A new basal promoter element recognized by RNA polymerase core enzyme

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Bacterial promoters are recognized by RNA polymerase (RNAP) σ subunit, which specifically interacts with the −10 and −35 promoter elements. Here, we provide evidence that the β′ zipper, an evolutionarily conserved loop of the largest subunit of RNAP core, interacts with promoter spacer, a DNA segment that separates the −10 and −35 promoter elements, and facilitates the formation of stable closed promoter complex. Depending on the spacer sequence, the proposed interaction of the β′ zipper with the spacer can also facilitate open promoter complex formation and even substitute for interactions of the σ subunit with the −35 element. These results suggest that there exists a novel class of promoters that rely on interaction of the β′ zipper with promoter spacer, along with or instead of interactions of the σ subunit with the −35 element, for their activity. Finally, our data suggest that sequence-dependent interactions of the β′ zipper with DNA can contribute to promoter-proximal σ-dependent RNAP pausing, a recently recognized important step of transcription control.

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Introduction

The formation of promoter complex, a step that commits bacterial RNA polymerase (RNAP) to transcribe a gene, requires the RNAP specificity (σ) subunit (Burgess and Anthony, 2001; Borukhov and Severinov, 2002). Within the context of the RNAP holoenzyme, σ regions 2 and 4 (σR2 and σR4) make specific interactions with promoter elements located, correspondingly, around positions −10 and −35 relative to the transcription start site (Helmann and deHaseth, 1999; Burgess and Anthony, 2001; Borukhov and Severinov, 2002). A minor class of extended −10 promoters, instead of the −35 motif, rely on a TG motif located immediately upstream of the −10 element (Barne et al, 1997). The TG motif is also recognized by the σ subunit. Region 1.2 was also shown to make sequence-specific interactions with non-template nucleotide downstream of the −10 element (region referred to as ‘discriminator’ in stable RNA promoters; Haugen et al, 2006).

The available medium-resolution structure of Thermus aquaticus RNAP σR holoenzyme (Enσ) complex with fork-junction DNA (Murakami et al, 2002), a synthetic nucleic acid substrate mimicking promoter DNA, as well as structural modelling results (Naryshkin et al, 2000; Murakami and Darst, 2003) show that in addition to the expected contacts with σ, promoter DNA is close to residues from various regions of large (β′ and β) RNAP core subunits throughout the complex. At the downstream ‘end’ of the open complex, the β′ jaw, a structural element that is part of a trough where the double-stranded DNA downstream of the catalytic centre binds, contributes to open complex stability by wrapping the downstream DNA and firmly securing it in the trough (Edert et al, 2002). These interactions were proposed to contribute to species specificity of promoter utilization (Artsimovitch et al, 2000). At the upstream end, both specific and non-specific interactions of the α subunit C-terminal domains (αCTDs) with DNA upstream of the −35 promoter element have a strong stimulatory effect on promoter complex formation. Non-specific interactions of RNAP core with a region between positions −30 to −40 were also proposed (Nechaev and Geiduschek, 2006). DNA around the transcription initiation start point makes intimate contacts with β and β′ residues that form the RNAP catalytic centre (Murakami and Darst, 2003). Several structural elements of RNAP core also come in close proximity with promoter spacer located between the −10 and −35 promoter elements (Murakami et al, 2002). These structural elements are depicted in Figure 1A and include the β′ zipper, the β′ zinc-binding domain, and the β flap. We previously showed the critical role of the β flap for promoter selectivity (Kuznedelov et al, 2002b). The β flap contributes to promoter recognition indirectly, by enabling σR4 interaction with the −35 promoter element. The functional role, if any, of other RNAP core elements proximal to the spacer in promoter recognition is presently unclear.

In this work, we show that the β′ zipper, which is part of the evolutionarily conserved segment B present in the largest subunits of all multi-subunit RNAPs, is directly involved in promoter recognition. Moreover, we show that, depending on the spacer sequence, interactions of the β′ zipper with the spacer can substitute for σR4 interactions with the −35 element during the open complex formation. These results highlight the unexpected complexity of the process of bacterial promoter recognition and suggest novel ways
in which promoter specificity of bacterial RNAP can be regulated.

