Single-strand promoter traps for bacterial RNA polymerase

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Besides canonical double-strand DNA promoters, multisubunit RNAPs (RNA polymerases) recognize a number of specific single-strand DNA and RNA templates, resulting in synthesis of various types of RNA transcripts. The general recognition principles and the mechanisms of transcription initiation on these templates are not fully understood. To investigate further the molecular mechanisms underlying the transcription of single-strand templates by bacterial RNAP, we selected high-affinity single-strand DNA aptamers that are specifically bound by RNAP holoenzyme, and characterized a novel class of aptamer-based transcription templates. The aptamer templates have a hairpin structure that mimics the upstream part of the open promoter bubble with accordingly placed specific promoter elements.

The affinity of the RNAP holoenzyme to such DNA structures probably underlies its promoter-melting activity. Depending on the template structure, the aptamer templates can direct synthesis of productive RNA transcripts or effectively trap RNAP in the process of abortive synthesis, involving DNA scrunching, and competitively inhibit promoter recognition. The aptamer templates provide a novel tool for structure–function studies of transcription initiation by bacterial RNAP and its inhibition.

Key words: abortive transcription, aptamer, DNA scrunching, promoter recognition, RNA polymerase, single-strand template.

INTRODUCTION

Proper expression of genetic information critically depends on the ability of RNAP (RNA polymerase) to specifically recognize promoters and initiate RNA synthesis in a highly regulated manner [1]. In bacteria, transcription initiation is performed by the RNAP holoenzyme containing a promoter-specificity factor, the σ subunit. The major σ subunit (σ^70 in Escherichia coli) is involved in recognition of most cellular promoters which contain a number of conserved promoter elements, including the −35 (TTGACA), TG, −10 (TATAAT) and GGGA (discriminator) elements recognized by conserved regions 4.2, 3.0, 2.3/2.4 and 1.2 of the σ subunit respectively [1–5] (Figure 1C). Initiation of transcription on double-stranded DNA requires local melting of the template around the starting point, promoted by specific interactions between the σ subunit and the non-template strand of the −10 and GGGA elements, as illuminated by recent structural analysis [6,7]. In addition, core RNAP was proposed to recognize the melted non-template DNA strand downstream of the GGGA element, with a particular preference for guanine at promoter position +2 [7]. The σ subunit is also implicated in later steps of transcription initiation, including priming of RNA synthesis, abortive transcription and promoter escape [8–10]. The process of abortive synthesis and promoter escape was shown to be accompanied by significant changes in the conformation of the DNA template, involving extension of the transcription bubble and ‘scrunching’ of the melted DNA chains, as a result of downstream DNA transcription without breaking upstream σ-mediated RNAP–promoter contacts [11,12].

Besides double-stranded DNA promoters, bacterial and eukaryotic RNAPs were shown to transcribe various types of single-strand templates, a possible relic of their functions in primordial world that existed before establishment of the genetic function of double-stranded DNA [13,14]. The best-studied examples of single-stranded DNA templates recognized by bacterial RNAP include minus-strand replication origins of filamentous phages (such as the phage M13 origin, oriM13 [15]) and lagging-strand origins of rolling-circle replication plasmids [16,17], where RNAP functions as a primase and synthesizes short pRNAs (priming RNAs) for replication initiation [18]. Structure analysis of phage and plasmid replication origins revealed the presence of −35- and −10-like elements located in partially double-stranded DNA regions, and some reported substitutions in these elements impaired pRNA synthesis by bacterial RNAP in vitro and origin function in vivo [16,17,19–21]. At the same time, in vitro pRNA synthesis on the oriM13 template was observed even in the absence of specific promoter elements, suggesting that core RNAP plays a major role in origin recognition [15].

