PERSPECTIVE

Promoter recognition by bacterial alternative σ factors: the price of high selectivity?

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A key step in bacterial transcription initiation is melting of the double-stranded promoter DNA by the RNA polymerase holoenzyme. Primary σ factors mediate the melting of thousands of promoters through a conserved set of aromatic amino acids. Alternative σs, which direct transcription of restricted regulons, lack the full set of melting residues. In this issue of *Genes & Development*, Koo and colleagues (pp. 2426–2436) show that introducing the primary σ melting residues into alternative σs relaxes their promoter specificity, pointing to a trade-off of reduced promoter melting capacity for increased promoter stringency.

Transcription initiation in bacteria is controlled primarily by σ factors, promoter specificity subunits for the catalytic core RNA polymerase (RNAP) (Gruber and Gross 2003; Paget and Helmann 2003). The primary σ ($σ^{70}$ in *Escherichia coli*) directs core RNAP to the majority of promoters active during log-phase growth. Alternative σs control restricted, specialized regulons in response to environmental conditions. Examples in *E. coli* (*Eco*) include $σ^S$ (general stress response, stationary phase) (Klauck et al. 2007), $σ^{32}$ (also called $σ^H$; cytoplasmic heat shock) (Guisbert et al. 2008), $σ^{28}$ (also called $σ^F$; starvation, flagellar synthesis) (Chilcott and Hughes 2000), $σ^E$ (envelope stress response) (Ades 2008), and FecI (iron deficiency) (Braun et al. 2003). Some bacteria have just one σ factor (*Mycoplasma sp.*), while some have at least 66 (*Streptomyces coelicolor*). The vast majority of σ factors, including all those described above, comprise a homologous family of proteins, the $σ^{70}$ family (Stragier et al. 1985; Gribskov and Burgess 1986), with four regions of conserved amino acid sequence (Lonetto et al. 1992; Gruber and Bryant 1997).

After binding to the core RNAP, the σ factor directs the resulting holoenzyme to a particular set of promoters dictated by sequence-specific recognition of the promoter

by σ (Murakami et al. 2002; Gruber and Gross 2003). Promoter motifs recognized by $σ^{70}$ family members include (from upstream to downstream) the −35 element (Gardella et al. 1989; Siegele et al. 1989; Campbell et al. 2002), the extended −10 element (Keilty and Rosenberg 1987; Barne et al. 1997; Koo et al. 2009a,b), the −10 element (Siegele et al. 1989; Daniels et al. 1990; Waldburger et al. 1990; Tatti et al. 1991), and the discriminator (Feklistov et al. 2006; Haugen et al. 2006). Primary σs, such as $σ^{70}$, recognize thousands of promoters constructed from subsets of these motifs that are poorly constrained in sequence. In contrast, alternative σs recognize only a handful of promoters—for example, *Eco* $σ^{32}$ directs the transcription of ~50 promoters, while *Eco* $σ^{28}$ transcribes ~25 promoters (Nonaka et al. 2006; Shen et al. 2006)—and these promoters require good matches to the cognate −35, extended −10, and −10 motifs (Koo et al. 2009a,b,c). In other words, primary σs tolerate a great deal of promoter sequence diversity (loose stringency), while alternative σs in general do not (high stringency). What are the sequence determinants of the σ factors that govern this dramatic range of promoter stringency, and what are the mechanistic underpinnings? In this issue of *Genes & Development*, Koo et al. (2009c) set out to answer these questions.

