

The relationship between the general transcription factor TFIIF and Gdown1 in promoter proximal pausing and the properties of TFIIFs truncated versions 1-217 and 1-356 within that relationship



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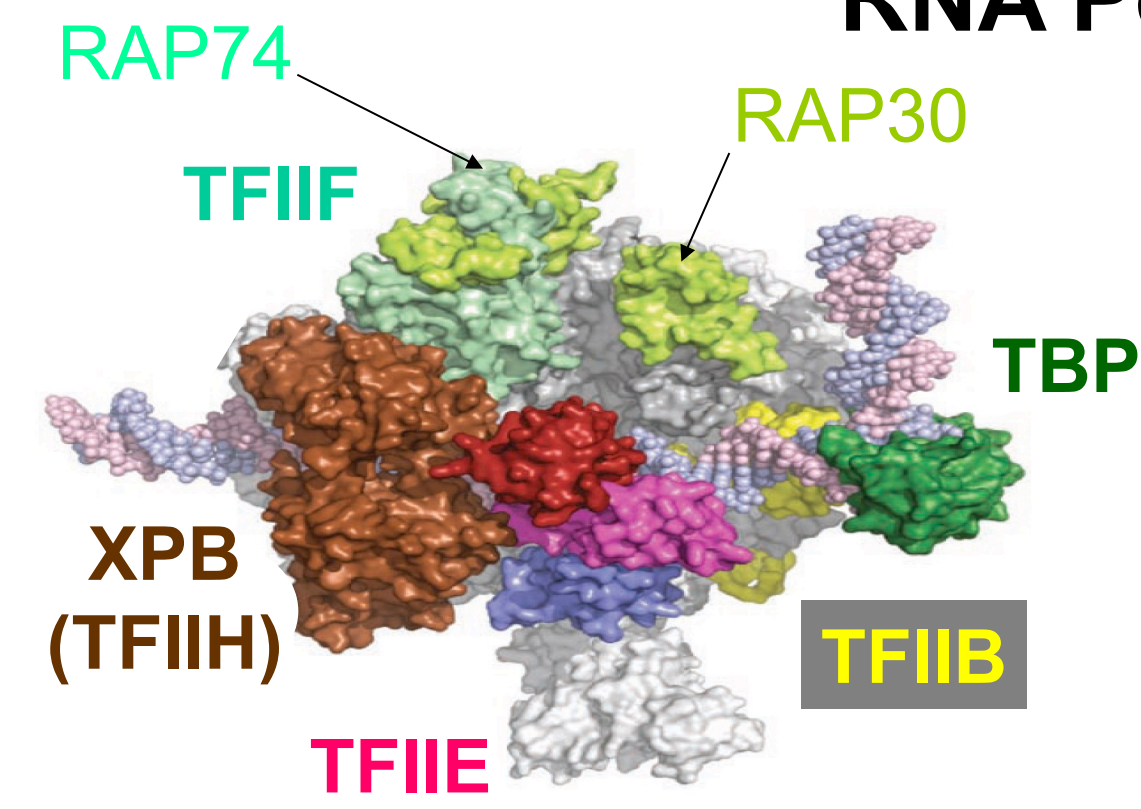
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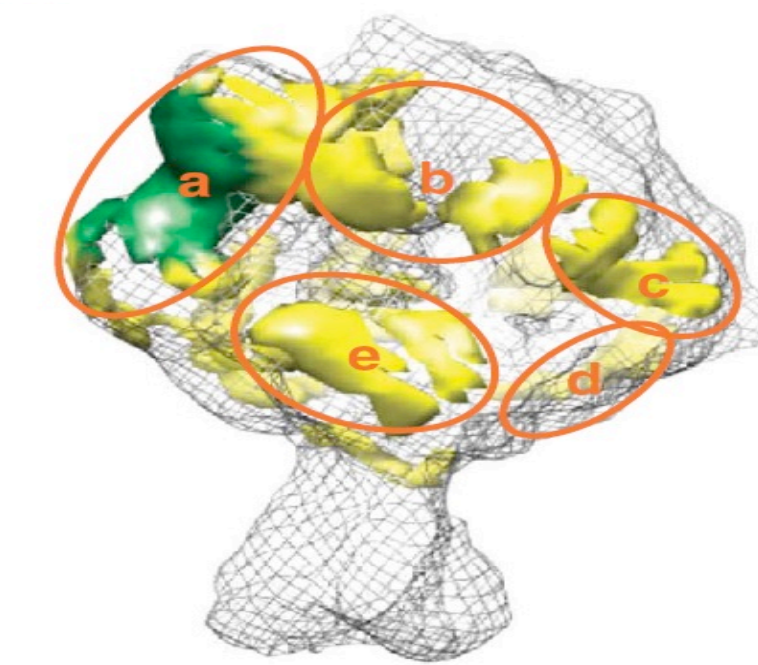
Abstract