Both bacterial and eukaryotic RNAPs were also shown to utilize RNA templates of various natures [22,23]. In a striking example of genetic regulation, bacterial 6S RNA was shown to serve as a specific inhibitor of σ^70 RNAP holoenzyme during stationary phase of growth, but to be released from RNAP during recovery from stationary phase as a result of its transcription by RNAP [24]. Remarkably, the secondary structure of 6S RNA resembles the structure of the open promoter with unpaired transcription bubble [24], and the primary determinants for specific 6S RNA binding by RNAP were shown to reside within regions corresponding to the −35 and −10 promoter elements [25–27]. Similarly to the oriM13 transcription, transcription of 6S RNA was shown to result in synthesis of short pRNAs, leading to structural rearrangements of the pRNA–6S RNA complex and its release from RNAP [24,28].

Analysis of different single-strand nucleic acid substrates recognized by RNAP demonstrated that, depending on their structure and genetic function, they can serve as transcription templates and direct synthesis of various types of RNA transcripts and/or specifically inhibit RNAP activity [15,17,24]. However,
in many cases, the mechanistic details of transcription initiation on these templates remain poorly understood. To investigate further possible ways of recognition of single-strand templates by bacterial RNAP, we obtained high-affinity single-strand DNA aptamers that are specifically bound by the RNAP holoenzyme, and characterized a novel class of templates based on the aptamer sequences. The aptamer templates share important properties with other single-strand RNAP substrates and can efficiently inhibit RNAP during the initiation step of transcription, thus providing a new tool for analysis of transcription initiation and for development of RNAP inhibitors.

EXPERIMENTAL

Proteins and DNA

_E. coli_ core RNAP and the σ^{70} subunit were purified as described previously [29]. Unmodified and fluorescently labelled DNA oligonucleotides were purchased from Syntol. The T7A1 promoter fragment was obtained as described in [29].

Aptamer selection

Aptamer selection was performed using a 75-nt-long oligonucleotide library with randomized 32-nt-long central region (Supplementary Figure S1A at http://www.biochemj.org/bj/452/bj4520241add.htm) essentially as described previously [2,30,31]. In each round of selection, RNAP holoenzyme (10–100 nM) was mixed with the oligonucleotide pool in binding buffer containing 40 mM Tris/HCl (pH 7.9), 10 mM MgCl2, 160 mM NaCl and 40 mM KCl at 37°C. RNAP holoenzyme (50 nM core RNAP plus 500 nM σ^{70} subunit) was incubated with the aptamer template (10–50 nM) for 5 min at 37°C followed by addition of NTP substrates. RNA primers and UTP (with

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**Figure 1  Aptamers to holoenzyme of bacterial RNAP**

(A) Sequences of the central stem–loop of the aptamers. Nucleotides from the random and constant library regions are shown in upper- and lower-case letters respectively. Sequence motifs that form double-stranded stems are shown in grey with underlined complementary nucleotides; the −10 and TG promoter elements are red and green respectively; pyrimidine-rich motifs at the right part of the loop region are highlighted in light/dark yellow. The sequence logo of the conserved promoter motif is shown below the aptamer sequences. (B) Proposed secondary structures of shortened aptamers hEcap1, hEcap5 and hEcap12. (C) The model of the open promoter complex of _T. aquaticus_ RNAP [40]. The active-site magnesium is pink; the promoter DNA is orange; relative positions of the −35, TG, −10 and GGGA promoter elements are indicated. The σ subunit is shown as a tube model, with regions 1.2, 2.3/2.4, 2.5 and 4.2 shown in brown, red, green and blue respectively.