Results

Interaction of RNAP with DNA around position −21 of the spacer

The strategy of our study was to search for previously unrecognized interactions of RNAP with DNA by sequentially removing known interactions. We analysed transcription by *T. aquaticus* holoenzyme, *E*σ*A*, on derivatives of strong −10/−35 class T7A1 promoter bearing the consensus −10/−35 element (here this promoter is referred to as −10; Figure 1B; Supplementary Figure S1A). We analysed both abortive and run-off transcription and obtained essentially the same results, indicating that promoter escape by *T. aquaticus* *E*σ*A* is independent on the strength of promoter interactions, at least on promoters studied here (see Supplementary data for details).
Transcription was performed under conditions of excess (fivefold) of promoter fragments over the enzyme. Given that *T. aquaticus* RNAP does not form stable open promoter complexes even on strong promoters (Kuznetsova et al., 2003; Kulbachinskaya et al., 2004; Schroeder and deHaseth, 2005), transcription in such conditions reported on the overall efficiency of closed and/or open complexes formation. To further distinguish between closed and open complexes formation, we used DNase I footprinting and KMnO4 probing.

Interactions of zCTDs with DNA 45–80 bp upstream of the transcription initiation start point strongly influence promoter utilization in *E. coli* (Estrem et al., 1999). In the case of *T. aquaticus* EroA, zCTDs do not have such a role (Wada et al., 2000). Consistently, the upstream truncation of the promoter fragment from position −85 to position −42 did not influence promoter activity (Figure 1C, lanes 1 and 2). Next, we removed the −35 promoter element by changing it to a non-functional sequence and generating the [−10] promoter fragment (Figure 1B). Curiously, removal of the −35 element had no effect on promoter activity (Figure 1C, lanes 2 and 3; see Supplementary data for details). Since the TG motif characteristic of extended −10 promoters (Barne et al., 1997) is absent from the [−10/−35] promoter and its derivatives, the result may be explained by postulating that non-specific favourable interactions of EroA with DNA suffice for [−10] promoter activity. To test this idea, experiments were repeated with RNAP holoenzyme reconstituted from the wild-type core and σA mutant lacking region 4.2 (σA[−35−390]). Unexpectedly, as seen from Figure 1C (lanes 4 and 5), the mutant holoenzyme was active on both [−10/−35] and [−10] promoters (see Supplementary data for details). Therefore, we conclude that interaction(s) other than the interaction of σA with DNA is responsible for [−10] promoter activity.

To identify DNA regions important for [−10] promoter utilization, derivatives progressively truncated from the upstream end (Supplementary Figure S1A) were tested in transcription with EroA[−35−390] (Figure 1D). As can be seen, the mutant enzyme was still able to transcribe from promoters containing as little as 21 bp of DNA upstream of the transcription start point ([−10]−21 promoter; Figure 1D, lane 8). Further deletion (to position −18) abolished promoter activity ([−10]−18 promoter; Figure 1D, lane 9). This effect was not due to altered promoter escape since the abortive initiation assay gave essentially the same result (Figure 1D, lanes 5′−10′). Therefore, the experiment indicates that RNAP interactions with DNA at/or immediately downstream of position −21 contribute to promoter utilization. The slight decrease in promoter activity upon the truncation to position −39 (Figure 1D, compare lanes 1 and 2) can be explained by the elimination of the proposed non-specific interaction of RNAP core with this region (Nechaev and Geiduschek, 2006).

**The β′ zipper is required for promoter utilization in the absence of specific interactions of σA with the −35 element**