RNA Polymerase II (Pol II) requires several additional factors, known as general transcription factors (GTFs), to recognize promoters and initiate the synthesis of pre-mRNA. It is now appreciated that a post-initiation step, involving extended pausing of Pol II at ~50 base pairs downstream of transcription start, is also a key regulatory step in gene expression. It is not well understood how the GTFs relate to the factors that control this pausing. Of particular interest, is the relationship between one GTF, TFIIF, and one of the pausing factors, Gdown1. Gdown1 associates very tightly with Pol II and it can be thought of as a 13th, substoichiometric subunit of polymerase. Surprisingly, Gdown1 shares binding sites on Pol II with TFIIF, blocks TFIIF association with free Pol II, and displaced TFIIF from Pol II during transcript elongation. An important question thus arises: How can transcription begin if a factor necessary in pausing blocks the association of a factor necessary for transcription to start? Here I show, using a well-characterized in vitro transcription system: 1) that once a pol II pre-initiation complex (PIC) is formed, Gdown1 cannot displace TFIIF from it and 2) that the truncated versions of TFIIF are less effective at rescuing transcription than full length TFIIF.

Gdown1 and TFIIF Have Overlapping Binding Sites on RNA Polymerase II

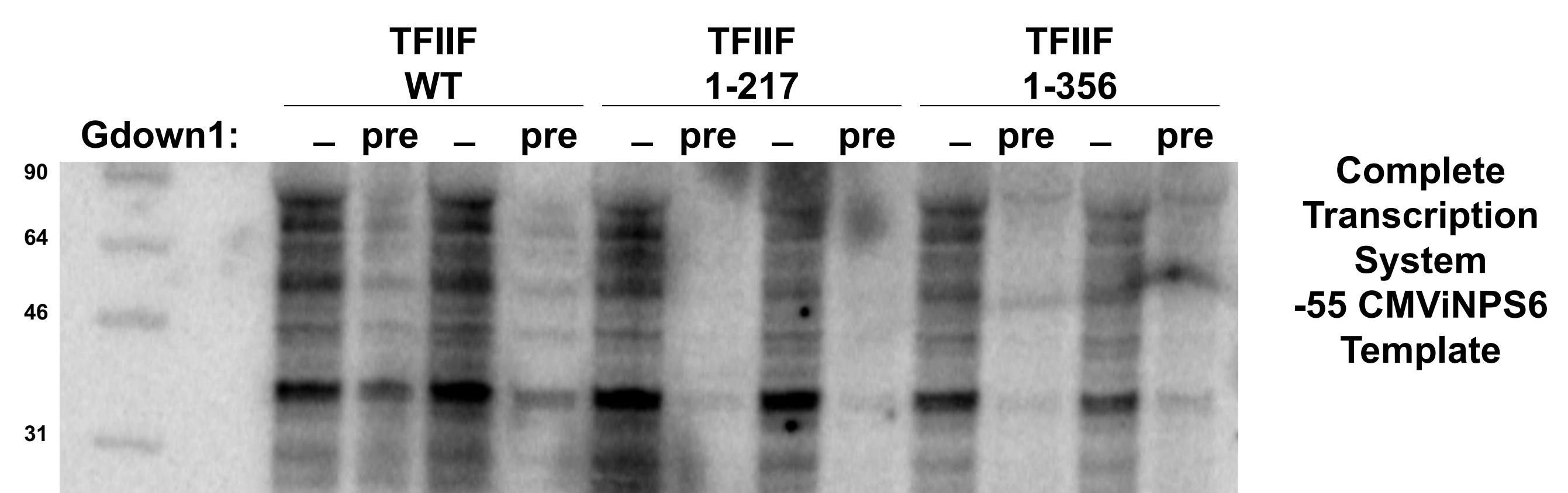
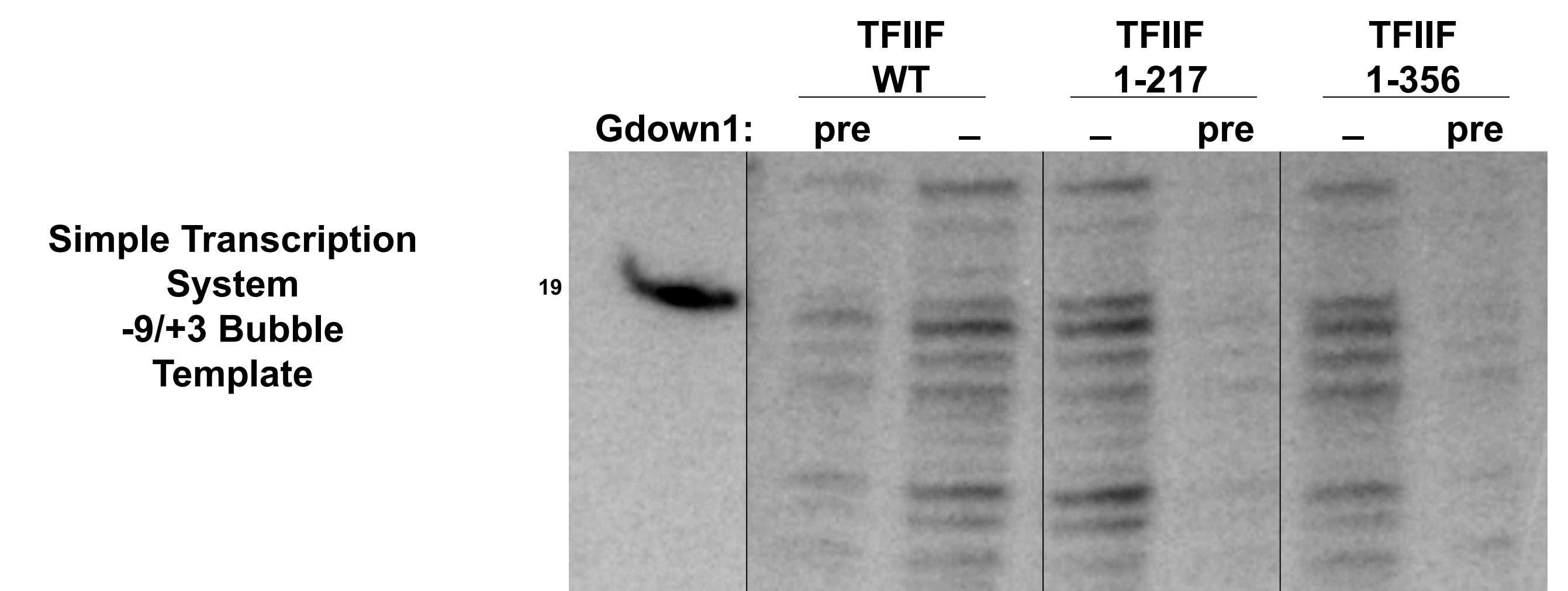


Structure of Pol II with General Transcription Factors⁵
The exact extent of RAP74 into the center of the structure is unknown.



Cryo-Electron Microscopy of Gdown1 Binding Regions⁶
Gdown1 has binding sites all over the polymerase, but most strongly associates with the area in green.