**In vitro transcription**

Most transcription reactions were performed in transcription buffer containing 40 mM Tris/HCl (pH 7.9), 10 mM MgCl2, 160 mM NaCl and 40 mM KCl at 37°C. RNAP holoenzyme (50 nM core RNAP plus 500 nM σ^{70} subunit) was incubated with the aptamer template (10–50 nM) for 5 min at 37°C followed by addition of NTP substrates. RNA primers and UTP (with
addition of [$\alpha$-32P]UTP) were at 25 $\mu$M, whereas concentrations of other NTPs were varied from 30 to 1000 $\mu$M each. The reactions were stopped after 5–10 min by addition of urea-containing buffer and RNA products were analysed by 15–23 % PAGE. In transcription inhibition experiments, the aptamers were mixed with the T7A1 promoter in transcription buffer containing 40 mM Tris/HCl (pH 7.9), 10 mM MgCl2 and 40 mM KCl, then RNAP holoenzyme was added and transcription reactions were performed as described above.

**K MnO4 footprinting of RNAP–aptamer complexes**

K MnO4 probing was performed as described previously [29]. RNAP holoenzyme (100 nM) was mixed with 5′-labelled aptamer template (10 nM in all reactions) in transcription buffer containing 40 mM Tris/HCl (pH 7.9), 10 mM MgCl2, 160 mM NaCl and 40 mM KCl, incubated at 25 °C for 5 min and treated with 2 mM K MnO4 for 30 s. NTPs (500 $\mu$M each) were added 2 min before K MnO4 where indicated. The reactions were processed as described in [29]. The variations in the total amounts of DNA in different reactions after sample processing did not exceed 10–15 %. The DNA cleavage products were separated by either 15 or 23 % PAGE to reveal long and short cleavage products respectively. The K MnO4 experiments were repeated three times resulting in highly reproducible modification patterns.

**Fluorescence measurements**

Analysis of aptamer fluorescence was performed in the Modulus Microplate Multimode Reader (Turner BioSystems) using the blue filter with the maximum excitation wavelength of 490 nm and emission wavelength of 510–570 nm. Reaction mixtures contained 500 nM RNAP holoenzyme and 250 nM hEcap1/51 aptamer derivatives in 60 $\mu$l volume of transcription buffer (40 mM Tris/HCl, pH 7.9, 10 mM MgCl2, and 40 mM KCl) at 25 °C in black-coloured microtitre plates, either in the absence of NTPs or in the presence of 500 $\mu$M ATP, UTP and GTP. Three measurements were performed in parallel and background fluorescence values were subtracted. Each experiment was repeated three to five times, followed by averaging of the relative fluorescence intensities.

**RESULTS**

**Aptamer-based mimics of the open promoter complex**

To explore the DNA-recognition potential of RNAP, we selected single-strand DNA aptamers that bind the *E. coli* $\sigma^{32}$ RNAP holoenzyme with high affinity and specificity (hEcaps, for holoenzyme *E. coli* aptamers). Aptamer selection was performed using a standard protocol from a 75 nt library containing a 32-nt-long random region (Supplementary Figure S1A). Sequencing of individual clones revealed that all aptamers contained promoter-like motifs corresponding to the TG and −10 promoter elements (Figure 1A and Supplementary Figure S1A). In most aptamers (i.e. aptamers 1–11), these two elements were separated by an additional nucleotide, reflecting their relative position in double-stranded promoters. In aptamers 12–15, the TG motif immediately preceded the −10 element, similarly to previously described single-stranded DNA aptamers recognized by free $\sigma$ subunits from *E. coli* and *Thermus aquaticus* [2,31]. Further analysis is needed to reveal possible differences in the recognition of the TG motif by RNAP holoenzyme at these two aptamer positions. Remarkably, the degree of nucleotide conservation at all positions of the aptamer promoter motifs (in particular, at the third, fourth and fifth positions of the −10 hexamer) was higher than in the case of natural promoters [33], probably because the aptamers were specifically selected for the optimal binding to RNAP.

