SPOTLIGHT

The nuclear pore complex as a platform for epigenetic regulation

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The nuclear pore complex (NPC) both mediates exchange of proteins and RNA between the nucleus and the cytoplasm and physically interacts with chromatin to regulate transcription. In this issue of *JCB*, Kumar et al. (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202207060) provide new insight into the molecular basis for NPC-mediated epigenetic silencing through loading of the replication processivity factor PCNA.

The nuclear envelope separates the nucleus from the cytoplasm, compartmentalizing the genome from the rest of the cell. Exchange of proteins and RNAs between these compartments is mediated by the nuclear pore complex (NPC). The NPC is a massive molecular assembly made up of multiple copies of ~30 different individual polypeptides and ranging in size from 50 to 100 megadaltons in different organisms (1, 2). Diffusion through the pore is inhibited by unstructured phenylalanine-glycine-rich domains that fill the volume of the channel, and transit is facilitated by transport factors that bind to these unstructured repeats. Passage of cargo-bound transport factors through this selective filter and regulation of cargo binding by a gradient of Ran-GTP between the cytoplasm (low) and nucleus (high) leads to directed movement into and out of the nucleus (3).

The NPC also serves other functions. In budding yeast, flies, worms, and mammals, NPCs physically interact with chromatin and impact transcription, chromatin structure, and DNA repair (4). Work in budding yeast suggests that several distinct mechanisms mediate interaction of chromatin with the NPC; while interaction of active genes with the NPC requires components of the nucleoplasmic basket, interaction of telomeres with the NPC requires a core channel protein, Nup170. These interactions have

different effects, as well. Whereas interaction of active genes with the NPC stimulates transcription, interaction of telomeres promotes transcriptional silencing (5). Similar effects have been observed in flies and mammals. However, the precise molecular mechanisms that mediate these effects have been unclear.

A paper in this issue of *JCB* employs sophisticated genomic, proteomic, and biochemical methods to provide new insight into how Nup170 promotes transcriptional silencing by regulating loading of a replication factor called proliferating cell nuclear antigen (PCNA; 6). PCNA is an essential ring-shaped structure that associates with replication forks and facilitates processivity of DNA polymerase (7). PCNA loading and unloading is regulated by several AAA+ ATPase complexes.

To explore the molecular basis for Nup170-stimulated transcriptional silencing, the authors employed a multi-pronged global approach. Comparing the proteins that co-purified with Nup170 with genes that show genetic interactions with *NUP170* and transcriptome profiles from null mutants that resembled *nup170Δ* identified Ctf18-RFC, a PCNA loading complex. Ctf18 had previously been implicated in telomere localization to the periphery (8). Ctf18-RFC co-purified with Nup170 and loss of either Nup170 or Ctf18 disrupted silencing of subtelomeric genes.

Using a split-GFP imaging system, Ctf18 was found to interact with Nup170 at the nuclear periphery. Ctf18 failed to reconstitute GFP with another nuclear pore protein (Nup49), highlighting the specificity of this interaction. This likely represents interaction of the NPC with replication forks since it peaked during DNA replication.

Because of their size and complexity, it has long been suggested that NPCs of different types, with distinct protein composition, may exist. Indeed, recent work has demonstrated that budding yeast cells possess (at least) three distinct types of NPCs: those without a basket, those with a basket, and those having a double basket (9). Which of these mediates the effect on gene silencing? To address this question, the authors tested for co-purification and colocalization of Ctf18 with basket proteins and found that Ctf18 neither co-localizes nor co-purifies with basket proteins. This suggests that NPCs lacking baskets interact with Ctf18-RFC to facilitate silencing of telomeres (Fig. 1).

Does interaction with Nup170 enhance the function of Ctf18-RFC in PCNA loading? To answer this question, the authors measured the association of PCNA with replication forks genome-wide. Loss of Nup170 led to a global decrease in PCNA loading. This suggests that the interaction

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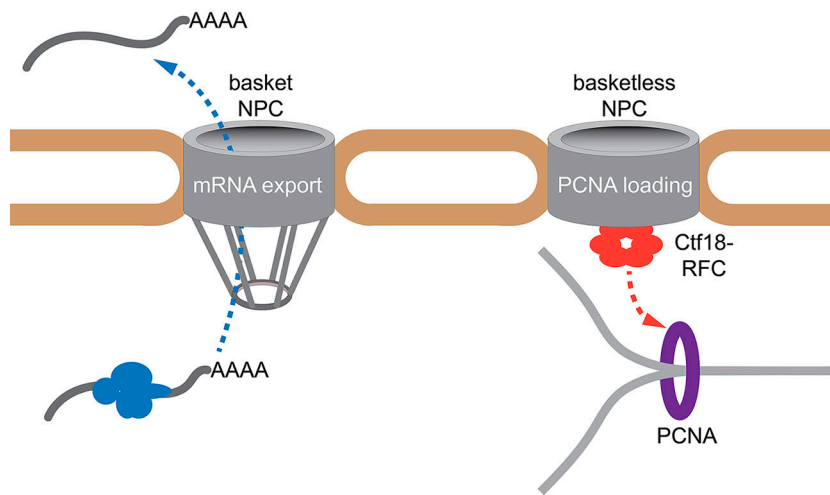Correspondence to Jason H. Brickner: j-brickner@northwestern.edu.

