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Rethinking the role of TFIIF in transcript initiation by RNA polymerase II

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TFIIF is considered to be a general transcription factor, based on the fact that it is essential for assembly of RNA polymerase II preinitiation complexes on fully double-stranded templates in vitro. Existing models assign various tasks to TFIIF during pre-initiation complex formation and transcript initiation. Recent results do not support all aspects of those models but they do emphasize the significance of the interaction of TFIIF and TFIIB. Other recent findings raise the possibility that a fraction of RNA polymerase II transcription complex assembly proceeds through a pathway that is independent of TFIIF.

Keywords: TFIIF, RNA polymerase II, initiation, general transcription factors, preinitiation complex, TFIIB, transcription bubble, Gdown1

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The Role of TFIIF within the Pre-Initiation Complex

It has long been appreciated that RNA polymerase II (RNP II) requires numerous additional factors to recognize promoters and initiate RNA synthesis.[1-3] The canonical minimal set of general transcription factors (GTFs) for pre-initiation complex (PIC) assembly at promoters containing a TATA box includes the TATA box binding protein TBP, TFIIB, TFIIF, TFIIE and TFIIH. The assembly pathway begins with binding of TBP and TFIIB to the template, followed by loading of RNP II along with TFIIF. The TFIIE and TFIIH factors complete the PIC,[1-3] which can convert to an open complex through the XPB helicase subunit of TFIIH.[4,5] Many reports link TFIIB to transcript initiation.[6-9] TFIIF is also thought to be important for the initiation step itself.[10-12] The role of mammalian TFIIF in initiation is emphasized by experiments employing pre-melted (bubble) templates. Such templates support transcription with TBP and TFIIB alone but RNA synthesis is very strongly stimulated by TFIIF.[13,14] Results using yeast RNP II and GTFs do not fully agree with findings in the mammalian system;[15] this point will be discussed further below.

Structural studies of yeast TFIIB-RNP II complexes show that a segment of TFIIB reaches into the active center of the polymerase, consistent with the importance of TFIIB in transcript initiation.[16,17] TFIIF was originally identified as a factor with very high affinity for RNP II.[18] Recent structural results place TFIIF and TFIIB adjacent to one another within the yeast PIC, bound to the Rpb2 subunit of RNP II.[15,19,20] This agrees with studies in yeast showing that TFIIF can influence start site selection through interactions with TFIIB.[12,21-24] The presence of TFIIF within a yeast open complex significantly alters the location of TFIIB within that complex.[15] It has also been reported that TFIIF causes human RNP II to assume a particular orientation in complexes with Mediator.[25] Thus, TFIIF may provide an important connection between the core transcriptional machinery and regulatory components. Metazoan TFIIF can also strongly stimulate transcript elongation,[26-29] a property which is unique among the GTFs.

The Use of Phosphorylated TFIIF Provides Surprising Insights into TFIIF Function and the Connection between TFIIF and TFIIB

TFIIF assembled from its recombinant RAP74 and RAP30 subunits is fully

functional in PIC assembly and stimulation of transcript elongation in vitro.[10] However, TFIIF purified from mammalian cells can be extensively phosphorylated.[18,30-34] There is no consensus on the function of these modifications.[33-35] At least some of the phosphorylation seen on TFIIF purified from nuclear extracts appears to result from casein kinase 2 (CK2),[34] consistent with the fact that among the GTFs, TFIIF is a preferred CK2 substrate in vitro.[35]

Our studies on the effects of CK2 phosphorylation of human TFIIF have demonstrated that this modification is a useful tool to probe TFIIF's various functions.[36-38] To facilitate discussion, I will refer to CK2-phosphorylated TFIIF as P-IIF and unmodified TFIIF as U-IIF. P-IIF remains competent to support PIC assembly and binds as well to free RNP II as U-IIF does.[36] However, P-IIF does not bind to RNP II elongation complexes and it has minimal effect on elongation rates.[36] The failure of P-IIF to bind to elongation complexes (without loss of affinity for free RNP II) raises interesting questions about how TFIIF is actually oriented in the elongation complex. Models of the yeast RNP II-TFIIF interaction in both the PIC and open complex[15,19,20] do not suggest an obvious basis for this difference, but it is important to note that a significant portion of the RAP74 subunit of TFIIF cannot be localized in those models.