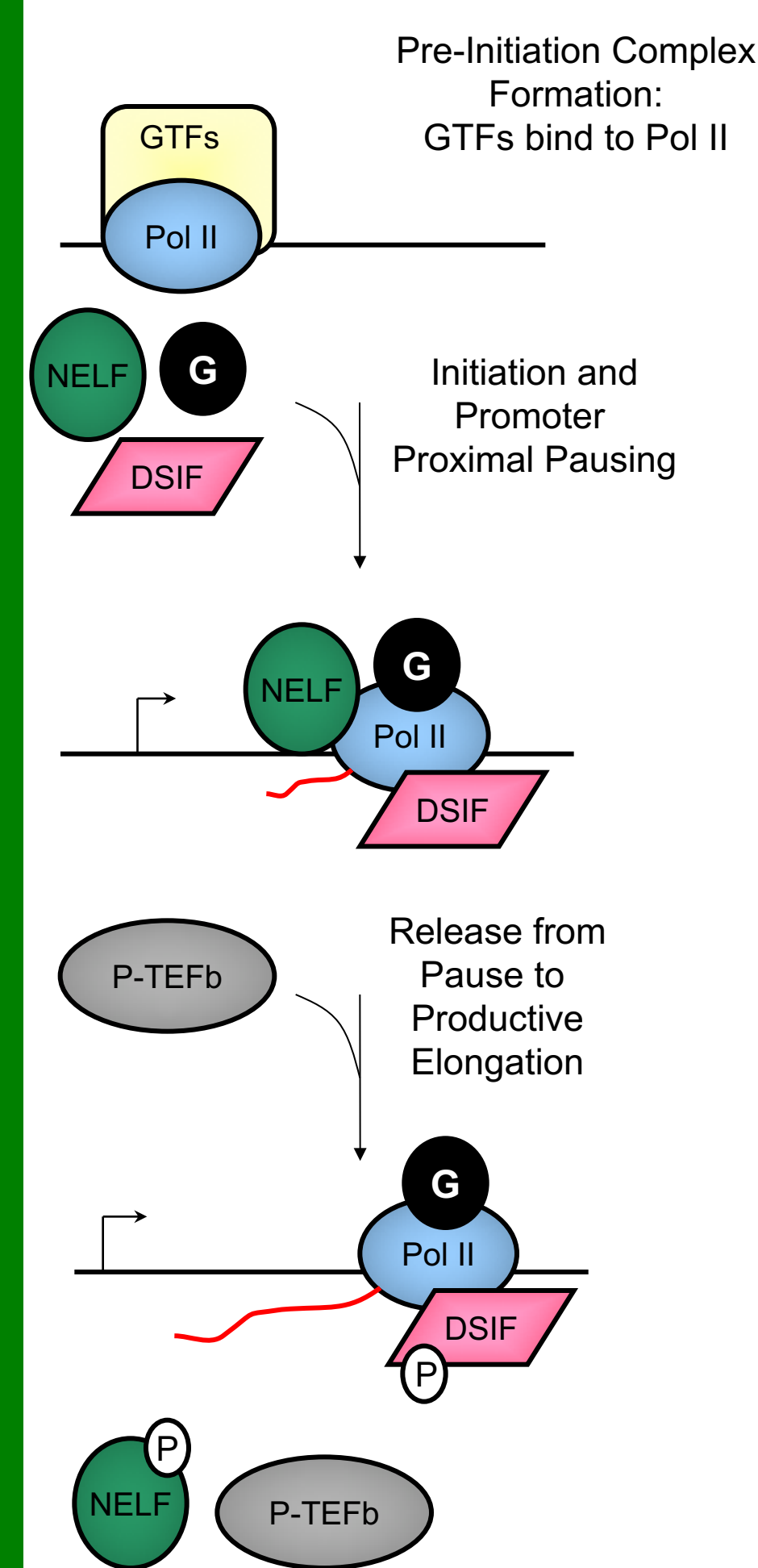
Neither TFIIF 1-217 nor TFIIF 1-356 Can Compete Effectively with Gdown1 When it is Added Pre-PIC Formation in Either Simple or Complete Transcription Systems