To explain our data, we hypothesized that there exists a domain of RNAP that contacts DNA on deeply truncated promoter fragments. This interaction appears to involve promoter spacer at/or around position −21, since truncation beyond this point destroys promoter activity, presumably by preventing the interaction. On the basis of structural considerations, the β flap, the β′ zinc-binding domain, or the β′ zipper could interact with spacer DNA (Figure 1A). We tested transcription by RNAPs lacking these domains: EroAflap, EroAZn, and EroAZipper, respectively (Figure 1E). As above, the experiment was done in the five-fold molar excess of the promoter over RNAP. Differences in transcription by various RNAPs can, therefore, reflect both the efficiency of promoter utilization and/or RNAP-specific activity. In order to compare transcription by mutant enzymes on different templates between each other, their activities were normalized to the activity of EroA (or EroA[−390]) on each template (Figure 1E, histogram). The activities of mutant holoenzymes reconstituted with σA on the [−10/−35] promoter are shown in Figure 1E (lanes 1–4). Next, wild-type and mutant RNAP cores were combined with σA[−35−390], and the resultant holoenzymes were tested using the shortest active truncated promoter [−10]−21 as a template (Figure 1E, lanes 1′−4′). As can be seen, deletion of the β flap or the β′ zinc-binding domain had no significant effect on transcription from [−10]−21 (lanes 3′ and 4′). In contrast, removal of the β′ zipper abolished transcription (lane 2′), indicating that the β′ zipper is essential for utilization of the truncated promoter. This result suggests that the β′ zipper is also responsible for transcription activity of EroA[−35−390] on full-length [−10/−35] promoter (Figure 1C, lane 4). Indeed, EroAZipper[−10/−390] but not other double mutant RNAP holoenzymes was inactive on [−10/−35] promoter (Figure 1E, compare lanes 1′−3′). The result further suggests that activity of EroA on promoter lacking the −35 element was also determined by the β′ zipper (Figure 1C, lane 3). Indeed, EroAZipper reconstituted with full-length σA was inactive on the [−10] promoter (Figure 1F).

Note that, as tested in elongation complexes, kinetics of nucleotide addition and specific activities of wild-type and EroAZipper core enzymes were similar (Supplementary Figure S2A). This indicates that the observed differences in activities of EroA and EroAZipper σA indeed reflect differences in promoter utilization.

Further mapping has revealed that highly conserved Y34 and R35 (Supplementary Figure S3A) of the β′ zipper are...
responsible for its function (Figure 1G). In the crystal structure of the promoter complex, Y34 and R35 are in contact distance from spacer DNA (Figure 1A), suggesting that the β′ zipper may indeed function through direct interactions with promoter DNA around position –21.

The β′ zipper stabilizes promoter complexes

We were interested to determine which step of transcription initiation is affected by the deletion of the β′ zipper. We examined closed and open complexes formation using DNAse I footprinting (Figure 2A) and probing with KMnO4 (Figure 2B),
respectively. KMnO₄ probing was done at equilibrium conditions, in the absence of heparin (recall that T. aquaticus RNAP open promoter complexes are relatively unstable). As can be seen, Er₈ formed closed and open promoter complexes on both [−10/−35] (Figure 2A, lane 3 and Figure 2B, lane 3, respectively) and [−10] (Figure 2A, lane 6 and Figure 2B, lane 7, respectively) promoters. E₈Zipperσ₈ was able to form open (Figure 2A, lane 4) and closed (Figure 2B, lane 4) complexes on the [−10/−35] promoter. However, in the absence of the −35 element, E₈Zipperσ₈ failed to form either open (Figure 2B, lane 8) or closed (Figure 2A, lane 7) promoter complex. These results are consistent with the results of transcription assays with Er₈ and E₈Zipperσ₈ on [−10/−35] and [−10] promoters (Figure 2C), and indicate that the lack of activity of E₈Zipperσ₈ in the absence of specific interactions of σR4 with the −35 element is due to the inability of mutant enzyme to form closed promoter complex.

We next tested whether the β’ zipper contributes to promoter complexes formation in the presence of specific interactions of σR4 with the −35 element. We challenged transcription by Er₈ and E₈Zipperσ₈ on the [−10/−35] promoter with DNA competitor heparin and increased ionic strength. Given that transcription elongation was not affected by the concentrations of heparin and KCl used (Supplementary Figure S2B), the assay directly addresses the stability of promoter complexes. As shown in Figure 2C and D, transcription by E₈Zipperσ₈ was much more sensitive to heparin and high ionic strength treatment than transcription by the wild-type Er₈. DNase I and KMnO₄ analysis of promoter complexes also revealed that both closed and open promoter complexes formed by E₈Zipperσ₈ on [−10/−35] were far less resistant to salt challenge than those formed by Er₈ (Figure 2E). However, due to instability of promoter open complexes formed by T. aquaticus RNAP we cannot distinguish whether the effect of increased salt concentration on open complexes was direct, or was caused by destabilization of closed complexes. The results indicate that the β’ zipper contributes to stabilization of closed (and possibly open) promoter complexes both in the presence and in the absence of specific interactions of σR4 with the −35 element.

