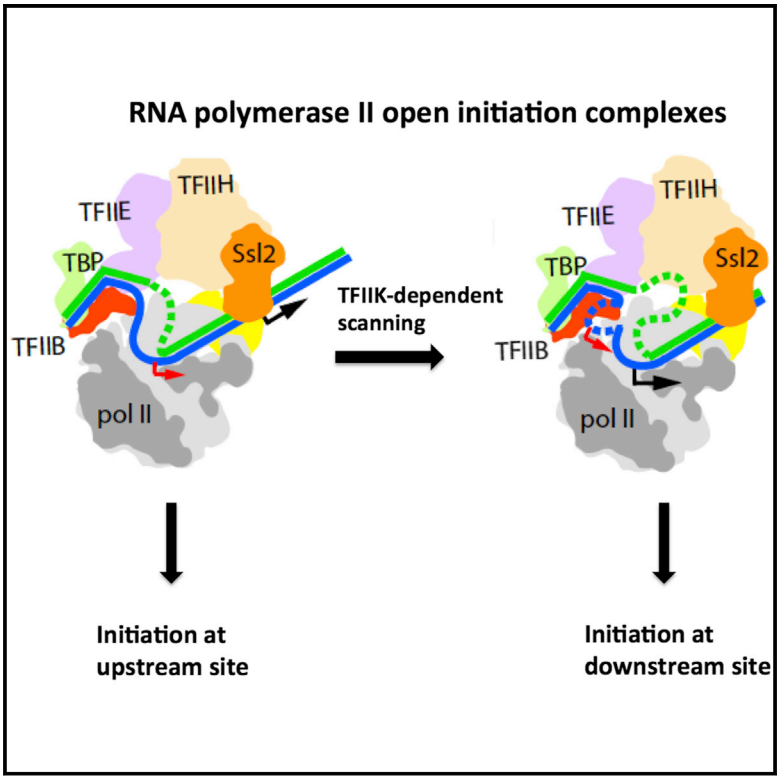
Short Article

Molecular Cell

Uncoupling Promoter Opening from Start-Site Scanning

Graphical Abstract



Highlights

- Omission of TFIIK shifts the TSS in a yeast system to a metazoan location
- Moving the TSS to a metazoan location enables transcription in the yeast system
- Omission of TFIIK uncouples promoter opening from start-site scanning

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In Brief

Murakami et al. find that yeast TFIIK complex is necessary for coupling two stages of transcription initiation mechanism: promoter opening and start-site scanning. Relocation of the transcription start site to an upstream position allows TFIIK-independent transcription initiation.

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Uncoupling Promoter Opening from Start-Site Scanning

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SUMMARY

Whereas RNA polymerase II (Pol II) transcription start sites (TSSs) occur about 30–35 bp downstream of the TATA box in metazoans, TSSs are located 40–120 bp downstream in *S. cerevisiae*. Promoter melting begins about 12 bp downstream in all eukaryotes, so Pol II is presumed to "scan" further downstream before starting transcription in yeast. Here we report that removal of the kinase complex TFIIK from TFIIH shifts the TSS in a yeast system upstream to the location observed in metazoans. Conversely, moving the normal TSS to an upstream location enables a high level of TFIIK-independent transcription in the yeast system. We distinguish two stages of the transcription initiation process: bubble formation by TFIIH, which fills the Pol II active center with single-stranded DNA, and subsequent scanning downstream, also driven by TFIIH, which requires displacement of the initial bubble. Omission of TFIIK uncouples the two stages of the process.

INTRODUCTION

RNA polymerase II (Pol II) assembles with five general transcription factors (GTFs) in a pre-initiation complex (PIC) on promoter DNA (Conaway and Conaway, 1993; Kornberg, 2007). The largest GTF, TFIIH, comprises the helicase Ssl2, a six-subunit core complex, and a three-subunit kinase termed TFIIK (Gibbons et al., 2012; Schaeffer et al., 1993; Schultz et al., 2000; Svejstrup et al., 1995). Ssl2 unwinds the promoter duplex to form a "transcription bubble" (Holstege et al., 1997; Kim et al., 2000; Pal et al., 2005; Fishburn et al., 2015), starting about 12 bp downstream from the TATA box, and in metazoans Pol II initiates transcription about 30 bp downstream; in *S. cerevisiae* Pol II scans further downstream and initiates 40–120 bp from the TATA box. Start site recognition is due in part to properties of Pol II (Li et al., 1994; Yang and Ponticelli, 2012; Zhang et al., 2014), but the basis for scanning is unknown. The initiation of transcription is accompanied by phosphorylation of the C-terminal domain (CTD) of Pol II (Feaver et al., 1994; Laybourn and Dahmus, 1990; Roy et al., 1994), due to the action of TFIIK. Kinase activity is, however, dispensable for basal transcription in vitro (Li and Kornberg, 1994; Serizawa et al., 1993). Here we report on a requirement for TFIIK for initiation at downstream sites, and thus a likely role of TFIIK in promoter scanning.

RESULTS

Omission of TFIIK Shifts Transcription Initiation Upstream

TFIIH bearing a TAP tag on the Tfb4 subunit was purified from a yeast strain lacking the *TFB6* gene as described (Murakami et al., 2012). TFIIH bound to IgG-Sepharose through the TAP tag was stripped of TFIIK by washing with high salt (300 mM potassium acetate plus 400 mM ammonium sulfate); following elution and gel filtration, essentially pure TFIIH devoid of TFIIK (TFIIH-ΔTFIIK) was obtained (Figure 1A). TFIIH-ΔTFIIK in combination with Pol II and other GTFs proved capable of assembly in a complete PIC on a *HIS4* promoter fragment as described (Murakami et al., 2013b), and also of transcription initiation (Figure 1B). In the presence of TFIIH-ΔTFIIK, however, run-off transcripts were longer than expected (indicated by red arrow in Figure 1B). Transcription required TFIIH-ΔTFIIK (Figure 1B), TBP (Figure 1D), and TFIIB (Figure S1B), confirming the promoter dependence of the reaction. When TFIIK was added back, the expected distal TSS utilization was restored (indicated by black arrows in Figure 1B), with a concurrent decrease in proximal site use and a 4-fold increase in total run-off transcription; the same result was obtained when TFIIK bearing a mutation in the Kin28 subunit (TFIIK-L83G) that confers sensitivity to the kinase inhibitor NA-PP1 (Liu et al., 2004) was added to the reaction in the presence of the inhibitor (Figures 1C and S1A). Evidently TSS determination is dependent on a feature of TFIIK other than the kinase activity. Similar results were obtained with two additional promoters tested (Figure 1D). Primer extension analysis showed that in the presence of TFIIK, transcription was initiated 63–103 bp downstream of the TATA box, whereas in the absence of TFIIK, transcription was initiated 31–34 bp downstream of the TATA box (summarized in Figure S2), a location characteristic of transcription initiation in metazoans rather than *S. cerevisiae* (Ponjavic et al., 2006). TFIIS had a slight effect on TSS utilization, and TFIIA and Mediator had no effect (Figures S1D and S1E). All the other GTFs were required.

Transcription from an Upstream Start Site Does Not Require TFIIK

If omission of TFIIK shifts transcription initiation to sites upstream, then it may be dispensable for transcription of a promoter whose TSS is normally located upstream. We created