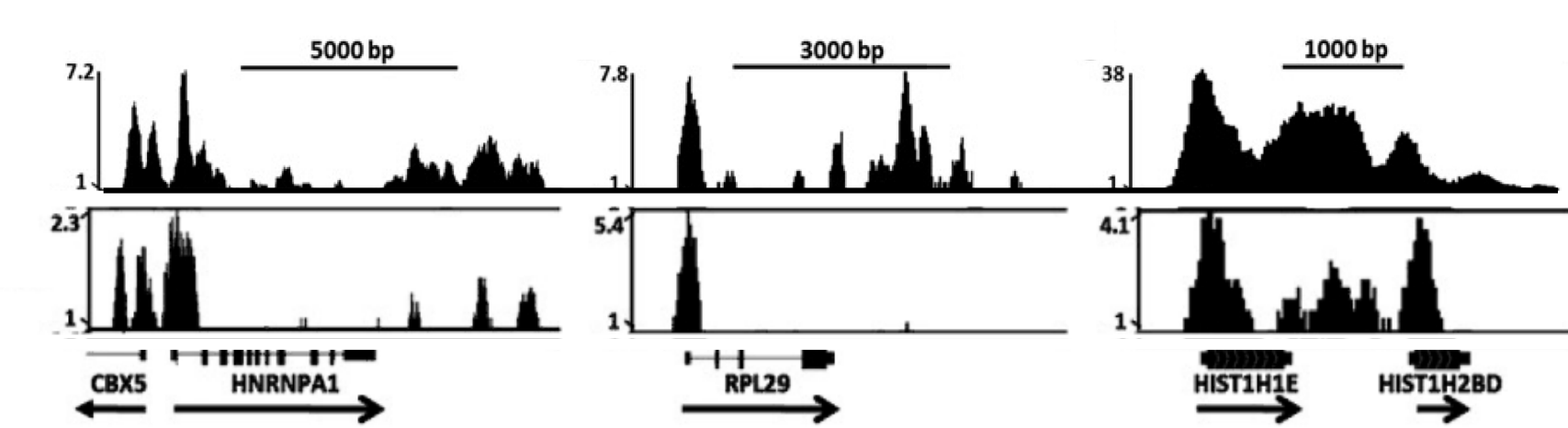
When Pol II is incubated with excess Gdown1 before PIC formation, transcription is inhibited in either assay. Wildtype TFIIF provides significantly better protection against this Gdown1 challenge than the 1-356 truncation, which was slightly more effective in the challenge assay than 1-217.