Taken together, the fact that the function of the β’ zipper is determined by two amino acids that face the promoter spacer (Figure 1G), the results on promoter shortening (Figure 1D and E), and the data on the role of the β’ zipper in stabilization of promoter complexes lend strong support to idea that the β’ zipper acts through direct contacts with promoter spacer. The β’ zipper may either provide additional favourable contacts with promoter spacer, or it may change the structure of promoter DNA, and thus optimize the recognition of the −10 promoter element by σ subunit and/or improve downstream RNAP-promoter interactions. In this regard, it is noteworthy that the DNase I footprint of closed complexes formed by Er₈, but not by E₈Zipperσ₈, contains a DNase I hypersensitive band upstream of the −10 element both in the presence and absence of the −35 element (Figure 2A, lanes 3 and 6), suggesting that, upon the interaction with the β’ zipper, promoter spacer indeed undergoes a conformational change.

The role of promoter spacer sequence in the β’ zipper function

Though our results suggest that the β’ zipper interacts with promoter spacer and participates in stabilization of promoter complexes, it is unclear if the contribution of the β’ zipper to promoter complex formation depends on the sequence of the spacer or is sequence independent. To address this issue, we changed (‘switched’) the sequence between positions −24 and −18 of [−10/−35] and [−10] promoters to a complementary sequence, and tested the activity of Er₈ and Er₈ZIP on resulting [−10/sw-24/−18/−10] and [−10/sw-24/−18] promoters (Figure 1B). As can be seen from Figure 2F, switching of the spacer sequence in the absence of either the −35 element (lanes 4’ and 4”) or σR4 domain (lanes 6 and 6’) decreased the level of transcription 4–5-fold. In the presence of the −35 element, transcription was not affected by the switch in the spacer sequence (Figure 2F, lanes 2 and 2’).

We examined what stage of transcription initiation is affected by a change in the spacer sequence in the absence of the −35 element. Permanganate probing and DNase I footprinting showed that Er₈ failed to open promoter DNA on [−10/sw-24/−18] promoter (Figure 2B, lane 15), while the formation of closed complex was not affected (Figure 2A, lane 12). Note that closed complex on [−10/sw-24/−18] persists in the conditions of permanganate probing (Supplementary Figure S4), indicating that the ‘switch’ of the spacer sequence affects only promoter opening. Though the switch in the spacer sequence did not affect the level of transcription from the [−10/−35] promoter, we found that in a background of non-consensus −10 element the spacer sequence between positions −24 and −18 contributed to promoter strength even in the presence of specific −35–σR4 interactions (Figure 2D, triangles). These results indicate that the sequence between positions −24 and −18 is important for open complex formation when RNAP interactions with promoter elements are weak. In the background of a strong −10 element, this sequence can substitute for the −35 element.

Switching of the spacer sequence could affect promoter strength by changing the curvature of promoter DNA, which, in turn, may influence the recognition of the −10 element or RNAP contacts with downstream DNA (Hook-Barnard and Hinton, 2009). However, in silico curvature prediction showed only small deviations in curvature upon the change of spacer sequence (Supplementary Figure SSA). Importantly, though E₈Zipperσ₈ formed very unstable promoter complexes (Figure 2C–E), its activity was the same on [−10/−35] and [−10/sw-24/−18/−35] promoters, that is, was not affected by the switch of the spacer sequence (Figure 2A, lanes 4 and 10; Figure 2B, lanes 4 and 12; Figure 2D, compare filled and empty circles; see also experiments with E. coli RNAP below). These results suggest that the change of the spacer sequence, on its own, does not seem to significantly influence the strength of promoter.

