Biochemistry (Moscow). Papers in Press. Published on February 11, 2019 as Manuscript BM18-301.

The σ^{24} Subunit of *Escherichia coli* RNA Polymerase Can Induce Transcriptional Pausing *in vitro*

A. B. Shikalov[1], D. M. Esyunina[1], D. V. Pupov[1], A. V. Kulbachinskiy[1], and I. V. Petushkov[1,a]*

SHIKALOV et al.

[1]Institute of Molecular Genetics, Russian Academy of Sciences, 123182 Moscow, Russia

[a]e-mail: telomer1@rambler.ru

Received November 2, 2018
Revised December 5, 2018
Accepted December 5, 2018

* To whom correspondence should be addressed.

Abbreviations: nt, nucleotides; RNAP, RNA polymerase; TEC, transcription elongation complex.

Abstract—The bacterium *Escherichia coli* has seven σ subunits that bind core RNA polymerase and are necessary for promoter recognition. It was previously shown that the σ^{70} and σ^{38} subunits can also interact with the transcription elongation complex (TEC) and stimulate pausing by recognizing DNA sequences similar to the –10 element of promoters. In this study, we analyzed the ability of the σ^{32}, σ^{28}, and σ^{24} subunits to induce pauses in reconstituted TECs containing corresponding –10 consensus elements. It was found that the σ^{24} subunit can induce a transcriptional pause depending on the presence of the –10 element. Pause formation is suppressed by the Gre factors, suggesting that the paused complex adopts a backtracked conformation. Some natural promoters contain potential signals of σ^{24}-dependent pauses in the initially transcribed regions, suggesting that such pauses may have regulatory functions in transcription.

Keywords: transcriptional pausing, RNA polymerase, alternative σ factors, Gre proteins

The σ factors play a crucial role in the process of transcription by allowing RNA polymerase (RNAP) to recognize different classes of promoters. All studied bacteria have the principal σ subunit (σ^{70} in *E. coli*) that is needed for transcription of housekeeping genes [1, 2].

Alternative σ subunits can recognize promoters that are significantly different from those recognized by the principal σ subunit and control gene expression in response to changes in the environment and stress factors. In *E. coli*, six alternative σ subunits were found, which could be divided into two families according to their protein sequence, σ^{70} and σ^{54} [2, 3]. The first family includes σ^{70}, σ^{38}, σ^{32}, σ^{28}, σ^{24}, and σ^{19}, while the second has only σ^{54}, which differs significantly from the others by the structure and mechanism of action; unlike σ^{70} family members, it needs an activator protein and ATP hydrolysis for transcription initiation [4]. The σ^{70} family contains four groups of σ factors [2, 5]. The principal σ^{70} subunit of *E. coli* is a member of group 1 and consists of four conservative regions (σ1, σ2, σ3, σ4) as well as a nonconserved region (NCR) between σ1 and σ2. Members of group 2 emerged from independent events of gene duplications in different bacteria [6]. A well-known member of group 2 in *E. coli* is σ^{38}, which lacks region σ1 and NCR. Analysis of the promoter complex structures and amino acid sequences revealed a high level of similarity between σ^{70} and σ^{38}, which allows them to recognize similar promoters [2, 7, 8]. The σ^{38} subunit is needed for transcription during stationary phase, plays a role in stress response (osmotic shock, high temperatures), and also participates in transcription at low temperatures [9, 10]. Group 3 is heterogeneous and includes multiple subgroups [6]. In *E. coli* the members of this group are σ^{32} and σ^{28}; their domain organization is similar to that of σ^{38}, but their sequences differ significantly and, accordingly, they recognize promoters with distinct sequences [11, 12]. The σ^{32} subunit recognizes promoters of genes during heat shock response [13, 14]. σ^{28} recognizes promoters of genes responsible for flagella synthesis [15-17]. The expression of this σ factor is also important for biofilm formation [18].

Members of group 4 have only regions σ2 and σ4, which are necessary for the recognition of the –10 and –35 promoter elements, respectively [1, 2, 6]. *Escherichia coli* has two representatives from this group, σ^{24} and σ^{19}. σ^{24} initiates transcription of heat shock genes as well as genes of the periplasmic stress response (triggered by

increased levels of misfolded proteins) [19-21]. This subunit is also necessary for cell resistance to Cu, Zn, and Cd ions [22]. The smallest σ^{19} participates in the transport of Fe ions at low intracellular Fe concentrations [23].