Analysis of predicted secondary structures revealed that all aptamers share a common structural motif containing a short hairpin formed by self-complementary aptamer regions, with the TG element located in the upstream double-stranded part and the −10 element placed in the loop region (Figure 1B and Supplementary Figure S1B) ([6] and references therein). In some aptamers, the whole −10 region was located in the loop region, demonstrating that RNAP holoenzyme can recognize all six positions of the −10 hexamer in fully single-strand form. Similarly, isolated $\sigma$ subunit was shown previously to recognize the fully single-stranded −10 element [2,6,31,34]. All aptamers also contained a pyrimidine-rich region in the bottom part of the single-stranded loop opposite the −10 element (Figure 1A), suggesting that the RNAP holoenzyme may have preference for such sequences in this part of the transcription bubble. To confirm that the aptamers are specifically recognized by RNAP, we analysed several minimized aptamer variants corresponding to the proposed secondary-structure motif (Figure 1B). The dissociation constants ($K_d$ values) of holoenzyme–aptamer complexes were shown to lie in the nanomolar range; in particular, hEcap1 and hEcap5 bound RNAP with $K_d$ values of 2.5 and 3.0 nM respectively, which is comparable with the binding affinities of natural double-stranded promoters (e.g. [3]).

**Aptamer templates guide specific transcription initiation**

The structural similarity of the aptamers to the open promoter bubble suggested that RNAP can use them as transcription templates. However, the original aptamers lacked the downstream DNA part that must be placed into the RNAP active-site cleft for transcription initiation, probably as a result of a limited length of the DNA library used for aptamer selection. Thus, to obtain aptamer-based transcription templates, we introduced short DNA inserts derived from the initially transcribed region of the T7A1 promoter into the downstream loop of hEcap1. To stabilize the template structure and to increase the strength of RNAP–aptamer interactions, we introduced additional G/C pairs into the aptamer stem region, made consensus changes in the −10 element and put the GGGG element downstream of it (Figures 2A and 2B).

Several aptamer variants were constructed that differed in the lengths of the inserted sequences (Figures 2A and 2B). The aptamer variant without the downstream DNA insert (hEcap1/43) did not support transcription initiation (Figure 2C, lane 1). However, aptamer-based templates containing inserts of 5–12 nt (templates hEcap1/48 to hEcap1/55, Figures 2A and 2B) promoted efficient synthesis of abortive RNA transcripts (Figure 2C, lanes 2–5). The abortive nature of the observed RNA products was confirmed in transcription experiments with immobilized RNAP. The lengths of synthesized RNA transcripts gradually increased (up to 12 nt) with the increase in the length of the downstream DNA inserts. However, the downstream loops present in the aptamer-based templates did not allow RNAP to proceed to RNA elongation, resulting in its trapping in the process of abortive synthesis (Figure 2C).
To localize the starting point of transcription on the aptamer templates, we performed transcription in the presence of different nucleotide substrate sets. The synthesis of labelled dinucleotide RNA product was observed from ATP and UTP when either \( [\gamma\text{-}^{32}\text{P}]\text{ATP} \) or \( [\alpha\text{-}^{32}\text{P}]\text{UTP} \) was used as a labelled substrate (Figure 2D, lanes 1 and 2), suggesting that transcription probably initiates with ATP at position +1 (indicated on Figure 2A). Indeed, efficient transcription initiation was observed in the presence of short primers corresponding to the proposed transcription start point. In particular, dinucleotide primers UpA (corresponding to positions −1/+1) and trinucleotide primer ApUpC (positions +1/+2/+3) could be used as initiating substrates (Figure 2D, lanes 3 and 4). Thus the likely starting point of transcription on the aptamer templates corresponds to the starting point of transcription in classical double-stranded promoters.

