptional control is poorly understood. In this issue, Schiklenk et al. (2018. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201711097>) reveal that the transcription factor *Zas1* mediates timely chromosome condensation and promotes transcription of several genes in *Saccharomyces pombe*, including the condensin subunit *Cnd1*.

Once DNA has been replicated during the cell cycle, the new chromosomes condense just before anaphase, which helps promote their accurate segregation to daughter cells. The kinetics of chromosome condensation are mediated by multiple factors, including mitotic kinases and a structural protein complex known as condensin. The condensin complex has received a lot of attention recently with the emergence of a new model to explain its function, the loop extrusion model (Yuen and Gerton, 2018). According to this model, the condensin ring complex pumps DNA loops through its lumen to condense chromosomes. However, it is not clear how various other additional factors might be required to condense chromosomes efficiently.

In this issue, Schiklenk et al. suggest that in *Saccharomyces pombe*, the transcription factor *Zas1* (Zinc fingers alternatively spliced) controls the kinetics of chromosome condensation. Notably, *Zas1* was identified in the study by a genetic screen for factors that impact the kinetics of chromosome condensation in a live cell assay. Mutations in the genes encoding *Zas1* and condensin subunits were identified multiple times, but no other mutations strongly influenced the kinetics of condensation, suggesting that this combination of structural proteins and the *Zas1* transcription factor may constitute the main components needed to achieve efficient chromosome condensation in *S. pombe*. Schiklenk et al. (2018) observed that *Zas1* is localized to the nucleus at every stage of the cell cycle and associates with chromatin. Chromatin immunoprecipitation (ChIP), followed by next-generation sequencing, revealed several potential targets for *Zas1* and the authors show that transcription of one of the genes encoding a subunit of the condensin complex, *Cnd1*, depends on *Zas1*. *Zas1*, via its role in promoting transcription of *Cnd1*, but also additional factors, is critical for the kinetics of chromosome condensation. Importantly, even though the level of *Cnd1* protein is reduced in the *zas1* mutant, chromosomes eventually become fully condensed, consistent with the model of condensin working as an extruding motor (Ganji et al., 2018).

Zas1 was first identified as a transcription factor in *S. pombe* that, depending on its splicing, contains either two or three zinc fingers (Okazaki and Niwa, 2000). Typically, transcription factors are composed of a DNA binding domain and a domain that activates transcription, often by recruiting additional factors including RNA polymerase. In addition to the predicted zinc fingers, the *Zas1* protein also contains a putative nuclear localization sequence, a transactivation domain, a C-terminal α -helical domain, and a short evolutionarily conserved motif termed a TAD motif. Essential to *Zas1* function in the timing of chromosome condensation are the nuclear localization sequence, the zinc fingers, and the TAD motif, but not the larger transactivation domain or the C-terminal helical domain. The zinc fingers presumably allow *Zas1* to bind DNA.

Using ChIP sequencing, Schiklenk et al. (2018) identified several genes with promoters bound by *Zas1*. The RNAs corresponding to many of these genes are present at lower levels in a *zas1* mutant, suggesting that they may be transcriptional targets. Many of the targets, including *Cnd1*, are involved in cell division. The protein levels of three other subunits of condensin are unaffected in the *zas1* mutant, so *Zas1* does not regulate all the condensin subunits as a group, nor does it seem to exert any cell cycle-specific regulation of *Cnd1*, but rather controls the general level of *Cnd1*. The delayed condensation appears to be a result of the effect of *Zas1* on multiple genes, since restoring *Cnd1* to normal levels did not rescue the phenotype. Using the promoter regions identified by ChIP sequencing, the authors identified a potential upstream activation sequence consisting of a 6-bp consensus sequence motif (5'-CCAY-3'), which is often present in more than one instance for some *Zas1*-bound promoters. To further examine whether *Zas1* binds to this motif, the authors purified recombinant *Zas1* protein and showed that it binds the motif in a DNA gel shift assay, and mutations in the DNA motif reduced binding. The zinc fingers are likely responsible for this DNA binding specificity, but additional experiments will be

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required to further explore the DNA binding capabilities of Zasl. An intriguing aspect of the genome-wide binding profile of Zasl is its restriction to chromosomes I and II, with rarely any sites on chromosome III or near centromeres. The gene encoding Zasl lies on chromosome II. It is not clear whether the observed chromosome specificity is simply an evolutionary accident or was selected for an unknown reason.