According to the classical model of transcription initiation, the σ subunit leaves the transcription complex during promoter escape. However, it was later found that the σ^{70} subunit could stay bound to RNAP during the elongation phase of transcription [24-29]. Moreover, σ^{70} is able to rebind free transcription elongation complex (TEC) [30-34]. When bound to the TEC, the σ subunit can recognize promoter-like sequences, which leads to transcriptional pausing [35, 36]. The key signal required for pause formation is a –10-like element in the transcribed DNA, which is recognized region σ2 of σ in the nontemplate DNA strand [30, 36-39]. The –35-like elements as well as DNA sequences around the RNA 3′-end, which can promote TEC backtracking, can also contribute to RNAP stalling [40-42]. Although σ^{70}-dependent pausing could be observed in both phage and bacterial genes [30, 36, 38, 43], the functional role of such pauses in most cases remains unknown (reviewed in [35, 44]).

Recently, we have shown that not only σ^{70} but also σ^{38} can induce transcriptional pauses, whose formation is dependent on the presence of –10-like elements [34]. However, it is not known if any of the other σ subunits in *E. coli* could induce transcriptional pausing. The goal of this work was to determine whether alternative σ^{32}, σ^{28}, or σ^{24} subunit could induce transcriptional pauses *in vitro*.

MATERIALS AND METHODS

Reagents. The following reagents and compounds were used: Tris, acrylamide, N,N′-methylene bis-acrylamide, urea, imidazole, magnesium chloride, sodium chloride, potassium chloride, phenylmethylsulfonyl fluoride (PMSF), and rifampicin (all from Sigma, USA; purity >99%); EDTA from Dia-M, Russia; 99.2%), isopropyl β-D-1-thiogalactopyranoside (IPTG) (Thermo Fisher Scientific, USA; >99%), potassium dihydrogen phosphate and potassium hydrogen phosphate (Roth, Germany; >99%); lysozyme (Amresco, USA; ultrapure); nucleotide triphosphates (NTPs) (Illustra, GE Healthcare, UK; >98%), kanamycin and ampicillin (Sintez, Russia).

Protein purification. *Escherichia coli* RNA polymerase (RNAP) core enzyme

with a 6× His-tag on the β′-subunit *N*-terminus was purified from the *E. coli* BL21(DE3) strain carrying the expression plasmid pVS10 containing all core RNAP genes under the control of T7 RNAP promoters. Protein expression and purification were performed as described previously [45].

The *rpoH* and *rpoF* genes, encoding the σ^{32} and σ^{28} subunits, respectively, were cloned into the pET29 vector between the NdeI and XhoI sites. The resulting plasmids pET29_proH and pET29_rpoF were used for transformation of *E. coli* strain 3013 (New England Biolabs, USA). An overnight culture of transformed cells (500 μl) was transferred to 500 ml of LB medium with kanamycin (50 μg/ml) and chloramphenicol (25 μg/ml, for *E. coli* 3013 pLys plasmid maintenance). IPTG was added to 1 mM at A_{600} = 0.6. In the case of σ^{32} expression, rifampicin (150 μg/ml) was added 30 min after IPTG to prevent transcription of chaperone genes to enhance formation of inclusion bodies. The induction was carried out for 2 h at 22°C, and the cells were collected by centrifugation. Inclusion bodies were isolated from the cells as described previously [46]. After renaturation, the protein mixture was purified by anion exchange chromatography using a 1-ml MonoQ column (GE Healthcare), which was pre-equilibrated with buffer containing 40 mM Tris-HCl, pH 7.9, 5% glycerol, 1 mM EDTA disodium salt, and 0.1 mM dithiothreitol. The sample was loaded at the rate of 0.5 ml/min and elution was carried out for 90 min using a linear NaCl gradient from 120 to 600 mM in the same buffer solution at 1 ml/min [47]. The σ^{32} and σ^{28} subunits were eluted at 17-22 and 38-43 min, respectively. These fractions were collected and concentrated with an Amicon Ultra-4 Ultracel-10K (Merck Millipore, USA) ultrafiltration system.