To reveal how transcription of the aptamer templates depended on the promoter elements, we analysed several variants of a representative template, hEcap1/51, containing different combinations of the elements. The starting template variant contained TG, −10 and GGGA consensus elements; in template hEcap1/51-GGGA, the GGGA motif was replaced with ATAT; in template hEcap1/51-TG, the TG element was replaced with AC, preserving the double-stranded stem structure; in template hEcap1/51−10, the −10 element was replaced with the TCACCA sequence, and the last two templates also lacked the GGGA motif (Figures 2A and 2B). The transcription activity of all four templates was analysed either in the presence of all four NTPs (upper panel) or UpA and UTP (lower panel), allowing for abortive synthesis of a trinucleotide RNA product. In agreement with the key role of the

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**Figure 2** Transcription properties of aptamer-based templates

(A) Sequences of the hEcap1-based templates. Positions of conserved promoter elements are indicated above the sequences. Sequences that form double-stranded stems are shown in light grey; the downstream DNA inserts are underlined. (B) The structure of the hEcap1/51 template. The specific promoter elements are indicated; the transcription start point is shown with an arrow. The downstream DNA insert is boxed; note that in the hEcap1/51–RNAP complex, the downstream hairpin is fully melted (see Figures 3A and 3B). The structures of downstream inserts in other hEcap1 variants are shown. (C) Transcription of hEcap1-based templates containing various downstream DNA inserts. The aptamer lengths are indicated. (D) Localization of the transcription start point in the hEcap1/51 aptamer template. The experiment was performed with various initiating nucleotides and primers, as indicated. (E) Effects of substitutions in consensus promoter elements on the activity of the hEcap1/51 template in the presence of all four NTPs (upper panel) or UpA and UTP (lower panel). Positions of characteristic RNA products are indicated (RO, run-off).
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Figure 3 DNA scrunching during abortive transcription on aptamer templates

(A) The scheme of the aptamer template showing positions of KMnO₄-modified thymines (numbered starting from the 5'-end) and fluorescent dyes, attached through thymines substituted for cytosines at indicated positions. The thymine bases that display differential activities in various KMnO₄ reactions (Figure 3B) are coloured as follows: grey, bases in the stem region that are cleaved only at high temperature (reaction 4); blue and red, bases in the RNAP–aptamer complex that are more reactive either in the absence (reaction 2) or in the presence of NTPs (reaction 3) respectively. (B) KMnO₄ footprinting of RNAP–aptamer complexes. The scanned modification profiles are shown on the right. All reactions contained equal total amounts of the DNA template, as confirmed by phosphorimaging. Reaction 1 (black) contained no RNAP, reaction 2 (blue) contained RNAP–aptamer complex, reaction 3 (red) contained RNAP–aptamer complex in the presence of NTPs, and reaction 4 (green) contained free aptamer template at 70°C. The ‘A + G’ is a Maxam–Gilbert sequencing reaction. The colouring of thymine bases corresponds to Figure 4(A).

(B) Relative fluorescence levels of the aptamer–RNAP complexes measured in the absence and in the presence of NTPs (250 μM each). The hEcap1/51 template either contained fluorescein (F) at position −12, or was double-labelled with fluorescein and dabsyl (F/Q) at positions −12 and −2 respectively. RNAP activity, measured on the control unlabelled hEcap1/51 template (‘C’) or fluorescently labelled templates in the presence of ATP, UTP and CTP, is shown on the right. (D) Schematics of the aptamer template transcription showing proposed changes in the DNA conformation, with positions of hyper-reactive thymines and fluorescent dyes indicated. The RNAP active centre is shown by a red dot.

−10 element in promoter recognition, substitution of this element completely abolished transcription initiation (hEcap1/51−10, Figure 2E, lanes 3). At the same time, removal of the GGGA motif alone only slightly reduced abortive RNA synthesis (compare templates hEcap1/51 and hEcap1/51-GGGA, Figure 2E, lanes 1 and 4). Notably, however, the hEcap1/51-GGGA template lacking the GGGA motif produced very small amounts of ~30 nt full-length RNA corresponding to the transcription to the 5'-end of the template (Figure 2E, upper panel, lane 4). This RNA product was completely absent in the case of templates containing the GGGA motif (Figures 2C and 2E), suggesting that, on these templates, RNAP is fully trapped in abortive complexes. Substitution of the TG element (hEcap1/51-TG) moderately decreased trinucleotide RNA synthesis (Figure 4E, lower panel, lane 2), but greatly reduced abortive transcription in the presence of all four NTPs and stimulated full-length RNA synthesis, suggesting that, in this case, RNAP can disrupt specific contacts with the template and escape to productive elongation (Figure 4E, upper panel, lane 2).