Although P-IIF does support PIC formation, a closer examination revealed several unanticipated observations. We found that P-IIF and U-IIF support equal levels of transcription from a TATA box promoter with optimal spacing.[37] However, on the 8g2D template, where the TATA to +1 spacing is shorter than optimal,[39] P-IIF supports only half as much transcription compared with U-IIF.[37] The basis for this difference apparently resides in differing levels of TFIIB: PICs assembled on the 8g2D template with P-IIF contain about half as much TFIIB in comparison with complexes assembled with U-IIF.[37] Thus, TFIIF is important in either recruiting or retaining TFIIB as a function of promoter architecture. This is consistent with recent structural work[15,19,20] but this result is not predicted by the conventional model, in which TFIIB is recruited to the template (along with TBP) prior to the loading of RNP II and TFIIF.[1,2]

The reduced recruitment of TFIIB on the 8g2D promoter in the presence of P-IIF suggested the possibility that P-IIF itself might not load effectively on that promoter. When we tested this idea, the results were quite surprising: we found that regardless of the spacing of the TATA box and transcription start, PICs assembled with P-IIF retain no P-IIF after rinsing.[37] It should be stressed that in our system, PIC assembly is absolutely dependent on TFIIF in some form. However, when P-IIF is used to support assembly, P-IIF does not remain stably associated with the resulting complexes. We found no difference in the amount of RNA synthesis obtained with PICs which retained TFIIF (originally assembled with U-IIF and rinsed) or which lacked TFIIF (originally assembled with P-IIF and rinsed). This was true when we measured transcription at initiation itself (in first bond formation assays) or at promoter escape.[37] Thus, TFIIF is not required for initiation or for promoter clearance by mammalian RNP II.

It is important to note that earlier findings in both mammalian and yeast systems[11,12] suggested a major role for TFIIF at initiation, which would appear to contradict our recent observations. One key point in reconciling our study and the earlier work is the difference in the type of template employed. The previous studies used bubble templates in order to study transcription with and without TFIIF (recall that TFIIF is required for any RNA synthesis by RNP II with conventional double-stranded templates). As noted, PICs assembled with P-IIF do not retain TFIIF. We showed that complexes lacking TFIIF lose TFIIB at open complex formation.[37] In contrast, when transcription initiates from PICs made with U-IIF, TFIIB is retained until +12 to +13.[37] Thus, if TFIIB is essential for initiation and TFIIF is critical to retain TFIIB once the template is opened, TFIIF would be expected to be essential for initiation with pre-melted templates. In agreement with this prediction (and in contrast to our results with double-stranded templates[37]) we obtained much less RNA synthesis in bubble template transcription reactions in which P-IIF was used, in comparison with otherwise-identical reactions containing U-IIF.[38] Thus, TFIIF's apparent role as an initiation factor in studies with bubble templates may reflect the importance of TFIIF in the recruitment and retention of TFIIB.

Is the Functional Relationship between TFIIF and TFIIB the Same for All Eukaryotes?

A recent report indicates that there are some differences in the functional relationship of TFIIF and TFIIB in yeast and mammalian PICs. Fishburn and Hahn[15] studied transcription with purified yeast RNP II and GTFs using a series of templates with 12 bp denatured regions located from 30 to 80 bp downstream of the TATA element of the *HIS4* promoter. Their findings varied with choice of location for the bubble, but it is particularly instructive to compare their results with a bubble 30 bp downstream of TATA (which is the normal TATA to +1 distance for a mammalian promoter) vs. a bubble 80 bp downstream of TATA (which encompasses a normal start site for *HIS4*). On the +80 bubble template, a strong transcription signal was obtained with TBP and TFIIB alone and transcription was only modestly stimulated by TFIIF.[15] While there is no initiation on double-stranded *HIS4* templates 30 bp downstream of TATA with the yeast transcriptional machinery, the +30 bubble was transcribed using TBP and TFIIB alone. However, the addition of TFIIF actually inhibited transcription on the +30 bubble template.[15] This last result is in strong contrast to findings with mammalian RNP II and GTFs on bubble templates, where transcription in the presence of TBP and TFIIB is very strongly stimulated by TFIIF.[13,14] This difference may be based in the different architectures of yeast and mammalian TATA box promoters. Initiation at mammalian TATA box promoters only occurs in a narrow range of 34 to 29 bp downstream of the TATA element (counting from the upstream T of TATA—see ref. 39) while in yeast initiation typically occurs farther downstream and with a much broader range of permissible distances from TATA (from 40 to

120 bp downstream; see ref. 40). The biochemical and structural studies reported by Fishburn and Hahn[15] reinforce the importance of the TFIIF-TFIIB interaction in the PIC but they also emphasize that this interaction may not be positive in all cases. This is particularly relevant in the context of the potential role of factors, which can antagonize TFIIF's action, as discussed in the following section.