The *rpoE* gene encoding the σ^{24} subunit was cloned into the vector pLATE52 (Thermo Fisher Scientific) using the manufacturer's protocol. The resulting vector was used for transformation of the *E. coli* strain BL21(DE3). Cells were allowed to grow in 500 ml of LB medium with 20 ml aminopeptide and ampicillin (200 μg/ml). The induction was carried out by the addition of IPTG to 1 mM. Cells were pelleted by centrifugation and suspended in lysis buffer containing 50 mM potassium phosphate, pH 7.4, 500 mM NaCl, 1 mM $MgCl_2$, 0.1 mM PMSF, 0.2 mg/ml lysozyme, and 80 U DNase I (Thermo Fisher Scientific). σ^{24} was extracted from cellular lysate by Co^{2+}-affinity chromatography using a 1-ml HiTrap TALON crude column (GE Healthcare). The column was pre-equilibrated with buffer containing 50 mM potassium phosphate,

pH 7.4, 500 mM NaCl, and 1 mM $MgCl_2$. The sample was loaded at the rate of 0.5 ml/min, and the column was rinsed with 5 ml of the buffer. σ^{24} was eluted by the same buffer containing 200 mM imidazole. GreA and GreB factors were purified from the soluble protein fraction as described previously [48]. The purity of all protein preparations was ≥98% and the protein yield was from one to several milligrams.

In vitro **transcription.** To investigate the ability of alternative σ subunits to induce transcriptional pausing, TECs were assembled from oligonucleotides and core RNAP. Oligodeoxyribonucleotides and oligoribonucleotides were synthesized by DNA-Synthesis (Russia) or Evrogen (Russia) (see all sequences in Figs. 1 and 2). Radioactive label was introduced at the 5′-end of RNA oligonucleotides with T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP. The labeled RNA (250 nM final concentration) was mixed with the template DNA strand (2.5 μM) in TK buffer containing 40 mM Tris-HCl, pH 7.9, 40 mM KCl. The sample was incubated at 65°C for 3 min and then cooled to 20°C at 1°C/min. After this, the sample was diluted 3-fold with the TK buffer, and then core RNAP was added to 190 nM concentration. The mixture was incubated for 15 min at 37°C followed by the addition of nontemplate DNA strand to 1 μM and incubation for an additional 15 min at 37°C. The resulting preparation was then split into two parts. To the first part, a σ subunit was added to 2.5 μM final concentration, followed by incubation for 10 min at 37°C. To the second part, the same volume of the protein storage buffer was added (40 mM Tris-HCl, pH 7.9, 100 mM NaCl, 50% glycerol). Next, the assembled TECs were diluted 10-fold by the TK buffer pre-heated at 37°C, and aliquots for control samples were taken. In the case of reactions with GreA or GreB factors, they were added to the reaction mix at 1 μM concentration. Transcription was initiated by the addition of NTPs (100 μM each, final concentration) and $MgCl_2$ (10 mM) and stopped after various time intervals by the addition of an equal volume of stop-buffer (7 M urea, 30 mM EDTA, 2× TBE). The transcription products were separated by 15% denaturing PAGE (19 : 1) and visualized by a Typhoon 9500 scanner (GE Healthcare). The pausing efficiency was calculated as the ratio of paused products to the sum of paused and longer RNA products. All experiments were performed in least in two replicates (3-5 times for quantitative analysis).

RESULTS AND DISCUSSION

To investigate the ability of the alternative σ^{32}, σ^{28}, and σ^{24} subunits to induce transcriptional pausing, we (i) obtained purified σ subunits, (ii) performed *in vitro* assembly of TECs containing potential pause signals for these σ subunits, and (iii) tested the influence of the σ factors on RNA synthesis in such complexes. For our experiments, we assembled TECs from synthetic oligonucleotides carrying consensus –10 promoter sequences for each of the alternative σ subunits. An analogous approach was previously successfully used for investigation of *E. coli* σ^{70}- and σ^{38}-mediated transcriptional pauses [33, 34].

At the beginning, we cloned the genes of all investigated σ subunits, expressed them in *E. coli* cells, and purified the proteins. Control experiments showed that all 3 σ subunits have transcriptional activity and are able to direct promoter-specific transcription initiation by the RNAP holoenzyme (data not shown).