For σ factors belonging to the $σ^{70}$ family, recognition of the promoter sequence is followed spontaneously by a series of isomerization steps yielding the transcription-competent open promoter complex (Fig. 1A), in which ~14 base pairs of DNA are melted in a region that includes most of the −10 element and extends downstream to the transcription start site (deHaseth et al. 1998). Studies on primary σs indicate that σ plays a key role in the initiation of the DNA melting process within the promoter −10 element. A set of four aromatic residues that are invariant in primary σs, corresponding to *Eco* $σ^{70}$ F427, Y430, W433, and W434 (Gruber and Bryant 1997; Campbell et al. 2002), have been implicated in this promoter melting function (Helmann and Chamberlin 1988; Juang and Helmann 1994, 1995), possibly by interacting with the exposed bases of the nontemplate strand within the −10 element at the upstream edge of the transcription bubble to stabilize the melted
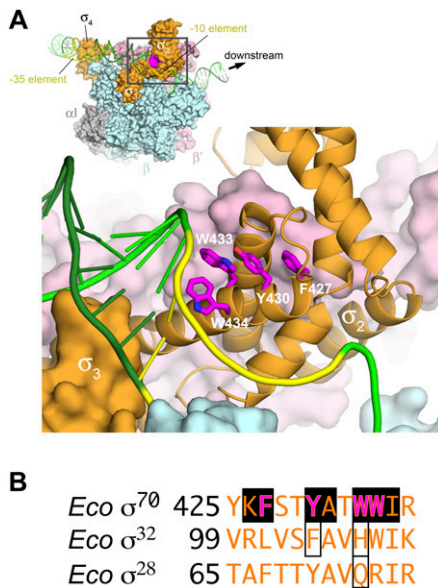
Figure 1. Primary σ promoter melting residues in the context of the RNAP holoenzyme open promoter complex. (*A*) Structural model of the RNAP holoenzyme open promoter complex (after Murakami et al. 2002). The RNAP is shown as a molecular surface, color-coded as follows: (gray) α; (cyan) β; (pink) β′; (orange) σ. The DNA is shown as phosphate backbone ribbons (template strand, dark green; nontemplate strand, light green), with the −35 and −10 elements colored yellow. The direction of transcription (downstream) is to the right. *The top left* (*inset*) shows an overview of the entire complex. The region in the gray box is magnified *below*. In the magnified view, σ domain 2 (σ2) is shown as a backbone ribbon. The side chains of the four, universally conserved "promoter melting" mutants of primary σs (see the text) are shown in magenta. The labels show residue numering according to *Eco* σ70. (*B*) Sequence context of the promoter melting residues. The "promoter melting" residues of the primary σ are bold and colored magenta. Absolutely conserved residues of the primary σs are shaded with a solid black box. The corresponding residues of the alternative σs that were substituted by Koo et al. (2009c) are boxed.

state of the DNA (Roberts and Roberts 1996; Huang et al. 1997; Marr and Roberts 1997). Structural studies of an RNAP holoenzyme/promoter fragment complex are consistent with this view (Fig. 1A; Murakami et al. 2002). Despite significant sequence variation, the structures of the conserved domains of primary and alternative σs are essentially identical (Malhotra et al. 1996; Campbell et al. 2002, 2003, 2007; Li et al. 2002; Sorenson et al. 2004), and their interactions with core RNAP and with promoter DNA are expected to be conserved. Nevertheless, the crucial "promoter melting" residues of the primary σs are not shared among the alternative σs (Lonetto et al. 1992).

The study by Koo et al. (2009c) focuses on two *Eco* alternative σ factors: σ32 and σ28 (encoded by *rpoH* and *rpoF*, respectively). σ32 is induced in response to the presence of unfolded proteins in the cytoplasm, which can result from heat shock or other stressful conditions. σ32 elicits transcription of regulons resulting in the expression of chaperones and proteases helping to refold or degrade damaged proteins (transcription of ~90 genes is initiated; e.g., *dnaK*, *groESL*, and *hslU*) (Nonaka et al. 2006). σ28 directs transcription from ~25 promoters controlling flagellin biosynthesis and chemotaxis (Chilcott and Hughes 2000).

The "promiscuous" primary σ, σ70, recognizes an extended −10/−10 element consensus of TGnTATAAT (Pribnow 1975; Shultzaberger et al. 2006). The extended −10 motif (TG) is recognized by σ domain 3 (Barne et al. 1997), while the −10 element is recognized by σ domain 2, which contains the "melting" residues F427, Y430, W433, and W434. σ32 and σ28 recognize −10 elements of CCCCATnT and GCCGATAA, respectively. Previous studies by Koo et al. (2009a,b) showed that these σ32 and σ28 −10 elements are, in fact, composite, and their recognition follows a general bipartite pattern similar to σ70, with an extended −10 element (upstream CC for σ32, GC for σ28) recognized by domain 3, and a −10 element recognized by residues in region 2.

The very first step in promoter opening probably involves flipping out of the consensus adenine residue, dubbed the "master base in promoter opening" (the −11A, the second adenine in the T**A**TAAT sequence), from the DNA duplex into a hydrophobic pocket in σ (Lim et al. 2001). This triggers recognition of the remaining bases of the −10 element, which is followed by extension of the melted region to the transcription start site. The universally conserved primary σ "melting residues" appear to act by promoting the −11A flipping, and in sequence-specific recognition of the −10 element nontemplate strand bases following the melting, with Y430 and W433 being the most crucial for the nucleation process (Tomsic et al. 2001; Schroeder et al. 2009). Using a combined in vivo/in vitro approach, Koo et al. (2009c) investigated the effects of substituting amino acid residues in the alternative σs at positions corresponding to σ70 Y430 and W433 to those present in primary σs (Fig. 1B). These substitutions rendered the alternative σs more promiscuous, in that they were able to tolerate promoters with less than the normally required complete set of promoter motifs (−35/extended −10/−10 elements), with nonoptimal motif sequences, and with nonoptimal spacing between the elements.