The Challenge Posed by Gdown1: Can the Pre-initiation Complex be Formed Without Any TFIIF?

A substantial minority (30–50%) of RNP II purified from mammalian tissue sources contains a 13th subunit, called Gdown1.[41] When RNP II bearing Gdown1 [RNP II(G)] was assayed in reactions with TFIID and other components, RNA synthesis was seen only when both an activator and Mediator were added. Under the same conditions, transcription by RNP II without Gdown1 also required an activator but was unaffected by Mediator.[41,42] The significance of these findings to the role of TFIIF in transcription was highlighted by two very recent reports that RNP II(G) cannot associate with TFIIF, either as free polymerase or as transcriptionally engaged polymerase.[42,43] This predicts that RNP II(G) should not be able to initiate RNA synthesis in reactions that depend on TFIIF. In agreement with this, it was shown in a simplified in vitro transcription system using supercoiled templates that the addition of Gdown1 inhibits transcription.[42] Template opening is driven by negative supercoiling,[44] which is functionally analogous to the use of permanently denatured bubble templates. Thus, based on our studies which indicate the importance of TFIIF in TFIIB loading/retention once the template has opened,[37,38] it is tempting to speculate that the transcription inhibition observed by Jishage et al. with Gdown1[42] reflects a failure to recruit/retain TFIIB when TFIIF is displaced by Gdown1.

In genome-wide ChIP assays, Gdown1 is found along with RNP II in the initially transcribed region of genes, throughout gene bodies and downstream of poly A addition sites, where RNP II terminates transcription.[43] Thus, RNP II(G) is apparently able to initiate transcription in vivo in spite of its inability to associate with TFIIF. Since Gdown1 provides a functional interface between Mediator and RNP II,[41] Mediator could offer an alternative mechanism to recruit RNP II(G) independently of TFIIF. However, if TFIIF is required to load TFIIB, how can this recruitment lead to transcription? It has been shown[45] that Mediator directly facilitates recruitment of TFIIB. Thus, Mediator may be able to substitute for TFIIF's function in TFIIB loading/positioning, particularly when Gdown1 is present to drive Mediator-RNP II interaction. Interestingly, while Gdown1 generally localizes with transcriptional-engaged RNP II, a peak of Gdown1 in genome-wide ChIP assays coincides with the site of transcript initiation.[43] This suggests the possibility that some RNP II(G) may be present at promoters in a form which is unable to efficiently initiate transcription, perhaps because of lack of interaction with TFIIF and/or TFIIB. While it might seem counter-intuitive to assemble RNP II complexes that are poorly effective in directing transcription, the existence of such complexes at genes that need only be transcribed infrequently could serve to prevent the potential silencing of those genes by nucleosome assembly. This is consistent with the finding that poorly expressed genes show the highest ratios of Gdown1 to RNP II in the region near the transcription start site.[43]

Conclusion and Perspectives

Our recent work has demonstrated that while mammalian TFIIF is needed for RNP II PIC formation on double-stranded templates, TFIIF is not an obligatory initiation factor.[37] We found that transcription complexes on double-stranded templates that retain or lack TFIIF are indistinguishable in their ability to initiate RNA synthesis and achieve promoter clearance.[37] Our studies indicate that a major role for TFIIF involves recruitment and retention of TFIIB.[37]

Looking forward, it should be emphasized that the studies defining the mammalian RNP II GTFs and the mechanisms of PIC formation, initiation and promoter clearance have all been performed using a very small number of promoters with well-defined consensus elements, principally the TATA box. However, the large majority of mammalian RNP II promoters do not have TATA sequences and a substantial fraction have no known promoter elements.[39,46,47] Even more unsettling is the fact that most of these non-consensus promoters support bidirectional initiation of transcription (reviewed in ref. 48). A major challenge for the future will be determining the applicability of the known set of GTFs and the current models of PIC assembly to the most common class of RNP II promoters.

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