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Get Back TFIIF, Don't Let Me Gdown1

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In this issue of *Molecular Cell*, papers by the Price and Roeder labs reveal how the Gdown1 protein antagonizes the general transcription factor TFIIF during RNAPII initiation and elongation and how the Mediator complex intervenes in this molecular tug-of-war to activate RNAPII.

In 1969, The Beatles recorded "Get Back" and "Don't Let Me Down," two songs that were later released as a single with "Get Back" on the A side. Paul McCartney and John Lennon composed mostly individually at this late stage, but these songs still represent their intertwined genius. McCartney penned "Get Back," but Lennon's three guitar solos pillar the song; Lennon wrote "Don't Let Me Down," but McCartney's backing vocals make it work. Fast forward 42 years to the January 2012 issue of *Molecular Cell*: the Price and Roeder labs release back-to-back papers providing major basic insights into the inner workings of the eukaryotic transcriptional machinery. Their reports contain important discoveries on the roles of the general transcription factor TFIIF, the protein Gdown1, and the Mediator coactivator complex in control of RNA polymerase II (RNAPII) activity. The two papers stand alone and may become classics on their own merit, but together they are better. They do not always agree, and the two labs reach some distinct conclusions. But, as is also true in art, disparate approaches and observations can reveal equally valid truths.

TFIIF is one of the general transcription factors (GTFs) required for formation of the preinitiation complex (PIC), the molecular assembly that recruits RNAPII to promoters and facilitates transcription initiation. Additionally, TFIIF stimulates RNAPII elongation. Previous work by the Price team showed that the positive effects of TFIIF on RNAPII elongation can be blocked by a factor present in nuclear extracts (Cheng and Price, 2007). In their new report (Cheng et al., 2012), they characterize Gdown1 as the "TFIIF resistance factor." Using elegant biochemical assays, they show that Gdown1 prevents binding of TFIIF to elongation complexes to cause RNAPII pausing, thus joining other "pausing factors" such as NELF and DSIF. They find that Gdown1 also inhibits the activity of the termination factor TTF2, thereby preventing the release of short transcripts and RNAPII dissociation. Importantly, the negative effects of Gdown1, NELF and DSIF are all relieved by P-TEFb, the positive elongation factor.

Interestingly, Gdown1 was first characterized by Roeder and colleagues as a polypeptide tightly bound to RNAPII that created a requirement for the Mediator coactivator complex during transcriptional activation (Hu et al., 2006). They found that transactivators stimulate RNAPII lacking Gdown1 in the absence of Mediator. However, if Gdown1 is present, RNAPII becomes refractory to the transactivators and Mediator becomes indispensable for activating transcription. In their current paper (Jishage et al., 2012), they expand their studies to show that Gdown1 also represses "basal" transcription (i.e., in the absence of transactivators), pointing to an effect on the general transcriptional machinery. Using a different set of biochemical assays than those employed by the Price team, they demonstrate that an excess of TFIIF relieves the negative effects of Gdown1 on basal transcription. Gdown1 assembles an alternative promoter complex containing TBP, TFIIB, and RNAPII, which is inert relative to the authentic PIC containing TFIIF. Thus, RNAPII exists in mutually exclusive complexes with either

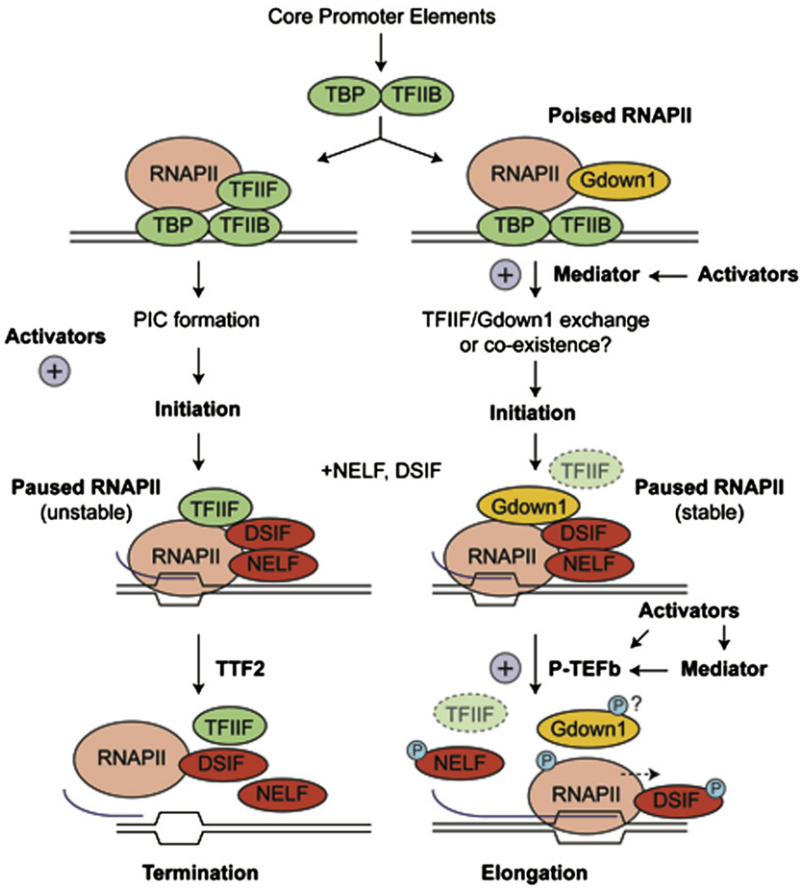

Figure 1. Gdown1 Antagonizes TFIIF during Both PIC Formation and Elongation, Thus Creating Poised and Stably Paused Forms of RNAPII, Respectively
The Mediator complex is required to activate transcription in the presence of Gdown1. Mediator helps RNAPII escape poised and paused states by favoring TFIIF inclusion and/or recruiting P-TEFb.

