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Post-initiation control by the initiation factor sigma

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Summary

Bacterial core RNA polymerase can initiate transcription at promoters only if guided by a σ subunit that directs the core enzyme to a subset of σ-specific promoters. Specific and stable interactions between the promoter DNA elements and σ are required for efficient promoter recognition. At the same time, persistent σ-DNA contacts can hinder RNA polymerase escape from a promoter or halt the enzyme downstream from the transcription start site, thereby reducing transcription of the affected genes. This microcommentary reviews recent data arguing that σ-dependent stalled transcription complexes form frequently *in vivo*, where they likely play important and diverse regulatory roles.

Bacterial core RNA polymerase (RNAP; subunit composition $\alpha_2\beta\beta'\omega$) relies on the specificity σ subunit to initiate RNA synthesis at promoters. Competition among different σ factors (as many as 65 species can coexist in a bacterial cell) for the core RNAP determines the pattern of gene expression. In a classical view of the transcription cycle, the only role of the σ subunit is to program the pool of core RNAP molecules to recognize a subset of σ-specific promoters, thereby turning on genes that control heat-shock, sporulation, nitrogen assimilation, and other regulatory pathways in response to changes in environment. Once RNAP productively initiates RNA synthesis, σ is thought to be dispensable. In the simplest scenario, σ is released to enter the next round of competition, whereas the core enzyme becomes a target for elongation factors such as NusA. This view was supported by the early *in vitro* studies demonstrating that the rapid and obligatory σ release is triggered by extension of the nascent RNA to 8–9 nucleotides (see Mooney *et al*., 2005 and references therein). However, σ/core contacts are extensive (10 000 $Å^2$ total interaction surface) and breaking them in a single step might turn out to be energetically costly (or even unnecessary). Indeed, the Ebright and Nudler groups (Bar-Nahum and Nudler, 2001; Mukhopadhyay *et al*., 2001) argued that σ might stay bound to the core throughout elongation. In contrast, Chip–chip analyses indicate that *in vivo* the vast majority of σ^{70} is rapidly released from the elongating RNAP (Raffaelle *et al*., 2005). It is important to note that the key question is not *when* the σ subunit is released from the core but *whether* it plays any regulatory role beyond promoter recognition or remains functionally silent even if not released upon escape.

Each σ factor recognizes promoter DNA elements and mediates melting of the double-stranded DNA to form the transcriptionally competent open complex (Fig. 1A). σ^{70} specifically binds to the −10 element located in the non-template DNA strand (Roberts and Roberts, 1996); the template strand becomes available for base pairing with the incoming substrates. The inherent affinity of σ for its DNA target is essential for its function. However, tight binding of σ to DNA can inhibit transcription: a perfect recognition sequence

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might 'lock' RNAP in abortive cycling at the promoter (Fig. 1B) or induce RNAP pausing when located downstream of the promoter (Fig. 1C and D). Both types of inhibitory events have been reported (see Hatoum and Roberts, 2008 for references) but their ubiquity *in vivo* has not been assessed.

In this issue of Molecular Microbiology, Hatoum and Roberts argue that RNAP stalling after open complex formation is widespread in bacteria, and might present a target for regulation. This elegant study extends the pioneering work from the Roberts lab demonstrating that σ can regulate transcription outside of the promoter context (Ring *et al*., 1996). Ring *et al*. have shown that not-yet-released σ^{70} induces RNAP pausing at a −10-like element located just downstream from the start site of the $P_{R'}$ promoter in the λ late operon. This pause is required to mediate recruitment of the λ Q protein to RNAP. After recruitment, Q becomes a 'subunit' of the transcription elongation complex and instructs RNAP to read through many consecutive termination signals, thereby ensuring the completion of the λ lytic cycle. For years, the $P_{R'}$ promoter remained the only known target of σ during elongation, until a similar promoter-proximal pause was characterized *in vitro* in the *lac*UV5 mutant variant of the *Escherichia coli lac* promoter (Brodolin *et al*., 2004; Nickels *et al*., 2004).

To ask whether σ-induced stalling occurs *in vivo* at natural *E. coli* promoters, Hatoum and Roberts (2008) surveyed randomly selected transcription units. They used $KMnO_4$ probing of the chromosomal DNA to detect transcription complexes stalled after initiation; in such complexes, the transcription bubble should be displaced downstream (by ~15 nt) relative to the region melted in the open promoter complex. $KMnO_4$ probing can only be used to assay those promoters that both form stable open complexes and are relatively strong – excluding, for example, the ribosomal *rrn* promoters that are highly active but form very short-lived open complexes. However, 34 out of 118 promoters examined were amenable to this analysis. Among these, a remarkably large fraction (seven promoters, or ~20%) gave rise to transcription intermediates stalled near the promoter! In addition, complexes stalled far downstream (~100 nt) from the start site were also detected in this study; σ-dependent pausing at a distal site has been observed *in vitro* (Mooney and Landick, 2003).