RNA Polymerase II Promoter Proximal Pausing

Model for Promoter Proximal Pausing¹

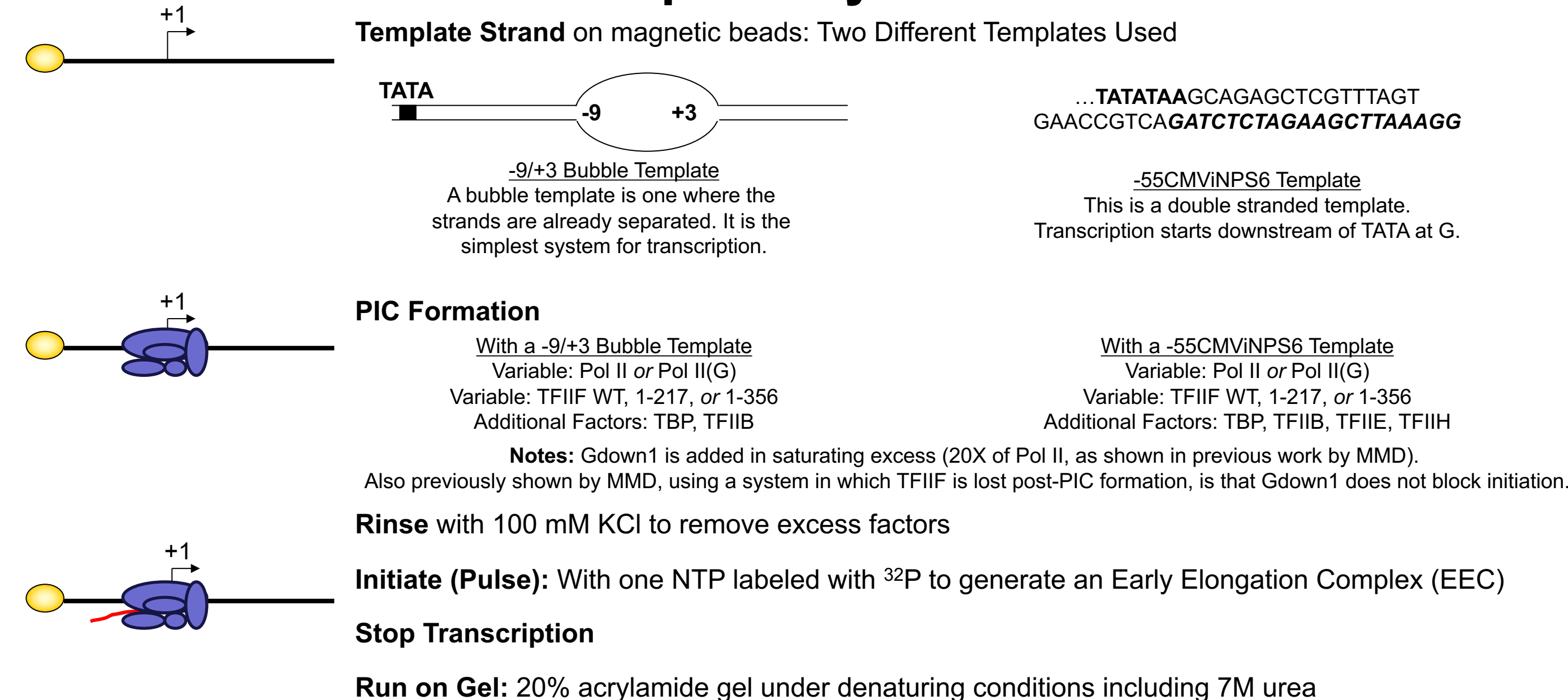


In eukaryotic transcription, rate-limiting steps help ensure proper regulation of gene expression. A major regulatory step in higher eukaryotes occurs when RNA polymerase II (Pol II) pauses around 50 bp downstream of the transcription start site (TSS). The accepted model for the induction of promoter proximal pausing involves two Pol II binding factors known as DSIF and NELF. Pausing is released when the factor P-TEFb phosphorylates both DSIF and NELF. pNELF and P-TEFb fall away, while pDSIF remains with Pol II. While DSIF and NELF are required for pausing, in vitro studies of the pausing process have shown that they alone are not sufficient at establishing pausing. Those studies have also indicated an important role in pausing for a 13th substoichiometric subunit of Pol II called Gdown1. ChIP-Seq data shows that Gdown1 is found primarily with Pol II around the site of promoter proximal pausing, consistent with a role for Gdown1 along with DSIF and NELF in establishing the paused state. Once Pol II is released from pausing, Gdown1 is thought to continue downstream along with the polymerase.

