Molecular Cell
Previews

Mediator: A Drawbridge across the Enhancer-Promoter Divide

Sohail Malik[1,*] and Robert G. Roeder[1]
[1]Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA
*Correspondence: maliks@rockefeller.edu
http://dx.doi.org/10.1016/j.molcel.2016.10.024

Two papers in this issue of *Molecular Cell* provide insights into how the multisubunit Mediator coactivator complex dynamically links enhancer-bound activators to the RNA polymerase II machinery at the core promoter.

The large multisubunit Mediator cofactor complex, which controls the transcriptional programs of most genes in eukaryotic cells, is composed of four modules (Allen and Taatjes, 2015). Recent work established that Mediator head and middle modules, together with a scaffold subunit, constitute a conserved core (Cevher et al., 2014; Plaschka et al., 2015) that carries out the most basic functions through interactions with RNA polymerase II (Pol II) and other preinitiation complex (PIC) components. Many tail subunits physically interact with enhancer-bound transcriptional activators, whereas the kinase module can have both repressive and stimulatory effects. Therefore, in contrast to the core, tail and kinase modules play modulatory, albeit critical, roles. However, the Mediator complex is functionally more than the sum of its constituent modules, and a main goal in the field has been to elucidate mechanisms whereby the over two dozen of its subunits (the exact number varies between species) work as a unit to regulate multiple aspects of gene transcription. Despite strong biochemical and structural evidence that the PIC—and hence the core promoter and the transcriptional start site—is the ultimate site of Mediator's action, an unanticipated paradox had been that Mediator was hard to visualize by chromatin immunoprecipitation (ChIP) at core promoters. Mediator appeared to be localized mainly to the activator binding site-bearing enhancer regions, which could be located at considerable distances from core promoters, especially in metazoans. 2 years ago, the Robert and Struhl labs independently showed that inactivation in yeast of the Kin28 (CDK7) subunit of general transcription factor TFIIH, which normally phosphorylates the CTD of the large subunit of Pol II to regulate promoter clearance in vivo, allowed Mediator to accumulate at the core promoter (Allen and Taatjes, 2015). Their studies showed that Mediator interaction with the PIC is transient and controlled at least in part by phosphorylation. Now, in significant extensions of these studies, which appear in the current issue of *Molecular Cell* (Jeronimo et al., 2016; Petrenko et al., 2016), the two groups provide detailed views of module dynamics as activator-recruited Mediator at the enhancer is delivered to the PIC to activate the target gene.

As in their earlier studies, these groups relied on their ability to freeze otherwise transient Mediator interactions at the promoter to gauge how Mediator partitions between the enhancer and the core promoter. The Robert lab (Jeronimo et al., 2016) uses analog sensitive Kin28 yeast strains in which the enzymatic activity can be inactivated by treating with ATP analogs. The Struhl lab (Petrenko et al., 2016) uses the "anchor away" approach to rapidly deplete nuclear Kin28. Both groups combined these approaches with ChIP to localize almost a dozen Mediator subunits representing each of the four modules. The data revealed that the Mediator that is "constitutively" associated with enhancers consists of all four modules. By contrast, when Mediator is detectible at core promoters upon Kin28 treatments, it no longer has the kinase module. This is reminiscent of metazoan Mediator studies that suggested that the kinase-deficient form of the Mediator (PC2/CRSP) is generated from the complete complex as part of the transcription process after the latter is initially recruited by activators (Malik et al., 2005; Pavri et al., 2005). Consistent with recent structural studies of Mediator-containing PICs (Plaschka et al., 2015; Robinson et al., 2016), the new ChIP analyses further revealed that it is the head and middle subunits that localize to core promoters of active genes; in general, the tail subunits remain enhancer associated throughout. Importantly, both groups further established that when Mediator subunits are detected at core promoters, it is a single Mediator complex that occupies the enhancer-promoter combination of any given gene.

The inescapable conclusion is that an enhancer-associated Mediator complex, through prior or concomitant loss of its kinase module, interacts with the PIC, thereby transiently linking the enhancer and the core promoter via a chromatin loop. These studies thus provide formal in vivo proof for earlier models that postulated just such a physical Mediator-dependent link between enhancer-bound activators and core promoter-bound PIC. At the same time, the studies emphasize the underlying dynamic aspects, both with regard to the compositional change in the Mediator and the transient nature of the enhancer-promoter bridge. In this regard, the manner in which enhancer-bound Mediator engages the PIC is more reminiscent of a drawbridge that can be extended and retracted on demand than of the more permanent bridge structures that normally come to mind.