Gdown1 [referred to as RNAPII(G)] or with TFIIF, and the two factors compete for binding to the RNAPII subunits RPB1 and RPB5. Surprisingly, they show that RNAPII(G) copurifies with several Mediator subunits and can be recruited to a promoter by transactivators in a Mediator-dependent fashion.

Although both teams characterize Gdown1 as a repressor of RNAPII that acts by antagonizing TFIIF, they point to different steps in the transcription cycle at which Gdown1 functions. The Price team studied RNAPII elongation and found that Gdown1 functions as a negative elongation factor. The Roeder team focused on basal transcription and found that Gdown1 blocks PIC formation. Taken together, their findings could be integrated into an intriguing stepwise model (see Figure 1):

(1) Core promoter elements initiate PIC formation by recruiting the TFIID and TFIIB general transcription factors.
(2) A fork in the road appears. If RNAPII(G) is recruited to the nascent PIC, TFIIF binding is blocked, true PIC formation is aborted, and initiation does not take place. I will refer to this as "poised" RNAPII. If RNAPII lacking Gdown1 is recruited, then TFIIF binding is permitted and PIC formation succeeds, thus allowing initiation.
(3) Soon after initiation, negative elongation factors such as NELF and DSIF stop RNAPII in its tracks after transcription of a short RNA. I will refer to this as "paused" RNAPII. If Gdown1 is present at this stage, it will repress TTF2, thus preventing termination and ensuring stable RNAPII pausing.
(4) Upon signaling, transactivators either bind to DNA or become activated on chromatin. They can stimulate RNAPII in the absence of Gdown1, but they must recruit Mediator to activate RNAPII(G).
(5) Upon recruitment, Mediator orchestrates enhanced RNAPII activity at multiple steps of the transcription cycle. Mediator overcomes the negative effects of Gdown1 on poised and paused RNAPII, perhaps by favoring the exchange of Gdown1 for TFIIF and/or facilitating the recruitment of P-TEFb. Of note, Mediator can form a stable complex with TFIIF and RNAPII (Bernecky et al., 2011) and also interacts directly with the super elongation complex that contains P-TEFb (Takahashi et al., 2011).
(6) Enter P-TEFb. Upon recruitment by Mediator or other factors, P-TEFb promotes elongation by phosphorylating various targets (Peterlin and Price, 2006). Intriguingly, Gdown1 is a phosphoprotein containing conserved phosphoacceptor sites, yet the kinase(s) involved and the impact of these phosphorylation events are not known.
(7) The grand finale. When activation signals dissipate, the transactivators are rapidly inactivated. Paradoxically, all along the transactivators have indirectly recruited "repressed" RNAPII(G) via Mediator, thus creating a constant requirement for the coactivator. This establishes a hard-wired shut down mechanism: activators recruit Mediator, which recruits RNAPII(G), which only responds to the transactivator for as long as Mediator is present. In the last round of transactivation, RNAPII(G) is left at the promoter either poised or paused, and it will not proceed further until activating signaling resumes.

This model ties together the in vitro observations made by both teams; however, to what degree these results

represent the in vivo scenario? To address this, both teams localized Gdown1 on a genome-wide scale via chromatin immunoprecipitation (ChIP) and tested the effects of Gdown1 knockdown. The data obtained show agreements but also discrepancies, which lead the authors to different conclusions. Some confusion is created by the fact that the Roeder lab uses the terms "poised" and "paused" as defined here, but the Price lab does not. Importantly, ChIP assays cannot adequately discriminate between poised and paused RNAPII. Although peaks of RNAPII occupancy slightly downstream of transcription start sites (TSSs) would suggest "pausing" rather than "poising," this is not conclusive. In their ChIP-seq experiments using HeLa cancer cells, the Price group observed that Gdown1 binds thousands of promoters occupied by RNAPII, leading to the conclusion that Gdown1 plays a global role in transcription. In contrast, the Roeder team performed their ChIP-seq in IMR90 human fibroblasts, and their analysis showed only 60 genes of high-confidence Gdown1 occupancy, suggesting a gene-specific effect. This discrepancy can be explained by technical differences in the ChIP-seq protocol, such as antibodies used, extent of crosslinking, sequencing depth, or peak-calling algorithms. More interestingly, this could reflect biological differences between cell types, a notion supported by the fact that Gdown1 knockdown is lethal in HeLa cells, but does not affect the growth of IMR90 fibroblasts. Despite the differences in the number of Gdown1 target loci, both teams make the interesting observation that while the average RNAPII signals peak slightly downstream of TSSs, Gdown1 signals peak around or upstream of TSSs. However, the teams differ in their interpretation of this result. The Roeder lab argues that the slightly upstream location of Gdown1 represents RNAPII(G) in a poised, preinitiated state and that Gdown1 does not cause proximal pausing. In contrast, the Price team focuses on the fact that the occupancy profiles for Gdown1 and RNAPII are largely overlapping, with significant Gdown1 presence at sites of paused RNAPII, thus concluding a role for Gdown1 in pausing. Readers will find the last chapters of each paper to be a clear example of healthy scientific debate around the meaning of subtle differences in ChIP peak positions. Most importantly, both teams concur on Gdown1 acting as a repressor in vivo, as they observed increase in RNAPII escape into coding regions (Price) and upregulation of mRNA levels (Roeder) upon Gdown1 knockdown.

Would the world today be a better place if The Beatles had not split up? Perhaps we should be grateful for the creative tension that produced gems like "Get Back" and "Don't Let Me Down." I for one am grateful that the Price and Roeder papers complement each other and agree on many, but not all, of their conclusions. I can hardly wait for their next releases.

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