The Z-element of promoters

Taking into account the proposed interaction of the β’ zipper with DNA around the position −21, our results suggest that the contribution of the sequence between positions −24 and −18 to promoter opening is mediated by the β’ zipper. The β’ zipper may either directly recognize the T7A1 spacer sequence around position −21 or ‘sense’ specific local structure of spacer DNA in this region. We name the spacer region that interacts with the β’ zipper ‘Z-element’ (for zipper). To determine if the β’ zipper and the Z-element contribute to promoter utilization by RNAPs other than T. aquaticus, we tested E. coli RNAP (EcEr₈) on the [−10/−35] promoter and
its derivatives. Only abortive initiation was analysed, since EcEcEs70 forms much more stable promoter open complexes than T. aquaticus enzyme, and biases arising at the stage of promoter escape were, therefore, expected. Transcription by EcEcEs70 was more sensitive to removal of the /C035 element, which led to B3-fold decrease in the level of transcription (Figure 3A). Interestingly, a switch of the Z-element also decreased transcription (B2-fold) even in the presence of the /C035 element (Figure 3A). Removal of both elements had a cumulative effect, and EcEcEs70 utilized [C010/C035]B6 times less efficiently than [C010/C035] (Figure 3A). Next, we tested if the Z-element affected EcEsY47A/R48A activity. Removal of the Z-element, either in the presence or in the absence of the /C035 element, had no effect on EcY47A/R48A activity. Removal of the −35 element decreased the level of transcription ~8-fold irrespective of the presence or the absence of the Z-element (Figure 3A). These results suggest that the Z-element participates in promoter utilization by EcRNAP and acts through the β0 zipper, supporting the results obtained with T. aquaticus RNAP. We also tested Bacillus subtilis holoenzyme, BsEsa, on T7A2 derivatives depicted below the histogram (promoter sequences are shown at the bottom of the panel). All activities by both enzymes were normalized to the activity of EcEcEs70 on T7A2[−10wt/−35]. (D) Tyr47 and Arg48 (corresponding to Tyr34, Arg35 of T. aquaticus) of β0 zipper are important in vivo. E. coli cells with chromosomal rpoC that is inactive at 43°C were transformed with plasmids carrying wild-type or mutant rpoC genes under IPTG-inducible promoters. Mutations in the β0 zipper are depicted to the left of the photographs of petri dishes (corresponding mutations of T. aquaticus β0 are in brackets). The serial dilutions of cells were grown at permissive (30°C) and restrictive (43°C) temperatures.

Figure 3 The Z-element of promoters. (A) Abortive transcription by E. coli EcEcEs70 and EcY47A/R48A on promoters depicted under histogram was quantified and normalized to the activity of each enzyme on [−10/−35] promoter. (B) Abortive transcription by B. subtilis holoenzyme, BsEsa, on promoters depicted under histogram was quantified and normalized to the activity on [−10/−35] promoter. (C) Transcription by EcEs and EcY47A/R48A on T7A2 derivatives depicted below the histogram (promoter sequences are shown at the bottom of the panel). All activities on EcEcEs70 activity. Removal of the −35 element decreased the level of transcription ~8-fold irrespective of the presence or the absence of the Z-element (Figure 3A). These results suggest that the Z-element participates in promoter utilization by E. coli RNAP and acts through the β0 zipper, supporting the results obtained with T. aquaticus RNAP. We also tested Bacillus subtilis holoenzyme, BsEsa. As can be seen from Figure 3B, BsEsa behaved similarly to EcEcEs70 with respect to the Z and the −35 elements contributions to promoter activity. These results suggest that participation of the Z-element and the β0 zipper in promoter utilization may be a ubiquitous phenomenon among bacteria, though the extent of the actual contribution may be species specific.

Changes of small blocks of the spacer sequence (Supplementary Figure S5B) allowed us to localize the Z-element to a region extending from position −22 to position −18 of T7A1 promoter (AACCT). We noted that another well-characterized strong promoter T7A2, designated here as T7A2[−10wt/−35] (Figure 3C, Supplementary Figure S1B), while having no sequence similarity with T7A1, has
an AACAT sequence in the −22 to −18 region, resembling the
Z-element of T7A1. We tested the functionality of putative
T7A2 Z-element with T. aquaticus RNAP. As can be seen from
Figure 3C, derivatives of T7A2[−10wt/−35] acted in the
same manner as [−10/−35] promoter derivatives with re-
spect to Z-element function: (i) The Z-element was able to
support transcription and was required on T7A2 derivative
lacking the −35 element (T7A2[−10wt]); (ii) EcZipperR48A,
while being as active as ErA in T7A2[−10wt/−35], failed to
transcribe from T7A2[−10wt]. These results suggest the
existence of a novel class of promoters that use interactions of
the β′ zipper with the Z-element instead of and/or along with
−35–σR4 interactions to facilitate promoter opening.

We analysed several single base-pair substitutions in the
T7A1 Z-element of the [−10] promoter with T. aquaticus
RNAP. Most of substitutions led to decreased level of trans-
scription by ErA in the absence of the −35 element (Supplemen-
tary Figure S5C), suggesting that the sequence of the
T7A1 Z-element may be close to consensus. However, the
effects were modest (<2-fold decrease), suggesting that
Z-element consensus may be ‘loose’. Though bioinformatics
analysis of E. coli and B. subtilis promoters with known
transcription start sites revealed that a significant proportion
(~10%) of them contain sequences that resemble the T7A1
Z-element (Supplementary data; Supplementary Tables S1
and S2), further analysis is required to determine function-
ality of these putative Z-elements.