Formation of transcriptional pauses was studied using an *in vitro* system with reconstituted TECs assembled from synthetic oligonucleotides and core RNAP. Each TEC contained the –10 element consensus sequence for one of the studied σ factors (Cons-σ^{28}, Cons-σ^{32}, Cons-σ^{24}; Figs. 1a and 2a) [11, 12, 49]. Previous studies of σ^{70}- and σ^{38}-dependent pauses showed that the distance between the 3′-end of nascent RNA and the –10-like element during its recognition by the σ subunit is similar to that between the –10 element and transcription start site (TSS) in the promoter complex [33, 34]. However, the pause is observed several nucleotides downstream since RNAP can add several nucleotides to RNA after pause recognition. Therefore, in reconstituted TECs the positions of the RNA 3′-end relative to the –10-like motifs corresponded to the distances between the –10 elements and TSS in natural promoters for corresponding σ subunits (Figs. 1a and 2a; the typical TSS position relative to the –10 promoter element is shown in yellow). Furthermore, known pause signals for the σ^{70} subunit can contain sequences favorable for RNAP backtracking at the pause site, which serve as an important component of the pause signal [41]. Accordingly, the reconstituted TECs contained such a sequence (shown in cyan at Figs. 1a and 2a) based on the initially transcribed sequence of the *lac*UV5 promoter known to induce a strong σ^{70}-dependent pause [30, 33, 38].

For TEC assembly, RNA was first annealed to the template DNA

oligonucleotide, then the hybrid was incubated with core RNAP, and an excess of nontemplate DNA was added, resulting in formation of a completely functional TEC [33, 34]. Figure 1b contains shows the pattern of transcription products synthesized in reconstituted TECs containing consensus –10 elements for the σ^{32} and σ^{28} subunits. A major part of the starting 20 nt RNA is elongated by RNAP, thus indicating successful TEC assembly. Even in the absence of σ factors, a transcriptional pause is observed for both TECs (25 nt RNA product). This can be explained by the presence of a backtrack-inducing DNA sequence in this region [33, 41, 42, 50]. At the same time, the addition of either σ^{32} or σ^{28} subunit does not increase the pause efficiency, and the major part of active complexes efficiently transcribe through the expected pausing region and synthesize full-length 52 nt RNA product (a minor fraction of RNAs have shorter lengths possibly due to premature TEC stalling near the template end). Thus, these two σ subunits are apparently unable to induce transcriptional pausing in the studied model system.

The same approach was then used for the σ^{24} subunit. As shown in Fig. 2b, in the absence of σ^{24}, pausing occurs with low efficiency and most transcription complexes synthesize full-length or near full-length products. The addition of σ^{24} leads to a significant increase in the amounts of 23 and 25 nt RNA products corresponding to the expected site of pause. Indeed, the calculated pausing efficiency is much higher in the presence of σ^{24} subunit than in its absence (~40 and 7%, respectively).

To understand the role of the –10-like element (consensus GTCAAA [49]) in pause formation, we replaced a conserved dinucleotide TC in this element with AG (TEC –10M; Fig. 3a). As shown in the figure, this TEC has a slightly increased efficiency of the pause formation in the absence of σ^{24}. At the same time, the addition of σ^{24} does not increase pause formation (Fig. 3b). Thus, pause formation depends on the TC dinucleotide in the –10-like element. The role of these nucleotides can be explained from the structural point of view. Recent structural analysis of a complex of σ^{24} with a ssDNA oligonucleotide corresponding to the nontemplate promoter strand revealed that the TC residues in the –10 element are involved in multiple specific contacts with amino acid residues from region 2 of σ^{24} [51]. The same interactions are likely to participate in the pause signal recognition.

It was shown previously that during σ^{70}- and σ^{38}-dependent pausing, the TEC backtracks along the DNA template thus making impossible further nucleotide addition

[30, 33, 38, 41, 52]. Such backtracking is stimulated by interactions of the σ subunit with DNA during pause signal recognition, while RNAP continues nucleotide addition. As a result, an unstable stressed complex is formed that can relax by backward translocation, and the RNA 3′-end loses its contacts with the active site. In *E. coli*, such complexes can be reactivated by the transcription factors GreA and GreB, which assist RNA cleavage in the active site of RNAP [53, 54]. The Gre factors reactivate stalled TECs during σ^{70}- and σ^{38}-dependent pausing [30, 33, 34, 41, 52]. We tested whether these transcription factors can also affect the σ^{24} pause. As shown in Fig. 3, both factors, GreA and GreB, decrease the efficiency of pause formation. Thus, similarly to the other σ factors, σ^{24}-dependent pausing is likely accompanied by TEC backtracking.

σ^{24} is the first member of the group 4 σ factors for which the ability to induce transcriptional pausing is shown. The properties of σ^{24}-pause are similar to σ^{70}- and σ^{38}-dependent pauses investigated previously. Thus, conserved regions σ1 and σ3, which are absent in all σ group 4 members including σ^{24}, are not crucial for pause formation. Indeed, it was shown that a fragment of σ^{70} lacking most of its region 1 and the whole region 4 is also able to induce σ^{70}-dependent pausing [39]. Thus, similarly to σ^{70}-dependent pausing, the pause formation depends on the interactions of σ^{24} region 2 with core RNAP and with the –10-like element in the nontemplate DNA strand [32].