Hatoum and Roberts show that, just as in the $P_{R'}$ case, stalling next to all seven promoters is σ dependent: an amino acid substitution at the binding interface with β′ (σLeu402Phe) that diminishes the promoter-proximal pausing at $P_{R'}$ also reduces the *in vivo* idling near promoters. These data support the hypothesis that the σ/β′ CH interaction (Fig. 1A) is critically important for the σ function, particularly after promoter escape when other σ/core contacts are lost (Mooney *et al*., 2005; Sevostyanova *et al*., 2008). Promoter-proximal pausing also relies on base-specific contacts between σ and the −10 element (Marr and Roberts, 2000; Brodolin *et al*., 2004; Nickels *et al*., 2004). To elucidate the mechanism of stalling, Hatoum and Roberts 'inactivated' the −10 element, either within the core promoter or downstream from the start site, and tested for the retention of stalled complexes. This mutational analysis indicates that some of these complexes (*lacZ, cspD, tnaA*) are likely paused after escape, whereas others (*rplK, rpsA*) are caught in reiterative abortive synthesis.

This study clearly demonstrates that the 'initiation' σ factor does not relinquish its effects on transcription even after it breaks the contacts with the promoter DNA and completes its 'primary' initiation job. Further, even though some classes of promoters (weak or forming unstable complexes) are absent from this set, these data strongly argue that RNAP stalling after initiation does not happen just at a couple of model promoters but is a frequent occurrence in *E. coli*. These complexes are not silent off-pathway intermediates. First, similarly to its role in the λ lytic cycle, stalling may be required for recruitment to the elongating RNAP of yet-unknown cellular proteins with diverse regulatory functions. Second, stalling limits transcription and might be modulated by accessory proteins such as

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GreA, which decreases promoter-proximal stalling up to 19-fold at *rpsU* (Hatoum and Roberts, 2008). Both promoter-proximal and distal stall sites could respond to transcription factors: although Hatoum and Roberts did not observe GreA effects at distal sites, another family of proteins that includes RfaH and its paralogues (NusG, ActX) would be expected to inhibit σ-dependent pausing during elongation. RfaH binds simultaneously to the non-template DNA strand and the β′ CH and abrogates σ-dependent pausing, at least *in vitro* (Sevostyanova *et al*., 2008). Future studies will undoubtedly uncover many specific examples of transcription regulation aimed at the stalled transcription intermediates.

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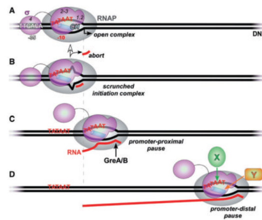

Fig. 1.

Regulatory targets of the σ subunit.

A. The 'classical' σ-subunit target, the open promoter complex. σ specifically recognizes core promoter sequences (shown here for the primary σ^{70} from *E. coli*) and, most importantly for all members of this family, mediates the melting of the DNA to expose the transcription start site (a bent arrow) on the template DNA strand to which the incoming initiating NTP substrate would base pair. At a typical promoter, σ^{70} region 4 binds to the −35 hexamer, $\sigma^{1.2}$ – to the discriminator DNA, and σ^{2-3} – to the −10 hexamer and the β′ subunit clamp helices (CH; blue cylinder), the principal σ-core interaction site (Haugen *et al*., 2006; Mooney *et al*., 2005). The interactions between $\sigma^{1.2-3}$ and the transcription complex can be maintained during elongation (Mooney *et al*., 2005).

B. In the initiation complex, the transcription bubble is enlarged upon RNA synthesis but the RNAP remains stationary because the σ-DNA contacts persist. The 'excess' DNA is scrunched (Kapanidis *et al*., 2006; Revyakin *et al*., 2006) to allow for translocation of the active site along the template. The accumulated stress can be relieved in two ways: (i) the enzyme reiteratively makes and releases (aborts) short, typically 2–8 nt long transcripts, thus reverting to the open complex state; or (ii) the σ-DNA bonds are broken; the core RNAP leaves the promoter (escapes) and enters the elongation phase.

C. The promoter-proximal pause. The (not yet released from the core RNAP) σ subunit recognizes the second −10 element located downstream from the start site. This interaction depends on the same set of contacts (regions 2–3 and perhaps 1.2) and induces a block to RNAP translocation (pause). An intermediate scrunched state induced by σ-DNA contacts (Marr and Roberts, 2000) is relaxed when RNAP moves back and extrudes the nascent RNA, disengaging the 3′ end from the active site. This backtracked complex is a target for Gre proteins (Marr and Roberts, 2000) that facilitate the endonucleolytic removal of the extruded RNA to allow for the next round of nucleotide addition.

D. The promoter-distal pause triggered by (most likely) *de novo* recruitment of the σ subunit to RNAP that transcribes through a −10 element located far from the start site (Brodolin *et al*., 2004). This complex likely undergoes the same structural rearrangements as the proximal pause. Auxiliary elongation factors that bind to the non-template DNA strand (X) and/or to the β′ CH (Y) would insulate RNAP from σ-induced pausing (Sevostyanova *et al*., 2008).

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