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Promoter melting by an alternative σ, one base at a time

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Abstract

Housekeeping σ factors are initiation factors for the bacterial RNA polymerase at most promoters, whereas alternative σs direct focused responses to specific environmental conditions. Structural and functional analysis of an alternative σ complexed with its cognate −10 motif elucidates the mechanism for initiation of strand opening, highlighting two critical properties: why alternative σs, compared to housekeeping σs, recognize so few promoters and how their promoter-recognition strategy was diversified during evolution.

The initiation factors (called σ factors) for bacterial RNA polymerase (RNAP) carry out promoter recognition and initiate strand opening[1]. Bacteria typically have a single essential, highly conserved housekeeping σ, which promotes the transcription of thousands of genes required during normal cell growth (represented by the founding member, σ^{70} from *Escherichia coli*), and multiple evolutionarily related alternative σs that each promote transcription of limited regulons for coping with stress or development[2]. The so-called extracytoplasmic-function (ECF) or group IV σs are the most divergent and also the most numerous and widespread alternative as (refs. [3–5]). The mechanism of strand opening by the housekeeping σs has been extensively studied[6,7], but the mechanism used by the ECF σs was previously completely unknown. Elegant new work by Campagne *et al*.[8] sheds light on the mechanism by which these σs initiate strand opening. Importantly, their work also provides insight into two critical properties of alternative σs: why they recognize such a restricted set of promoters compared to those recognized by the housekeeping σs and how they have diversified their promoter-recognition strategy through evolution.

The bacterial promoter recognized by RNAP holoenzyme containing the housekeeping σ has two major motifs: the −35 motif (TTGACA), located ~35 bp upstream of the transcription start site and recognized as double-stranded DNA, and the −10 motif (TATAAT), located ~10 bp upstream (at positions −12 to −7) of the transcription start site. Strand opening, which is

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mediated by σ, begins within the −10 motif. A recent study of the interaction between the housekeeping σ and its −10 motif, coupled with rigorous biochemical analysis, showed that recognition of the −10 element and strand opening are coupled[7]. Crystal structures revealed that the σ_2 domain, responsible for −10-motif interaction, recognizes the two most highly conserved bases in the −10 motif, A_{-11} and T_{-7} (ref. 9), which are flipped out of a single-stranded base stack and captured in two protein pockets (Fig. 1). The σ_2 thus uses the binding energy to drive initial strand separation. Rigorous biochemical studies then established that −10-motif recognition by σ_2 is possible only with unwound DNA. Therefore, the pockets in σ_2 must recognize and capture A_{-11} and T_{-7} during transient strand opening, thus indicating that recognition and strand opening are part of the same event. Once the bases are captured, this initial step of transcription- bubble formation is locked in, and the combination of RNAP and σ (i.e., the holoenzyme) can then proceed to complete the transcription bubble and initiate transcription.

Campagne *et al.*[8] started their studies knowing that the housekeeping strategy was not likely to be precisely replicated in the ECF σs. First, the σ_2 domains of ECF σs lack some of the conserved residues implicated in the recognition strategy of the housekeeping σs (ref. 3). Second, in stark contrast to the housekeeping σs, which recognize the same −10 motif in all bacteria[9,10], ECF σs have diverged into many clades, each exhibiting distinct −10 motifs[5]. Therefore, ECF σs must possess a mechanism that enables them to diversify recognition. The recognition strategy of ECF σs was solved with a combination of elegant structural, biochemical and functional studies of *E. coli* σ^E, an ECF σ responsible for responding to envelope stress[11–13]. Using NMR to probe recognition between $\sigma^E{}_2$ and both double-stranded and nontemplate-strand −10-motif DNA, as well as X-ray crystallographic analysis, Campagne *et al.* show that ECF σs use new elements that preserve the logic of housekeeping-σ recognition while key differences in mechanism confer the unique properties characteristic of this highly diverse group of σs.

Within the complex of $\sigma^E{}_2$ bound to its cognate nontemplate-strand −10 motif (TGTCAAA at positions −13 to −7), a nearly absolutely conserved base (analogous to A_{-11} within the −10 motif of housekeeping σ) is flipped out of a single-strand base stack and buried in the $\sigma^E{}_2$ pocket that corresponds to the A_{-11} pocket of housekeeping σ_2. Although, in the case of σ^E, the flipped-out base is a C (C_{-10}; Fig. 1), σ^E recognizes all possible features of the flipped C_{-10} to form an extensive net of interactions similar to those anchoring A_{-11} in the housekeeping σ (ref. 7). However, a key difference is that there is no structural equivalent of the T_{-7} pocket of housekeeping σs; in σ^E only one base is flipped and specifically captured in a protein pocket. All of the σ^E-recognition determinants come from a ten-residue loop (L3) connecting two α-helices, which is highly flexible in the absence of the DNA but is 'tightened' up around the flipped C_{-10} base in the single-stranded DNA complex. Importantly, the amino acid sequence of this loop is not conserved among a wide variety of ECF σs, thus leading the authors to surmise that loop variation among different ECF σs governs the recognition of variable −10 motifs. Elegant swapping experiments proved this to be the case[8]. For instance, the −10-motif consensus for promoters recognized by *Bacillus subtilis* ECF σ^W suggests that A is the flipped base. Swapping in the sW specificity loop for the sE specificity loop gave rise to a hybrid σ that recognizes A rather than C as the flipped base.