The principal finding of the Koo et al. (2009c) study comes from the results of in vitro binding assays comparing σ binding to promoter templates made of dsDNA with so-called fork junction templates (Guo and Gralla 1998). Fork junction DNA, which contains a double-stranded −35 element but mostly only the nontemplate strand of the −10 element, forms stable complexes with the RNAP holoenzyme that mimic many properties of the promoter open complex (Tsujikawa et al. 2001; Murakami et al. 2002). Thus, comparison of RNAP holoenzyme binding to a double-stranded versus a fork junction promoter relates to the stability of the initial, closed promoter complex versus the melted, open promoter complex. Koo et al. (2009c) observed that the mutated alternative σs were much more proficient then their wild-type counterparts in forming fork junction promoter complexes relative to double-stranded promoter complexes.

The investigators concluded that the primary σ "melting residues," placed in the context of the alternative σs, made the mutant alternative σs more efficient in the DNA strand separation (melting) step of promoter utilization, and that this was also the explanation for the increased tolerance toward imperfect promoters.

One can think of an evolutionary trade-off between melting capacity of a σ factor and its promoter specificity. A σ factor with strong melting capability would serve as a global regulator, serving many, poorly conserved promoters. σ Factors with weak melting capability would act as local regulators, confined to a more restricted set of promoters. In a broad sense, the results of Koo et al. (2009c) provide an interesting corollary to the recent observation that transcription factors with high DNA-binding specificity tend to mediate a more focused response on a genome-wide scale, while lower DNA-binding specificity enables them to control a broad range of targets in a global manner (Lozada-Chavez et al. 2008).

The region of σ harboring the "melting residues" (σ^{70} F427, Y430, W433, and W434), the so-called σ conserved region 2.3 (σ$_{2.3}$) (Lonetto et al. 1992), is thought to act, at least in part, as a sequence-specific, ssDNA-binding element. In this view, σ$_{2.3}$ functions by binding the nontemplate strand bases in the melted −10 element, thereby stabilizing the melted state (Roberts and Roberts 1996; Huang et al. 1997; Marr and Roberts 1997). Thinking about σ$_{2.3}$ function in this way, the results of Koo et al. (2009c) may seem to present a paradox, since substitution of the primary σ melting residues into the alternative σ improves melting function on the cognate promoter of the wild-type alternative σ. However, this can be understood since, in the primary σ cognate −10 element, the −11A (T<u>A</u>TAAT) is extremely highly conserved (Shultzaberger et al. 2006) and is thought to be the initial base that flips out of the double helix to interact with σ and nucleate promoter melting (Lim et al. 2001). Alignment of the σ^{70}, σ^{32}, and σ^{28} promoter sequences indicates that the −11A is retained in each of the promoters (see Fig. 1B of Koo et al. 2009c). The conservation of an A at this promoter position for the primary and alternative σ promoters; the apparent specificity of the primary σ melting residues for this A; and the dominant, nucleating effect of this A on the promoter melting process resolves the paradox.

The ability of the primary σ melting residues to improve the melting capabilities of the mutant alternative σs was inferred by comparing binding of the mutant σs to double-stranded and fork junction promoter fragments. It will be informative to investigate the mechanisms of melting by the alternative σs (both mutant and wild type) using the battery of biophysical and biochemical tools that have been brought to bear on the primary σ (deHaseth et al. 1998).

While primary σs on their own direct transcription from a wide range of poorly conserved promoters, the range of promoters is broadened even further by the influence of *trans*-acting factors (i.e., transcription activators), which allow transcription from promoters too poor to be used by σ^{70} on its own. The influence of *trans*-acting regulatory factors on the promoter selectivity of holoenzymes containing alternative σs has not been investigated to any extent.

While alternative σ factors should clearly know their allowed territory, housekeeping σs can act on behalf of alternative σs in certain situations. For example, bacterial strains lacking σ^{32} can still transcribe heat-shock genes, possibly through the involvement of σ^{70} (Zhou et al. 1988). It was also shown that the majority of σ^{32} promoters can be used by the σ^{70} holoenzyme in vivo and in vitro. Some σ^{E} promoters are recognized by σ^{70} as well (Wade et al. 2006). The biological role of σ^{70} in specific responses, normally thought to be the exclusive purview of alternative σs, remains to be understood.

Alternative σs usually mediate responses to environmental stresses, so these responses need to be rapid, focused, and confined to a tightly defined regulon. The work of Koo et al. (2009c) indicates that this is achieved, at least in part, through the strict promoter recognition requirements of the alternative σs. This is governed by the reduced promoter melting capability of the alternative σs due to the loss of "promoter melting" aromatic residues in σ region 2.3, pointing to an evolutionary trade-off between promoter melting capability and promoter stringency.

Acknowledgments

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