Two mechanisms of σ-dependent pausing formation were proposed previously. In the first case, the pause signal can be recognized by the same σ molecule that participated in transcription initiation and remained bound to RNAP during promoter escape (*in cis* mechanism; Fig. 4a) [37]. In the second case, the σ factor can bind free TEC and induce pausing *in trans* (Fig. 4a). *In trans* pause formation was shown for both σ^{70} and σ^{38} subunits [30, 31, 33, 34]. In this work, we tested only *in trans* pausing, by adding the σ factors to TECs carrying consensus –10 element sequences.

In contrast to the σ^{24} subunit, σ^{28} and σ^{32} (group 3 σ factors) do not induce transcriptional pausing in this model system. The σ subunit concentrations used in this work (2.5 μM) greatly exceed previously measured dissociation constants for binding of σ^{70} and σ^{38} to the TEC (~150 and 300 nM, respectively) [34] and are comparable to or greater than intracellular concentrations of σ^{28} and σ^{32} [55, 56]. It is difficult to understand why group 3 σ factors do not induce transcriptional pausing, since no information on the structure of RNAP holoenzymes containing these alternative σ

subunits is available to date. Furthermore, it is known that pausing signals for σ^{70}-dependent pauses can include not only –10-like elements but also additional motifs such as backtrack-inducing sequence around the RNA 3′-end or –35-like element [40-42, 50]. It is possible that formation of σ^{28}- and σ^{32}-dependent pauses may also require additional DNA sequences that were not present in the TECs analyzed in this study.

Analysis of the ability of alternative σ subunits to induce transcriptional pausing after promoter-dependent transcription initiation is an important task for future studies. Indeed, analysis of known promoters for σ^{32}, σ^{28}, and σ^{24} reveals that some of them contain in the initially transcribed region sequences similar to the –10 element consensus for these σs (Fig. 4b). Thus, σ-dependent pauses might occur by an *in cis* mechanism during promoter-dependent transcription if the σ subunits could remain bound to RNAP after promoter escape and recognize these sequences. It is possible that σ^{28}- and σ^{32}-dependent pauses may be detected in the future in such experimental systems. Furthermore, it would be interesting to investigate the influence of temperature on σ^{32}-dependent pausing, since this subunit is responsible for transcription under heat shock conditions.

Although σ-dependent pauses were discovered long ago in bacteriophages and in several phylogenetically distant bacterial species [30, 36, 38, 57], their prevalence and functional significance in genome transcription are still unknown even for model organisms such as *E. coli* [44]. Up to date, there are only rough estimates that σ^{70}-dependent pauses can occur during transcription of 10-20% of operons [43, 58]. However, many genes are transcribed with the participation of alternative σ subunits that may also induce pausing, suggesting that the frequency of such pauses may be underestimated. Indeed, it was demonstrated that the σ^{70} subunit can bind TEC after transcription initiation by the RNAP holoenzyme containing the σ^{28} subunit (Fig. 4a) [31]. As we have shown, pauses can be induced by multiple σ factors, and their exchange could increase the complexity of transcriptional pausing and potentially participate in fine tuning of gene expression.

Acknowledgements

We thank I. Artsimovich for the pVS10 plasmid, A. Oguienko for testing the σ^{28}

activity, and anonymous reviewers for valuable comments.

Funding

This work was supported by the Russian Science Foundation (grant 16-14-10377).