Given that our results suggest that the β′ zipper is
required for stabilization of promoter complexes, we ex-
pected these interactions to be important for cell viability.
We, therefore, investigated the importance of proposed inter-
action of β′ zipper with promoter spacer in vivo. Mutations
introducing single-alanine substitutions of β′ Lys41, Tyr47,
Arg48, Thr49, Phe50 (corresponding to Ec248, 249, 250, 251,
252, and Leu37 of T. aquaticus; Figure 1G), were
created in plasmid-borne E. coli rpoC (codes for RNAP β′).
Plasmids expressing mutant β′ subunits from an IPTG-
inducible promoter were transformed into E. coli cells har-
bouring a chromosomal copy of rpoC coding for tempera-
turesensitive β′ (which is inactivated at 43°C). We analysed the
ability of these plasmids to complement a temperature-
sensitive phenotype of host cells (Figure 3D). As can be
seen, growth of cells expressing β′ with Y47A substitution
was strongly diminished at restrictive temperature.
Complementation by β′ with R48A substitution was weaker
than by the wild-type β′, though the effect was more moder-
ate than that of the Y47A substitution. The β′ subunits
with remaining substitutions complemented the temperature-sen-
tive phenotype as efficiently as wild-type β′ subunit (com-
pare with the in vitro results in Figure 1G). Promoter escape,
elongation, and termination by E. coli RNAP were not
affected by Y47A and R48A substitutions in vitro
(Supplementary Figure S6A and B), supporting the idea that
in vivo effects of these mutations were caused by deficiencies
in promoter utilization.

**β′ Zipper–Z-element interactions affect σ-dependent
promoter-proximal pausing**

In addition to its essential role in transcription initiation,
the RNAP σ subunit has a role in transcription elongation
by causing transcriptional pausing in initially transcribed region
of some promoters (Ring et al., 1996; Brodolin et al., 2004;
Nickels et al., 2004; Hatoum and Roberts, 2008). The pause
occurs when σ binds to elongating RNAP core and σ2
establishes specific contacts with the −10-like sequence in
promoter-proximal transcribed DNA. Thus, the paused com-
plex partially resembles the promoter open complex
(Brodolin et al., 2004). We hypothesized that the β′ zipper
may interact with DNA upstream of pause-inducing −10-like
element during σ-dependent promoter-proximal pausing, and
thus affect the pause efficiency.

We used a transcription template carrying the lacUV5
promoter, which contains a −10-like σ-dependent pause-
inducing sequence between positions +1 and +6
(Brodolin et al., 2004; Nickels et al., 2004; Figure 4A).
Intriguingly, 4 bp upstream of the −10-like element, a se-
quence that resembles the Z-element (AATGT) is present. To
test if the Z-like sequence is functional, we prepared a lacUV5
derivative that lacked this element (lacUV5-Z, Figure 4A)
and measured efficiency of σ-dependent pausing on lacUV5 and
lacUV5-Z by T. aquaticus and E. coli RNAPs. ErA paused two
times (Figure 4B), while EcErAthree times less efficiently
on lacUV5-Z than on lacUV5 (Figure 4C). To test if the action
of the Z-like element is mediated by the β′ zipper, we
examined pause formation by EcZipperR48A and EcEcZip-
perR48A-Z. Pause efficiencies by mutant T. aquaticus
(Figure 4B) and E. coli (Figure 4C) enzymes on lacUV5
were similar to those of corresponding wild-type RNAPs on
lacUV5-Z, indicating that the effect of the Z-like element
of pause-inducing sequence is abolished by removal of or
mutations in the β′ zipper. We could not check pausing by
mutant RNAPs on the lacUV5-Z template, because the mutant
enzymes were inactive on this promoter due to downmuta-
tion in the −10 promoter element introduced by alterations in
the Z-like sequence. The moderate effect of the Z-like element
on pause efficiency may have been caused by the fact
that this element lacked the equivalent of −20C, which
could not be introduced into the sequence since the change
destroyed the lacUV5 promoter activity (Supplementary
Figure S7).