Thus, depending on the strength of RNAP–DNA interactions, the single-strand templates can direct synthesis of either abortive or full-length RNA transcripts.

DNA scrunching during transcription initiation on aptamer templates

Transcription initiation on double-stranded promoters was shown to be accompanied by the scrunching of the DNA template within the RNAP molecule [11,12]. The resulting strain can be relieved either through release of abortive RNAs or through disruption of σ-mediated contacts with the promoter and transition to productive elongation. The trapping of RNAP in the process of abortive synthesis on the consensus aptamer templates suggests that, in this case, RNAP retains persistent contacts with the promoter elements, thus favouring the first scenario and preventing further RNA elongation. To detect structural changes of the aptamer template that occur during abortive RNA synthesis, we performed probing of the RNAP–hEcap1/51 complex with KMnO₄ which modifies single-stranded thymine bases, allowing their further visualization by piperidine cleavage. Comparison of the modification pattern of the free aptamer template obtained in the absence of RNAP with the control pattern observed at high temperature (70°C, to disrupt the secondary structure) showed that thymines in the proposed stem region...
The activity of RNAP holoenzyme on DNA template containing the T7A1 promoter and the aptamer template in the reaction were 30, 100 and 50 nM respectively. The promoter recognition by bacterial RNAP holoenzyme [24].

Since holoenzyme-specific aptamers mimic natural promoter substrates, we expected them to also inhibit promoter-dependent transcription.

To reveal the effects of the aptamer templates on promoter-dependent transcription we analysed T7A1-dependent RNAP activity in the presence of hEcap1/51 at different NTP concentrations. The aptamer template strongly inhibited synthesis of full-length T7A1 RNAs and switched transcription to the synthesis of short abortive RNAs, even in the presence of a 2-fold excess of the promoter (Figure 4). Remarkably, efficient inhibition was observed even at high NTP concentrations (up to 1 mM), suggesting that the transcription of the aptamer template does not relieve its inhibitory effect on promoter recognition. This contrasts with 6S RNA that was shown to dissociate from RNAP upon pRNA synthesis [24,28]. Thus, unlike 6S RNA, the aptamers probably do not undergo RNA-dependent secondary-structure rearrangements and remain bound to RNAP during transcription.

**DISCUSSION**

Transcription of single-strand DNA and RNA templates by cellular RNAPs plays important biological functions in both bacteria and eukaryotes [13,16–19,23,24]. Analysis of single-strand aptamer templates specifically recognized by bacterial RNAP, performed in the present study, suggests general principles in the recognition and function of various types of single-strand promoters. The aptamer structure reveals a striking similarity to the structure of the open promoter bubble containing the unpaired −10 promoter element and double-stranded TG motif upstream of it. Thus the in vitro selection experiment demonstrated that bacterial RNAP holoenzyme has a strong preference for such DNA templates over other structural and sequence DNA motifs. This preference probably underlies RNAP DNA melting activity during formation of the open complex on double-stranded promoters. Previous studies have identified additional elements...
in the melted promoter bubble that are specifically recognized by RNAP holoenzyme, including the GGGA/discriminator element immediately downstream of the −10 element [2–5] and the ‘core-recognition element’ at promoter positions −4/+2 [7]. However, these elements were not revealed in the holoenzyme-specific aptamers, which may be explained by strong RNAP–aptamer interactions that are formed even in the absence of these motifs or by limited aptamer size that might preclude their selection.