REFERENCES

1. Feklistov, A., Sharon, B. D., Darst, S. A., and Gross, C. A. (2014) Bacterial sigma factors: a historical, structural, and genomic perspective, *Annu. Rev. Microbiol.*, **68**, 357-376.
2. Gruber, T. M., and Gross, C. A. (2003) Multiple sigma subunits and the partitioning of bacterial transcription space, *Annu. Rev. Microbiol.*, **57**, 441-466.
3. Paget, M. S. (2015) Bacterial sigma factors and anti-sigma factors: structure, function and distribution, *Biomolecules*, **5**, 1245-1265.
4. Zhang, N., Darbari, V. C., Glyde, R., Zhang, X., and Buck, M. (2016) The bacterial enhancer-dependent RNA polymerase, *Biochem. J.*, **473**, 3741-3753.
5. Lonetto, M., Gribskov, M., and Gross, C. A. (1992) The sigma 70 family: sequence conservation and evolutionary relationships, *J. Bacteriol.*, **174**, 3843-3849.
6. Iyer, L. M., and Aravind, L. (2012) Insights from the architecture of the bacterial transcription apparatus, *J. Struct. Biol.*, **179**, 299-319.
7. Maciag, A., Peano, C., Pietrelli, A., Egli, T., De Bellis, G., and Landini, P. (2011) *In vitro* transcription profiling of the sigmaS subunit of bacterial RNA polymerase: re-definition of the sigmaS regulon and identification of sigmaS-specific promoter sequence elements, *Nucleic Acids Res.*, **39**, 5338-5355.
8. Liu, B., Zuo, Y., and Steitz, T. A. (2016) Structures of *E. coli* sigmaS-transcription initiation complexes provide new insights into polymerase mechanism, *Proc. Natl. Acad. Sci. USA*, **113**, 4051-4056.
9. White-Ziegler, C. A., Um, S., Perez, N. M., Berns, A. L., Malhowski, A. J., and Young, S. (2008) Low temperature (23°C) increases expression of biofilm-, cold-

shock- and RpoS-dependent genes in *Escherichia coli* K-12, *Microbiology*, **154**, 148-166.

10. Battesti, A., Majdalani, N., and Gottesman, S. (2011) The RpoS-mediated general stress response in *Escherichia coli*, *Annu. Rev. Microbiol.*, **65**, 189-213.
11. Zhao, K., Liu, M., and Burgess, R. R. (2007) Adaptation in bacterial flagellar and motility systems: from regulon members to "foraging"-like behavior in *E. coli*, *Nucleic Acids Res.*, **35**, 4441-4452.
12. Nonaka, G., Blankschien, M., Herman, C., Gross, C. A., and Rhodius, V. A. (2006) Regulon and promoter analysis of the *E. coli* heat-shock factor, sigma32, reveals a multifaceted cellular response to heat stress, *Genes Dev.*, **20**, 1776-1789.
13. Neidhardt, F. C., VanBogelen, R. A., and Lau, E. T. (1983) Molecular cloning and expression of a gene that controls the high-temperature regulon of *Escherichia coli*, *J. Bacteriol.*, **153**, 597-603.
14. Grossman, A. D., Erickson, J. W., and Gross, C. A. (1984) The htpR gene product of *E. coli* is a sigma factor for heat-shock promoters, *Cell*, **38**, 383-390.
15. Komeda, Y. (1986) Transcriptional control of flagellar genes in *Escherichia coli* K-12, *J. Bacteriol.*, **168**, 1315-1318.
16. Komeda, Y., Kutsukake, K., and Iino, T. (1980) Definition of additional flagellar genes in *Escherichia coli* K12, *Genetics*, **94**, 277-290.
17. Arnosti, D. N., and Chamberlin, M. J. (1989) Secondary sigma factor controls transcription of flagellar and chemotaxis genes in *Escherichia coli*, *Proc. Natl. Acad. Sci. USA*, **86**, 830-834.
18. Barrios, A. F., Zuo, R., Ren, D., and Wood, T. K. (2006) Hha, YbaJ, and OmpA regulate *Escherichia coli* K12 biofilm formation and conjugation plasmids abolish motility, *Biotechnol. Bioeng.*, **93**, 188-200.
19. Lipinska, B., Sharma, S., and Georgopoulos, C. (1988) Sequence analysis and regulation of the htrA gene of *Escherichia coli*: a sigma 32-independent mechanism of heat-inducible transcription, *Nucleic Acids Res.*, **16**, 10053-10067.
20. Wang, Q. P., and Kaguni, J. M. (1989) A novel sigma factor is involved in expression of the rpoH gene of *Escherichia coli*, *J. Bacteriol.*, **171**, 4248-4253.
21. Rouviere, P. E., De Las Penas, A., Mecsas, J., Lu, C. Z., Rudd, K. E., and Gross, C. A. (1995) rpoE, the gene encoding the second heat-shock sigma factor, sigma E, in *Escherichia coli*, *EMBO J.*, **14**, 1032-1042.

