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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′-CCCAY-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 Zas1. An intriguing aspect of the genome-wide binding profile of Zas1 is its restriction to chromosomes I and II, with rarely any sites on chromosome III or near centromeres. The gene encoding Zas1 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 Zas1 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 Zas1, and they further suggest that Zas1 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). Zas1 was reportedly present in affinity capture experiments with two other transcription factors, Cbf11 (Pancaldi et al., 2012) and Klf1 (Shimanuki et al., 2013), a Zas1 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 Zas1 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 Zas1 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 Zas1. 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 Zas1 consensus sequence such as the one used in the gel shift experiments could reveal whether Zas1 can similarly recruit the KIX-containing Med15 subunit of Mediator to promoter regions. Whether Zas1 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 Zas1 identifies a broadly conserved transactivation domain motif for future study in this model.

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