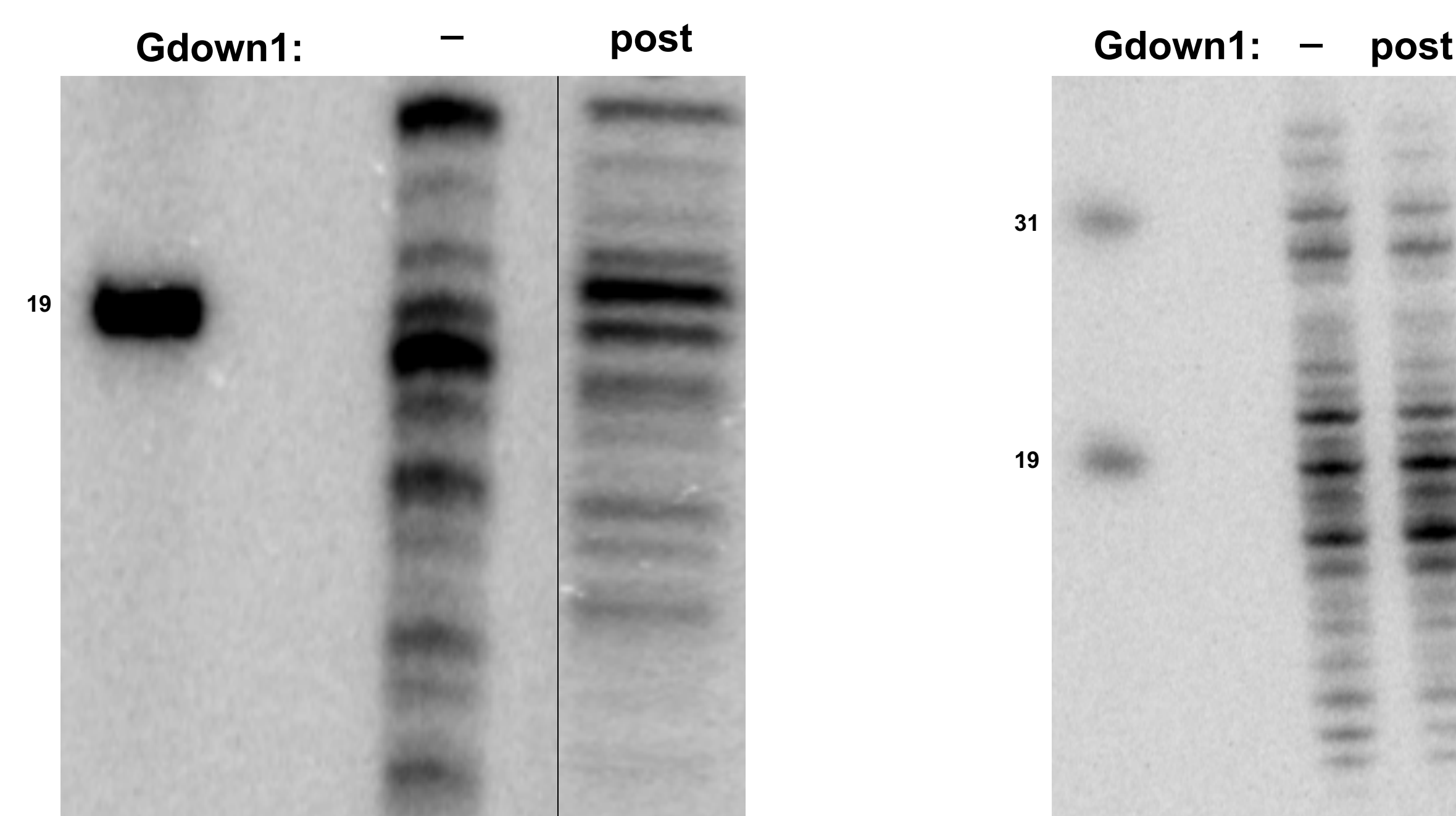


ChIP-Seq for Pol II (top) and Gdown1 (bottom) in HeLa cells. Gdown1 maps across the spectrum, but is primarily associated with Pol II around the site of promoter proximal pausing.²

Transcription Systems¹



Addition of Gdown1 Post-PIC Formation Does Not Diminish Transcription Signal in Either Simple or Complete Transcription Systems



Simple Transcription System -9/+3 Bubble Template

Complete Transcription System -55CMVINPS6 Template (MMD)

When Gdown1 is added post-PIC formation, no inhibition of transcription was seen for either the simple or the complete transcription system. This is somewhat surprising since TFIIF, which is a required transcription factor, is displaced from free Pol II and from Pol II engaged in transcription by Gdown1.

Conclusions

1. Gdown1 cannot displace TFIIF effectively when added post-PIC formation in the simple or complete transcriptions systems.
2. When Gdown1 is added pre-PIC formation, wildtype TFIIF can partially compete to support to transcription; however, neither TFIIF 1-217 nor TFIIF 1-356 can compete effectively with Gdown1 in these assays.

Future Directions

This research is ongoing. Questions that may be explored in future research include:

1. Do the survivors, those PICs that elongate despite saturation with Gdown1, have Gdown1 on them? What exactly are they composed of?
2. Is there co-residency of TFIIF, truncated or not, and Gdown1 at any point?