22. Egler, M., Grosse, C., Grass, G., and Nies, D. H. (2005) Role of the extracytoplasmic function protein family sigma factor RpoE in metal resistance of *Escherichia coli*, *J. Bacteriol.*, **187**, 2297-2307.
23. Angerer, A., Enz, S., Ochs, M., and Braun, V. (1995) Transcriptional regulation of ferric citrate transport in *Escherichia coli* K-12. Fecl belongs to a new subfamily of sigma 70-type factors that respond to extracytoplasmic stimuli, *Mol. Microbiol.*, **18**, 163-174.
24. Bar-Nahum, G., and Nudler, E. (2001) Isolation and characterization of sigma(70)-retaining transcription elongation complexes from *Escherichia coli*, *Cell*, **106**, 443-451.
25. Kapanidis, A. N., Margeat, E., Laurence, T. A., Doose, S., Ho, S. O., Mukhopadhyay, J., Kortkhonjia, E., Mekler, V., Ebright, R. H., and Weiss, S. (2005) Retention of transcription initiation factor sigma70 in transcription elongation: single-molecule analysis, *Mol. Cell*, **20**, 347-356.
26. Mukhopadhyay, J., Kapanidis, A. N., Mekler, V., Kortkhonjia, E., Ebright, Y. W., and Ebright, R. H. (2001) Translocation of sigma(70) with RNA polymerase during transcription: fluorescence resonance energy transfer assay for movement relative to DNA, *Cell*, **106**, 453-463.
27. Mooney, R. A., Davis, S. E., Peters, J. M., Rowland, J. L., Ansari, A. Z., and Landick, R. (2009) Regulator trafficking on bacterial transcription units *in vivo*, *Mol. Cell*, **33**, 97-108.
28. Raffaelle, M., Kanin, E. I., Vogt, J., Burgess, R. R., and Ansari, A. Z. (2005) Holoenzyme switching and stochastic release of sigma factors from RNA polymerase *in vivo*, *Mol. Cell*, **20**, 357-366.
29. Harden, T. T., Wells, C. D., Friedman, L. J., Landick, R., Hochschild, A., Kondev, J., and Gelles, J. (2016) Bacterial RNA polymerase can retain sigma70 throughout transcription, *Proc. Natl. Acad. Sci. USA*, **113**, 602-607.
30. Brodolin, K., Zenkin, N., Mustaev, A., Mamaeva, D., and Heumann, H. (2004) The sigma 70 subunit of RNA polymerase induces lacUV5 promoter-proximal pausing of transcription, *Nat. Struct. Mol. Biol.*, **11**, 551-557.
31. Goldman, S. R., Nair, N. U., Wells, C. D., Nickels, B. E., and Hochschild, A. (2015) The primary sigma factor in *Escherichia coli* can access the transcription elongation complex from solution *in vivo*, *eLife*, **4**, e10514.

32. Mooney, R. A., Darst, S. A., and Landick, R. (2005) Sigma and RNA polymerase: an on-again, off-again relationship? *Mol. Cell*, **20**, 335-345.
33. Zhilina, E., Esyunina, D., Brodolin, K., and Kulbachinskiy, A. (2012) Structural transitions in the transcription elongation complexes of bacterial RNA polymerase during sigma-dependent pausing, *Nucleic Acids Res.*, **40**, 3078-3091.
34. Petushkov, I., Esyunina, D., and Kulbachinskiy, A. (2017) Sigma38-dependent promoter-proximal pausing by bacterial RNA polymerase, *Nucleic Acids Res.*, **45**, 3006-3016.
35. Perdue, S. A., and Roberts, J. W. (2011) Sigma(70)-dependent transcription pausing in *Escherichia coli*, *J. Mol. Biol.*, **412**, 782-792.
36. Ring, B. Z., Yarnell, W. S., and Roberts, J. W. (1996) Function of *E. coli* RNA polymerase sigma factor sigma 70 in promoter-proximal pausing, *Cell*, **86**, 485-493.
37. Marr, M. T., Datwyler, S. A., Meares, C. F., and Roberts, J. W. (2001) Restructuring of an RNA polymerase holoenzyme elongation complex by lambdoid phage Q proteins, *Proc. Natl. Acad. Sci. USA*, **98**, 8972-8978.
38. Nickels, B. E., Mukhopadhyay, J., Garrity, S. J., Ebright, R. H., and Hochschild, A. (2004) The sigma 70 subunit of RNA polymerase mediates a promoter-proximal pause at the lac promoter, *Nat. Struct. Mol. Biol.*, **11**, 544-550.
39. Zenkin, N., Kulbachinskiy, A., Yuzenkova, Y., Mustaev, A., Bass, I., Severinov, K., and Brodolin, K. (2007) Region 1.2 of the RNA polymerase sigma subunit controls recognition of the –10 promoter element, *EMBO J.*, **26**, 955-964.
40. Devi, P. G., Campbell, E. A., Darst, S. A., and Nickels, B. E. (2010) Utilization of variably spaced promoter-like elements by the bacterial RNA polymerase holoenzyme during early elongation, *Mol. Microbiol.*, **75**, 607-622.
41. Perdue, S. A., and Roberts, J. W. (2010) A backtrack-inducing sequence is an essential component of *Escherichia coli* sigma(70)-dependent promoter-proximal pausing, *Mol. Microbiol.*, **78**, 636-650.
42. Strobel, E. J., and Roberts, J. W. (2014) Regulation of promoter-proximal transcription elongation: enhanced DNA scrunching drives lambdaQ antiterminator-dependent escape from a sigma70-dependent pause, *Nucleic Acids Res.*, **42**, 5097-5108.