Transcription factors are enriched for short amino acid motifs that can be both dynamic and adaptable for interacting with many different proteins needed for transcription (Staby et al., 2017). Deletion of the transactivation domain or C-terminal α -helical domain of Zasl did not have overt effects on proliferation, but deletion or point mutations in an evolutionarily conserved short motif profoundly reduced cell growth. Using biochemical approaches, Schiklenk et al. (2018) demonstrate that this TAD motif binds to the C-terminal domain of Zasl, and they further suggest that Zasl may normally function as a dimer. The TAD motif is present in some well-known transcription factors such as the cell cycle checkpoint regulator E2F, where it binds to the retinoblastoma tumor suppressor protein Rb (Lee et al., 2002). Zasl was reportedly present in affinity capture experiments with two other transcription factors, Cbfl1 (Pancaldi et al., 2012) and Klf1 (Shimanuki et al., 2013), a Zasl paralog that functions during cellular quiescence. It is not clear whether the TAD motif is involved in these interactions. In some cases, the activity of short motifs may be regulated by posttranslational modifications. The identification of this short evolutionarily conserved motif in Zasl begs for the identification of interacting partners and possible regulatory posttranslational modifications.

TAD motifs often bind to the KIX domain of the Gal11/Med15 subunit of the Mediator complex (Piskacek et al., 2016; Staby et al., 2017). Mediator is a large, multisubunit protein complex that promotes transcription via recruitment of RNA polymerase II. Although Mediator did not emerge as an interacting protein complex for Zasl in the study by Schiklenk et al. (2018), its size may have presented a technical hurdle for pull-down experiments. Pull downs with the Med15 subunit as bait may be more likely to reveal an interaction between Mediator and Zasl. The three-helix bundle KIX domain in Med15 engages the pleiotropic drug resistance transcription factor Pdr1, a key regulator of multidrug resistance in the clinically important human pathogen *Candida glabrata*. Targeting this interaction with a small molecule resensitized drug-resistant *C. glabrata* to azole antifungals (Nishikawa et al., 2016). Immobilized templates with a Zasl consensus sequence such as the one used in the gel shift

experiments could reveal whether Zasl can similarly recruit the KIX-containing Med15 subunit of Mediator to promoter regions. Whether Zasl operates in this established TAD motif-KIX interaction paradigm with Med15 or another KIX domain-containing interacting partner awaits future research.

In summary, the new study by Schiklenk et al. (2018) reveals the major genetic factors required for chromosome condensation in *S. pombe* and the characterization of the transcription factor Zasl identifies a broadly conserved transactivation domain motif for future study in this model.

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References

- Ganji, M., I.A. Shaltiel, S. Bisht, E. Kim, A. Kalichava, C.H. Haering, and C. Dekker. 2018. Real-time imaging of DNA loop extrusion by condensin. *Science*. 360:102–105. <https://doi.org/10.1126/science.aar7831>
- Lee, C., J.H. Chang, H.S. Lee, and Y. Cho. 2002. Structural basis for the recognition of the E2F transactivation domain by the retinoblastoma tumor suppressor. *Genes Dev.* 16:3199–3212. <https://doi.org/10.1101/gad.1046102>
- Nishikawa, J.L., A. Boeszoermyenyi, L.A. Vale-Silva, R. Torelli, B. Posteraro, Y.J. Sohn, F. Ji, V. Gelev, D. Sanglard, M. Sanguinetti, et al. 2016. Inhibiting fungal multidrug resistance by disrupting an activator-Mediator interaction. *Nature*. 530:485–489. <https://doi.org/10.1038/nature16963>
- Okazaki, K., and O. Niwa. 2000. mRNAs encoding zinc finger protein isoforms are expressed by alternative splicing of an in-frame intron in fission yeast. *DNA Res.* 7:27–30. <https://doi.org/10.1093/dnares/7.1.27>
- Pancaldi, V., O.S. Saraç, C. Rallis, J.R. McLean, M. Převorovský, K. Gould, A. Beyer, and J. Bähler. 2012. Predicting the fission yeast protein interaction network. *G3 (Bethesda)*. 2:453–467. <https://doi.org/10.1534/g3.111.001560>
- Piskacek, M., M. Havelka, M. Rezacova, and A. Knight. 2016. The 9aaTAD Transactivation Domains: From Gal4 to p53. *PLoS One*. 11:e0162842. <https://doi.org/10.1371/journal.pone.0162842>
- Schiklenk, C., B. Petrova, M. Kschonsak, M. Hassler, C. Klein, T.J. Gibson, and C.H. Haering. 2018. Control of mitotic chromosome condensation by the fission yeast transcription factor Zasl. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201711097>
- Shimanuki, M., L. Uehara, T. Pluskal, T. Yoshida, A. Kokubu, Y. Kawasaki, and M. Yanagida. 2013. Klf1, a C2H2 zinc finger-transcription factor, is required for cell wall maintenance during long-term quiescence in differentiated G0 phase. *PLoS One*. 8:e78545. <https://doi.org/10.1371/journal.pone.0078545>
- Staby, L., C. O'Shea, M. Willemoës, F. Theisen, B.B. Kragelund, and K. Skriver. 2017. Eukaryotic transcription factors: Paradigms of protein intrinsic disorder. *Biochem. J.* 474:2509–2532. <https://doi.org/10.1042/BCJ20160631>
- Yuen, K.C., and J.L. Gerton. 2018. Taking cohesin and condensin in context. *PLoS Genet.* 14:e1007118. <https://doi.org/10.1371/journal.pgen.1007118>