What causes the enhancer-anchored Mediator drawbridge to extend out to the core promoter? Is the eviction of the kinase module a cause or a consequence of bridge formation? Petrenko et al. (2016) suggest that simple competition between

the kinase module and Pol II for association with the Mediator may furnish a mechanistic basis for this key event in transcriptional activation. This would imply that following activator recruitment of Mediator, the remaining series of events would transpire stochastically. However, as illustrated by PARP1, which effects interconversion of Mediator forms in metazoans (Pavri et al., 2005), one suspects that subsequent interactions might potentially be more deterministically regulated, and at least in some cases, in a gene-specific manner. When Jeronimo et al. (2016) mutated the active site of CDK8, the eponymous kinase of the module, Mediator residence time at the enhancer was affected, just as when MED13, which anchors the kinase module to the core complex, was deleted. This suggests that CDK8-dependent phosphorylation (and degradation) of enhancer-bound transcription factors or of unidentified (tail?) Mediator subunits might be important. However, complicating matters is the observation that while the kinase module is important in determining how long the Mediator resides at the enhancer, eviction of the kinase module is apparently not rate-limiting with regard to Mediator-PIC interaction, at least at the global level of the current analyses. Perhaps reflecting the dual positive and negative roles of the kinase module, Mediator bridging of the enhancer and promoter could be the net result of multiple competing and potentially regulatable phenomena, including the enhancer residence time of the kinase module-containing Mediator and partitioning between the kinase-containing Mediator and the kinase-lacking Mediator. Hopefully, recapitulation of a functional Mediator-dependent enhancer-promoter bridge in cell-free in vitro assay systems (which thus far have mainly focused on templates in which activator binding sites are juxtaposed very close to the core promoter) will soon allow the contributions of these various parameters to be rigorously assessed.

Recent work has highlighted the critical roles that enhancers, including super-enhancers, play in metazoan development and human disease (Hnisz et al., 2013). Super-enhancers in particular appear to be significant Mediator reservoirs. Although enhancer-promoter chromatin looping models entailing Mediator and associated cohesin, as well as ncRNAs, have been proposed (Allen and Taatjes, 2015), the details of the underlying mechanisms in higher organisms remain unknown. It also is emerging that transcription in metazoans occurs in bursts of activity and that enhancers function in this process to increase not the "burst size" of promoters but the frequency at which they fire (Fukaya et al., 2016). Given the high degree of conservation between the yeast and metazoan Mediator complexes, as well as indications of Mediator interconversion during the activation process (Malik et al., 2005; Pavri et al., 2005), the findings of Jeronimo et al. (2016) and Petrenko et al. (2016) should extrapolate to higher organisms. But given further that metazoan Mediator contains many subunits not found in the yeast complex, as well as significant sequence divergence even in subunits that are conserved through evolution, the precise details of how Mediator might be involved in episodic enhancer-promoter interactions in metazoans, if that is how they occur, might yet turn to be quite divergent. An increasing number of human diseases, including cancer and neurological disorders, have been traced to mutations in subunits of the kinase module (Allen and Taatjes, 2015). The new findings raise the possibility that some aspect of dynamic enhancer-promoter looping might be compromised in these diseased states. This further underscores the need to devise methods to trap Mediator at various locations and monitor its compositional dynamics in metazoan cells.

REFERENCES

Allen, B.L., and Taatjes, D.J. (2015). Nat. Rev. Mol. Cell Biol. *16*, 155–166.

Cevher, M.A., Shi, Y., Li, D., Chait, B.T., Malik, S., and Roeder, R.G. (2014). Nat. Struct. Mol. Biol. *21*, 1028–1034.

Fukaya, T., Lim, B., and Levine, M. (2016). Cell *166*, 358–368.

Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-André, V., Sigova, A.A., Hoke, H.A., and Young, R.A. (2013). Cell *155*, 934–947.

Jeronimo, C., Langelier, M.-F., Bataille, A.R., Pascal, J.M., Pugh, B.F., and Robert, F. (2016). Mol. Cell *64*, this issue, 455–466.

Malik, S., Baek, H.J., Wu, W., and Roeder, R.G. (2005). Mol. Cell. Biol. *25*, 2117–2129.

Pavri, R., Lewis, B., Kim, T.K., Dilworth, F.J., Erdjument-Bromage, H., Tempst, P., de Murcia, G., Evans, R., Chambon, P., and Reinberg, D. (2005). Mol. Cell *18*, 83–96.

Petrenko, N., Jin, Y., Wong, K.H., and Struhl, K. (2016). Mol. Cell *64*, this issue, 443–454.

Plaschka, C., Larivière, L., Wenzeck, L., Seizl, M., Hemann, M., Tegunov, D., Petrotchenko, E.V., Borchers, C.H., Baumeister, W., Herzog, F., et al. (2015). Nature *518*, 376–380.

Robinson, P.J., Trnka, M.J., Bushnell, D.A., Davis, R.E., Mattei, P.J., Burlingame, A.L., and Kornberg, R.D. (2016). Cell *166*, 1411–1422.e16.