The role of the essential housekeeping σ is to promote initiation at the majority of bacterial promoters, regardless of their strength. Indeed, this is an essential level of control built into the cell during evolution. The ability to recognize and initiate at promoters that diverge extensively from the consensus promoter sequence (and that thus direct very weak expression) also enables the cell to use activators to boost expression from such promoters, thus facilitating dynamic responses to changing conditions. All housekeeping σs contain two protein pockets that specifically capture two flipped-out bases of the nontemplate strand. This gives housekeeping σs a powerful DNA-melting capacity, allowing them to function in the face of highly non-optimal promoter sequences.

Possibly as a consequence of their efficiency at DNA melting, the housekeeping σs require a check on their function in the form of a unique inhibitory domain, $\sigma_{1.1}$, not found in the other σ groups (Fig. 1). In addition to autoinhibiting promoter recognition by the free σ (in the absence of RNAP)[14], $\sigma_{1.1}$ may act as a gatekeeper by sitting in the RNAP active site cleft and blocking the access of random DNA to the active site. Strong and specific interactions with promoter DNA cause the displacement of $\sigma_{1.1}$, licensing access to the active site[15,16].

In contrast to the generalized role of the housekeeping σs, the alternative σs mount concerted, focused responses to specific environmental conditions and initiate transcription exclusively from promoters that correspond closely to a particular consensus. Their weakened DNA-melting capacity relative to that of the housekeeping σs has a significant role in their increased specificity.

At the time that ECF σs were identified, it was noted that the regions of σ implicated in initiating melting and in recognizing the −10 motif (σ regions 2.3 and 2.4) were highly divergent from housekeeping σs in sequence[3]. The current work of Campagne *et al.*[8] clearly reveals that the ECF σs also diverge from the housekeeping-σ paradigm in that they contain a single protein pocket to capture a flipped base of the −10 motif rather than the two pockets of the housekeeping σs (Fig. 1).

These new observations establish the structural basis for two key features of ECF σ function. First, the use of a single flipped-out base, recognized by a single protein pocket of the ECF σ, contributes to the weakened DNA-melting capacity, which is in turn the likely basis for the high specificity (and thus the focused response) characteristic of ECF σs. Second, recognition of the crucial flipped-out base of the −10 motif by a modular recognition motif permits the rapid evolutionary diversification of ECF σs and their cognate −10 motifs.

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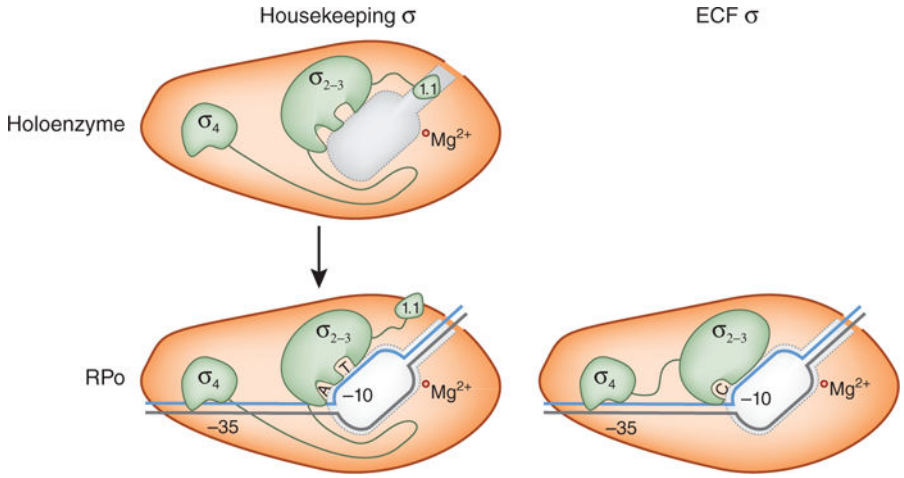

Figure 1.

A schematic view of RNAP holoenzymes. Core RNAP (orange), active site channel (light gray) and disposition of σ domains (green, with subscripts denoting domain numbers) are shown. Bottom, as bound to their cognate −10 and −35 promoter elements within the RNAP open complex (RPo). The housekeeping σ (top and bottom left) orchestrates promoter melting by capturing two highly conserved bases of the −10 element, A_{-11} and T_{-7} in RPo (bottom left). Nonspecific interactions of nucleic acids with the active site are prevented by $\sigma_{1.1}$ in the downstream duplex channel of the holoenzyme (top left). Entry of promoter DNA in RPo displaces $\sigma_{1.1}$ Alternative σs such as *E. coli* σ^E (bottom right) lack $\sigma_{1.1}$ and use a simplified strategy that relies on a single pocket for capturing C_{-10}.