Sources

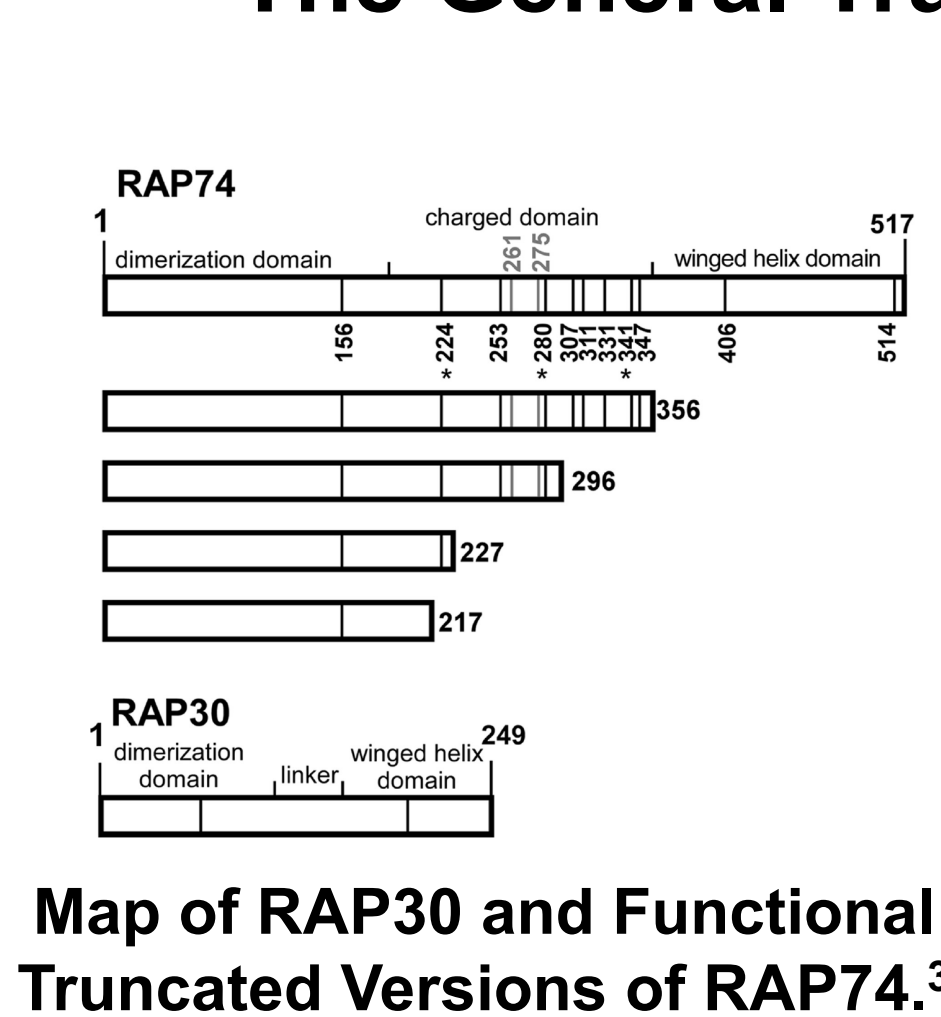
- ¹Axson and Mullen Davis 2013
²Cheng 2012 Mol Cell 45:38 (Partial Figure 5)
³Újvári 2011 J Bio Chem 286:26
⁵Grunberg 2012 Nat Struc Mol Biol 29: 788
⁶Wu 2012 EMBO J 31: 3575

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The General Transcription Factor TFIIF and Gdown1



The transcription factor TFIIF is required for pre-initiation complex (PIC) assembly, but once the complex is assembled it is no longer required. TFIIF also promotes elongation if present in the productive elongation complex (PEC). Wildtype TFIIF has two subunits, RAP30 and RAP74, each with three domains. The RAP74 subunit also contains multiple phosphorylation sites. The subunit RAP74 can be truncated at various points, eliminating phosphorylation sites and/or domains; these truncated TFIIFs are functional. These experiments focus on TFIIF 1-217 and TFIIF 1-356. TFIIF 1-217 is the shortest functional truncation, removing the charged domain and the winged-helix domain. TFIIF 1-356 is the shortest truncation that still has all of the phosphorylation sites of the wildtype.

Further research revealed something troubling: Gdown1 is antagonistic towards the essential TFIIF in binding with Pol II. It turns out that TFIIF and Gdown1 have overlapping binding sites on Pol II (see top center). In competitive binding assays using either free Pol II or Pol II engaged in transcript elongation, Gdown1 always displaces TFIIF from the polymerase. Thus, Pol II elongation complexes that contain Gdown1 are not responsive to stimulation by TFIIF.