We cannot exclude a possibility that the Z-like element acts
independently of the β′ zipper by influencing the recognition
of the −10-like sequence by σ subunit. The results on
promoter utilization, however, favour the possibility that
the Z-element acts through interaction with the β′ zipper.
As in the case of promoter utilization, during pausing,
the proposed interaction of the β′ zipper with the Z-like
element may increase the pause efficiency by either
providing additional contacts of RNAP with the pause signal,
or optimizing recognition of the −10-like element of the
pause-inducing sequence by σ2 (via structural changes in
DNA). Though the molecular details remain to be under-
stood, our results suggest that interactions of the β′ zipper
with DNA can influence transcription elongation rate by
affecting the efficiency of σ-dependent pausing by bacterial
RNAPs.

Note that the half-life of σ-dependent pausing by
EcZipperR48A was longer than that of ErA (3.5 times,
Figure 4B). This suggests that the β′ zipper may influence
stabilization of the pause. The half-life of pausing by EcEcZip-
perR48A-Zo increased only slightly, suggesting that deletion of
β′ zipper amino acids other than conserved tyrosine and
arginine is responsible for the observed effect. At present,
we cannot explain the mechanism behind increased pause
half-life of E\textsuperscript{D zipper}\textsubscript{0}, but the finding that the σ-dependent pause on lacUV5 promoter is stabilized via backtracking (Brodolin et al., 2004), suggests that E\textsuperscript{D zipper}\textsubscript{0} may be more prone to backtracking on the pause signal.

Discussion

Sequence-specific interactions with promoters are thought to be a prerogative of bacterial RNAP promoter specificity factor, the σ subunit, and the only known sequence-specific interaction of the core enzyme with promoters is that of CTDs of the σ subunits of RNAP with the Upstream Promoter element. RNAP core parts contacting downstream DNA of promoter were suggested to contribute to specificity in promoter utilization (Artsimovich et al., 2000). Non-specific interactions were reported for various parts of RNAP core (see Introduction). The principal result of this work is the demonstration that a structural element of the RNAP core largest subunit, the β’ zipper, interacts with the promoter spacer and the outcome of this interaction can be modulated by the sequence of the spacer.

The β’ zipper contributes to promoter closed (and possibly open) complex stabilization. By interacting with the spacer around position −21, the β’ zipper either provides additional contacts between RNAP and promoter DNA, or optimizes interactions of σ and/or core with DNA downstream of the spacer. The presence of the Z-element in the region of spacer facilitates promoter opening and can even substitute for the canonical interaction of σ with the −35 element. The mechanism of the Z-element contribution to promoter opening remains to be understood. Since Z-element does not seem to have any significant effect on promoter structure or activity on its own but requires intact β’ zipper, direct favourable protein–DNA interaction is possible. Structural analysis supports this hypothesis. The β’ zipper may either directly recognize Z-element sequence or, instead, ‘sense’ local DNA conformation determined by this sequence. It is also possible that the Z-element is not recognized directly, but its sequence modulates the outcome of the β’ zipper interaction with the spacer on promoter opening. In the latter mechanism, the Z-element may determine the properties of the spacer (bendability, kinking, etc.) which, upon interaction with the β’ zipper, promote localized melting downstream of the Z-element. The effect of spacer sequence on the promoter strength was demonstrated for the lac promoter by selecting spacer sequences that made the activity of promoter activator independent (Liu et al., 2004). Our results open a possibility that differences in the spacer region may be either sensed by the β’ zipper, or may modulate the β’ zipper contribution to promoter activity.

In the presence of a strong −10 element, the proposed β’ zipper–Z-element interaction can substitute for the σR4 interaction with the −35 element during the promoter open complex formation. In the background of a weak −10 promoter element, both the −35 and the Z-elements contribute to promoter utilization. In this sense, the Z-element is similar to the TG motif of the ‘extended −10’ class promoters in that it facilitates open complex formation in the absence of or along with the −35 element. Therefore, the results suggest an existence of promoters of a new type that rely on the Z-element instead of and/or along with the −35 element (−10/Z and/or −10/Z/−35). Further investigations are required to determine the consensus sequence(s) of the Z-element and the mode of its recognition by the β’ zipper.