Figure 1. Distinct NPCs mediate distinct functions. In budding yeast, NPCs exist with or without nucleoplasmic baskets. Those with baskets seem to play an important role in mediating mRNA export (left). Kumar et al. (6) demonstrate that the Ctf18-RFC PNCA loading factor interacts specifically with NPCs lacking baskets (right), stimulating PCNA loading onto replication forks. PCNA facilitates transcriptional silencing of sub-telomeric genes.

of Ctf18-RFC with the NPC stimulates its function as a loader of PCNA (6).

Is the decrease in PCNA observed in strains lacking Nup170 responsible for the loss of telomeric silencing? To address this question, the authors exploited a mutation in a PCNA unloading factor. Mutants lacking this factor have a higher concentration of PCNA on chromatin. If the level of PCNA is the critical factor controlling telomeric silencing, then combining a mutation that reduces it with a mutation that enhances it might lead to recovery of telomeric silencing. Indeed, combining the loss of Nup170 with the loss of the PCNA unloading factor resulted in normal transcriptional silencing of sub-telomeric genes. Therefore, increasing PCNA levels on chromatin by inactivation of an unloading factor is sufficient to bypass the requirement for NPC-stimulated loading of PCNA for maintaining telomeric silencing.

PCNA has been suggested to promote silencing of chromatin by facilitating reincorporation of histones nearby during DNA replication (10). Silencing of sub-telomeres and the mating type loci is epigenetically inherited by a read-write mechanism. Unacetylated nucleosomes within silent loci are preferentially reintroduced at these loci following DNA replication. Unacetylated nucleosomes are "read" by the silencing factor Sir3, which facilitates the spreading and re-establishment of silencing through recruitment of the histone deacetylase complex Sir2/Sir4 (i.e., the writers). The reincorporation of unacetylated nucleosomes is facilitated by histone chaperones that physically interact with PCNA during DNA replication (11). This suggests a model for the function of NPC-stimulated loading of PCNA in silencing: increasing PCNA on replication forks increases the concentration of histone chaperones at the replication fork, which enhances the efficiency of the local reincorporation of nucleosomes.

If this model were correct, then combining mutations in the histone chaperones with loss of Nup170 should enhance the silencing defects associated with either. However, when the authors combined mutations in a critical histone chaperone CAF-1 with loss of Nup170, they observed a more profound loss of telomeric silencing. This indicates that the histone chaperone and Nup170 contribute in parallel to silencing and suggests that PCNA levels on chromatin alone is playing an important role in silencing, independent of the recruitment of chaperones.

This paper also raises several fascinating questions that will drive future work in this field. For example, how does Nup170 interact with specific chromosomal loci? Although the transcriptional effects of inactivation of Nup170 are most obvious at telomeres, the protein physically interacts with sites throughout the genome (5). Upon loss of Nup170, PCNA levels decrease genome-wide, rather than only at telomeres, which suggests that Nup170 has a general role in loading PCNA. While the interaction of Nup170 with telomeres requires the Sir4 silencing factor, the interactions with chromosome arms does not. The telomeres and chromosome arms may have distinct mechanisms of interaction with Nup170 or a unifying mechanism that is regulated by factors such as Sir4.

Nup170 at NPCs lacking baskets interacts with Ctf18-RFC. This raises the possibility that distinct NPCs are either stable or interchanging. Recent work has shown that formation of the basket requires ongoing export of mRNAs, suggesting that baskets can be assembled or lost over time (12). But the dynamics of NPC interconversion is not well documented. If NPC subtypes interchange, this could serve to regulate chromatin tethering interactions that are subtype specific. If NPC subtypes are stable and do not readily interchange, individual NPCs could serve as stable functional sites on the nuclear envelope.

Future work will continue to expand our understanding of the functions of the NPC in regulating eukaryotic genomes. Given the complexity and diversity of this structure and its interactions with chromatin, such work will certainly reveal additional ways that this cellular landmark serves as a platform for myriad regulatory functions.

References

1. Alber, F., et al. 2007. *Nature*. https://doi.org/10.1038/nature06405
2. Hampoelz, B., et al. 2019. *Annu. Rev. Biophys.* https://doi.org/10.1146/annurev-biophys-052118-115308
3. Wing, C.E., et al. 2022. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/s41580-021-00446-7
4. Sumner, M.C., and J. Brickner. 2021. *Cold Spring Harb Perspect Biol.* https://doi.org/10.1101/cshperspect.a039438
5. Van de Vosse, D.W., et al. 2013. *Cell.* https://doi.org/10.1016/j.cell.2013.01.049
6. Kumar, S., et al. 2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202207060
7. Moldovan G.-L., et al. 2007. *Cell.* https://doi.org/10.1016/j.cell.2007.05.003
8. Hiraga, S. I., et al. 2006. *EMBO J.* https://doi.org/10.1038/sj.emboj.7601038
9. Akey, C.W., et al. 2022. *Cell.* https://doi.org/10.1016/j.cell.2021.12.015
10. Brothers, M., and J. Rine. 2019. *Genetics.* https://doi.org/10.1534/genetics.119.302452
11. Gartenberg, M.R., and J.S. Smith. 2016. *Genetics.* https://doi.org/10.1534/genetics.112.145243
12. Bensidoun, P., et al. 2022. *Mol. Cell.* https://doi.org/10.1016/j.molcel.2022.09.019