Subtle modification of the structure of aptamers converted them into efficient transcription templates, specifically recognized by RNAP holoenzyme. Depending on the downstream template structure and the presence of consensus promoter elements, we were able to obtain template variants capable of synthesis of RNA transcripts of various lengths and types. We therefore propose that the specific aptamer part can be considered as a recognition module for specific initiation of RNA synthesis on single-stranded DNA templates of various structures and origins, which may have various practical applications.

Comparison of the aptamer templates with other single-strand RNAP substrates characterized reveals common features in their recognition by RNAP. Similarly to the aptamer templates, single-strand phage and plasmid replication origins recognized by bacterial RNAP contain promoter-like motifs, including −10 and −35-like elements, that were shown to be important for their function in vitro [16,17,19–21]. Furthermore, similarly to the aptamers, all natural single-strand DNA and RNA substrates of RNAP fold into partially double-stranded structures containing hairpins and single-stranded bulges that are important for their binding to RNAP [16,19,20,23,24,36].

Transcription initiation on double-stranded promoters was shown to involve scrunching of the DNA strands in the melted region within the transcription complex, a process that is believed to facilitate promoter escape by RNAP [11,12,35]. Similarly, the DNA scrunching was proposed to occur during σ-dependent pausing in elongation complexes [37,38]. Our data suggest that transcription initiation on single-strand templates proceeds via a similar mechanism, involving scrunching of the template DNA segment (Figure 3D). Using the aptamer templates, we directly detected changes in the conformation of the template DNA strand during initiation. It should be noted that, owing to the specific hairpin structure of the template, the ‘non-template’ DNA segment is unlikely to be scrunched, but should rather enter the RNAP active centre for transcription, which makes RNA extension dependent on the size of the downstream hairpin.

Indeed, we observed that the aptamer templates promoted highly efficient abortive RNA synthesis that depended on the downstream DNA length. In the case of fully consensus aptamer templates, the σ-mediated contacts of RNAP with specific promoter elements cannot be broken and the resulting stress is relieved through dissociation of abortive RNA transcripts (Figure 3D). As a result, the templates act as suicidal promoter substrates for RNAP that sequester the enzyme in the process of abortive initiation and inhibit its interactions with promoters. At the same time, we propose that adjusting the strength, respective position of the promoter elements and the sequence of the initially transcribed region (in particular, its G/C-content) may allow efficient transition to elongation on the aptamer templates and may help to obtain single-strand promoters for in vitro and in vivo RNA production.

In conclusion, the holoenzyme-specific aptamers and aptamer-based templates described in the present paper provide a tool for structural analysis of different steps of promoter recognition and transcription initiation by bacterial RNAP, including the processes of DNA scrunching and abortive cycling, and for analysis of RNAP inhibition. We also propose that an aptamer-based approach can be useful to probe sequence specificity of the plethora of alternative σ factors in various bacteria for which only a very limited information on promoter specificity is currently available [39]. Detailed understanding of how these σ factors recognize their promoter elements will provide important insights into the mechanisms of transcription regulation and will allow rational design of novel σ–promoter pairs for controlled gene expression in synthetic biology applications.

AUTHOR CONTRIBUTION
Andrey Kulbachinskiy and Danil Pupov designed the research. Danil Pupov, Daria Esyunina and Andrey Feklistov performed the research. All authors analysed the data. Andrey Kulbachinskiy wrote the paper with the help of all other authors.

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SUPPLEMENTARY ONLINE DATA

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Supplementary Figure S1 is on the following page.

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Figure S1 Sequences and proposed secondary structures of aptamers to *E. coli* holoenzyme RNAP

(A) Full-length aptamer sequences are shown. The colour code corresponds to Figure 1(A) of the main text. The −10 and TG promoter elements are shown in red and green respectively. (B) The secondary aptamer structure was predicted using the mfold Web Server (http://mfold.rna.albany.edu) in the DNA folding mode [1]. The predicted wobble G:T base pairs are indicated with dotted lines. The aptamers are grouped on the basis of similarities of their sequences and proposed secondary structures.

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