Mechanism of Promoter Opening by TFIIH

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A critical step in the initiation of gene transcription is the formation of the open complex (OC). OC formation occurs when an RNA polymerase and its associated transcription factors bind to promoter DNA, resulting in the unwinding of ~11 base pairs (bp) of DNA upstream of the transcription start site. Opening of promoter DNA depends on the general transcription factor TFIIH, which contains two subunits (XPD/Rad3 and XPB/Ssl2, human/yeast protein names) that hydrolyze ATP in the presence of DNA, but only XPB/Ssl2 is required for DNA unwinding in the OC. Both XPB and XPD also play crucial roles in nucleotide excision repair. Moreover, mutations in several TFIIH subunits cause various xeroderma pigmentosum (XP) complementation groups (XPB and XPD), XP associated with Cockayne syndrome (XP/CS), and trichothiodystrophy (TTF) by disrupting transcription and/or DNA repair (Singh et al., 2015). Thus, understanding the mechanisms of TFIIH action is of great interest. However, how TFIIH functions in DNA unwinding during OC formation has remained controversial. While the XPD/Rad3 subunit acts as a helicase, it is dispensable for transcription, and several properties of XPB/Ssl2 indicate that it cannot function as a traditional helicase. However, it may function as a DNA translocase, tracking along DNA and leaving unwound DNA in its wake.

"Our motivation for this work was to understand the mechanism of why transcription initiation by RNA polymerase II is uniquely dependent on ATP hydrolysis – an observation first made in 1982. Why is Pol II different from all other Pols in this ATP requirement? How does Ssl2 work and, does this tell us something about the mechanism of DNA unwinding for all Pols?" said Dr. Steven Hahn (Basic Sciences Division). "[Hahn lab postdoc] Sebastian Grünberg first proposed that Ssl2/XPB might be a DNA translocase, rotating and inserting DNA into the Pol II active site cleft, leading to tortional strain and DNA unwinding. Previously, the only biochemical activity observed with this protein was DNA helicase activity. In this work, [Hahn lab research assistant] James Fishburn, working with our biophysicist collaborators in the Galburt lab [at Washington University in St. Louis], showed that Ssl2 is a DNA translocase with properties that can explain many previous observations such as the instability of Pol II open complexes, the weak ATPase function of Ssl2, and its ability to stimulate Pol II escape from the promoter after transcription initiation."

The authors first tested potential helicase and translocase functions of TFIIH components using in vitro assays. They assayed TFIIH helicase activity by testing the ability of purified TFIIH containing

wild-type (WT) or a predicted helicase-deficient Rad3 mutant. TFIIH containing mutant Rad3 was indeed defective in helicase activity but, interestingly, was able to support wild-type levels of transcription by Pol II, suggesting that the helicase activity of TFIIH is dispensable for Pol II OC formation. The ability of TFIIH to function as a translocase was next assayed, and revealed a robust, ATP-dependent translocase activity.

DNA translocases are thought to track in a particular direction along the strand of DNA, leading to DNA unwinding behind them. Ssl2 binds DNA downstream of where DNA unwinding occurs in the OC, and so the directionality of its translocase activity has important implications for the mechanism of DNA unwinding. To investigate the directionality of Ssl2's translocase activity, the authors tested the ability of Ssl2 to displace a short triplex-forming oligonucleotide (TFO) bound to a longer stretch of double-stranded DNA (dsDNA). Ssl2 displaced the TFO when it was bound to the top but not the bottom strand of the dsDNA, suggesting that Ssl2 tracks in the 5' → 3' direction. The authors further tested this by introducing gaps in the top and bottom strands. This showed that a gap in the top strand strongly inhibited TFO displacement, while a bottom strand gap had little effect, a result consistent with 5' → 3' translocase activity of Ssl2. Furthermore, blocking Ssl2 translocation using dye-modified nucleotides on the top strand resulted in inhibition of in vitro transcription.

These results strongly support the a idea model that in which TFIIH functions as a translocase in transcriptional initiation. "Our results have important implications for understanding how Pol II can scan for downstream transcription start sites, the molecular basis for mutations that cause human disease (xeroderma pigmentosum) and for its role in nucleotide excision repair where it acts in the first step of DNA unwinding during removal of DNA lesions," said Dr. Hahn.

Fishburn J, Tomko E, Galburt E, Hahn S. 2015. Double-stranded DNA translocase activity of transcription factor TFIIH and the mechanism of RNA polymerase II open complex formation. *Proc Natl Acad Sci USA* 112(13):3961-3966.

Also see: Singh A, Compe E, Le May N, Egly J-M. 2015. TFIIH Subunit Alterations Causing Xeroderma Pigmentosium and Trichothiodystrophy Specifically Disturb Several Steps During Transcription. *Am J Hum Genet* 96(2):194-207.

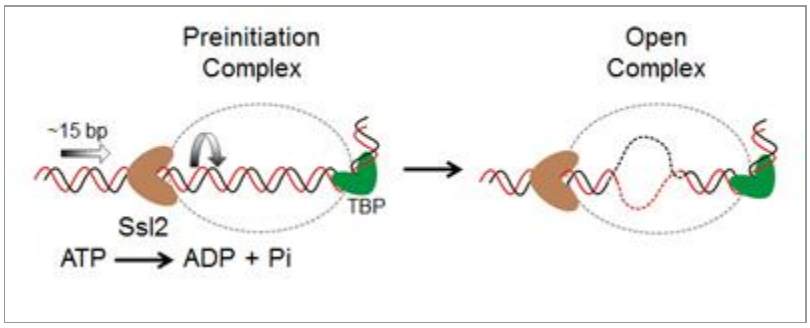

Image provided by Dr. Steven Hahn

(Left) Ssl2 binds DNA downstream of the site of DNA opening and translocates 5' ? 3' in an ATP-dependent manner, leading to (right) unwinding of promoter DNA.