The interplay of specific interactions of the β’ zipper and σR4 with their respective elements may potentially be a target for genetic regulation. Curiously, in the structure of holoenzyme bound to a promoter DNA fragment containing a sequence similar to the Z-element, AAATT, 4 bp upstream of the −10 element, the σR4 recognition helix is shifted 6Å upstream and does not interact with the −35 element specifically (Murakami et al., 2002).
RNAP stalling at promoter-proximal sequences is now believed to be a common way of transcription regulation in bacteria (Artsimovitch, 2008; Hatoum and Roberts, 2008). Though known σ-dependent pauses are close to promoters, the existence of distantly located σ-dependent pauses has also been proposed (Mooney and Landick, 2003; Deigan et al., 2011). Our results indicate that specific interactions of the β’ zipper with DNA can influence elongation rates by contributing to the efficiency of σ-dependent pauses.

The β’ zipper is highly conserved among bacteria (Supplementary Figure S3A), suggesting the conservation of the mechanisms of its interactions with DNA and its roles in transcription. In spite of a low level of sequence identity, the structural similarity of zipper domains of the largest subunits of bacterial, eukaryotic, and archaeal RNAPs (Hirata and Murakami, 2009; Supplementary Figure S3B) may indicate that interactions of zipper domain with promoter DNA may be utilized by RNAPs from all domains of life.

Materials and methods

Templates and proteins

Transcription templates were made by PCR and purified from agarose gel. Wild-type and mutant recombinant T. aquaticus core RNAPs and σ70 were purified as described (Kuznedelov et al., 2003). RNAPs lacking the β’ zipper (β’ residues 27–42) or the lid (β residues 526–539) domains were obtained as described (Zenkin et al., 2006). RNAP lacking the β flap domain (β residues from 757–786 were substituted for LeuGlu) was kindly provided by K Kuznedelov. RNAP lacking β’ zipper-binding domain was made by genetic substitution of β’ residues 54–82 for Gly-Gly linker. RNAPs with mutations in the β’ zipper were constructed by substitutions of single amino acids with alanines. T. aquaticus σ70 lacking domain 4.2 (σ704–130) was kindly provided by A Kulbachinsky. Wild-type and mutant E. coli RNAP core enzymes were isolated according to Kashlev et al. (1996). E. coli σ70 was isolated as described (Borukhov and Goldfarb, 1993). To obtain E. coli RNAP cores bearing single-alanine substitutions of β’ Lys41, Tyr47, Arg48, Thr49, Phe50, Lys51, Tyr52, Arg53, or 30 C; Kuznedelov 1996), bearing genomic rpoC that codes for a temperature-sensitive β’ subunit. The transformants were grown in LB at 30°C (permissive temperature) in the presence of 100 μg/ml of ampicillin. Exponentially growing cells (A600 of ~0.2) were diluted serially and plated by 2 μl spots on the minimal media (M9) agar, containing 100 μg/ml arginine, 0.5 μg/ml thiamine, 100 μg/ml ampicillin, with or without 1 mM IPTG, and were grown at 43°C (restrictive temperature) or 30°C overnight.

σ-Dependent pausing

Pausing on lacUV5 promoter was analysed as described in Brodolin et al. (2004) except for +16 stalled elongation complex by T. aquaticus RNAP was formed at 65°C, and +16 complexes were chased by addition of 100 μM NTPs at 40°C. Products of reactions were analysed as above, and pause efficiency (fraction of transcribing RNAP that pause at +17) was determined by nonlinear regression analysis using SigmaPlot software.

In vivo experiments

pRL663 plasmids carrying wild-type and mutant rpoC genes (from above) were used to transform E. coli 957C cells (Christie et al., 1996), bearing genomic rpoC that codes for a temperature-sensitive β’ subunit. The transformants were grown in LB at 30°C (permissive temperature) in the presence of 100 μg/ml of ampicillin. Exponentially growing cells (A600 of ~0.2) were diluted serially and plated by 2 μl spots on the minimal media (M9) agar, containing 100 μg/ml arginine, 0.5 μg/ml thiamine, 100 μg/ml ampicillin, with or without 1 mM IPTG, and were grown at 43°C (restrictive temperature) or 30°C overnight.

Supplementary data

Supplementary data are available at The EMBO Journal Online (http://www.embojournal.org).

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Author contributions: NZ and YV designed and performed the experiments and analysed the data, VT performed bioinformatics analysis; KS and NZ conceived the project and wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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