43. Deighan, P., Pukhrambam, C., Nickels, B. E., and Hochschild, A. (2011) Initial transcribed region sequences influence the composition and functional properties of the bacterial elongation complex, *Genes Dev.*, **25**, 77-88.
44. Petushkov, I., Esyunina, D., and Kulbachinskiy, A. (2017) Possible roles of sigma-dependent RNA polymerase pausing in transcription regulation, *RNA Biol.*, **14**, 1678-1682.
45. Svetlov, V., and Artsimovitch, I. (2015) Purification of bacterial RNA polymerase: tools and protocols, *Methods Mol. Biol.*, **1276**, 13-29.
46. Pupov, D., Kuzin, I., Bass, I., and Kulbachinskiy, A. (2014) Distinct functions of the RNA polymerase sigma subunit region 3.2 in RNA priming and promoter escape, *Nucleic Acids Res.*, **42**, 4494-4504.
47. Anthony, L. C., Foley, K. M., Thompson, N. E., and Burgess, R. R. (2003) Expression, purification of, and monoclonal antibodies to sigma factors from *Escherichia coli*, *Methods Enzymol.*, **370**, 181-192.
48. Laptenko, O., and Borukhov, S. (2003) Biochemical assays of Gre factors of *Thermus thermophilus*, *Methods Enzymol.*, **371**, 219-232.
49. Rhodius, V. A., Suh, W. C., Nonaka, G., West, J., and Gross, C. A. (2006) Conserved and variable functions of the sigmaE stress response in related genomes, *PLoS Biol.*, **4**, e2.
50. Strobel, E. J., and Roberts, J. W. (2015) Two transcription pause elements underlie a sigma70-dependent pause cycle, *Proc. Natl. Acad. Sci. USA*, **112**, E4374-4380.
51. Campagne, S., Marsh, M. E., Capitani, G., Vorholt, J. A., and Allain, F. H. (2014) Structural basis for –10 promoter element melting by environmentally induced sigma factors, *Nat. Struct. Mol. Biol.*, **21**, 269-276.
52. Marr, M. T., and Roberts, J. W. (2000) Function of transcription cleavage factors GreA and GreB at a regulatory pause site, *Mol. Cell*, **6**, 1275-1285.
53. Borukhov, S., Sagitov, V., and Goldfarb, A. (1993) Transcript cleavage factors from *E. coli*, *Cell*, **72**, 459-466.
54. Laptenko, O., Lee, J., Lomakin, I., and Borukhov, S. (2003) Transcript cleavage factors GreA and GreB act as transient catalytic components of RNA polymerase, *EMBO J.*, **22**, 6322-6334.
55. Grigorova, I. L., Phleger, N. J., Mutalik, V. K., and Gross, C. A. (2006) Insights into transcriptional regulation and sigma competition from an equilibrium model

of RNA polymerase binding to DNA, *Proc. Natl. Acad. Sci. USA*, **103**, 5332-5337.

56. Jishage, M., Iwata, A., Ueda, S., and Ishihama, A. (1996) Regulation of RNA polymerase sigma subunit synthesis in *Escherichia coli*: intracellular levels of four species of sigma subunit under various growth conditions, *J. Bacteriol.*, **178**, 5447-5451.
57. Zhilina, E., Miropolskaya, N., Bass, I., Brodolin, K., and Kulbachinskiy, A. (2011) Characteristics of sigma-dependent pausing in RNA polymerases from *E. coli* and *T. aquaticus*, *Biochemistry (Moscow)*, **76**, 1348-1358.
58. Hatoum, A., and Roberts, J. (2008) Prevalence of RNA polymerase stalling at *Escherichia coli* promoters after open complex formation, *Mol. Microbiol.*, **68**, 17-28.

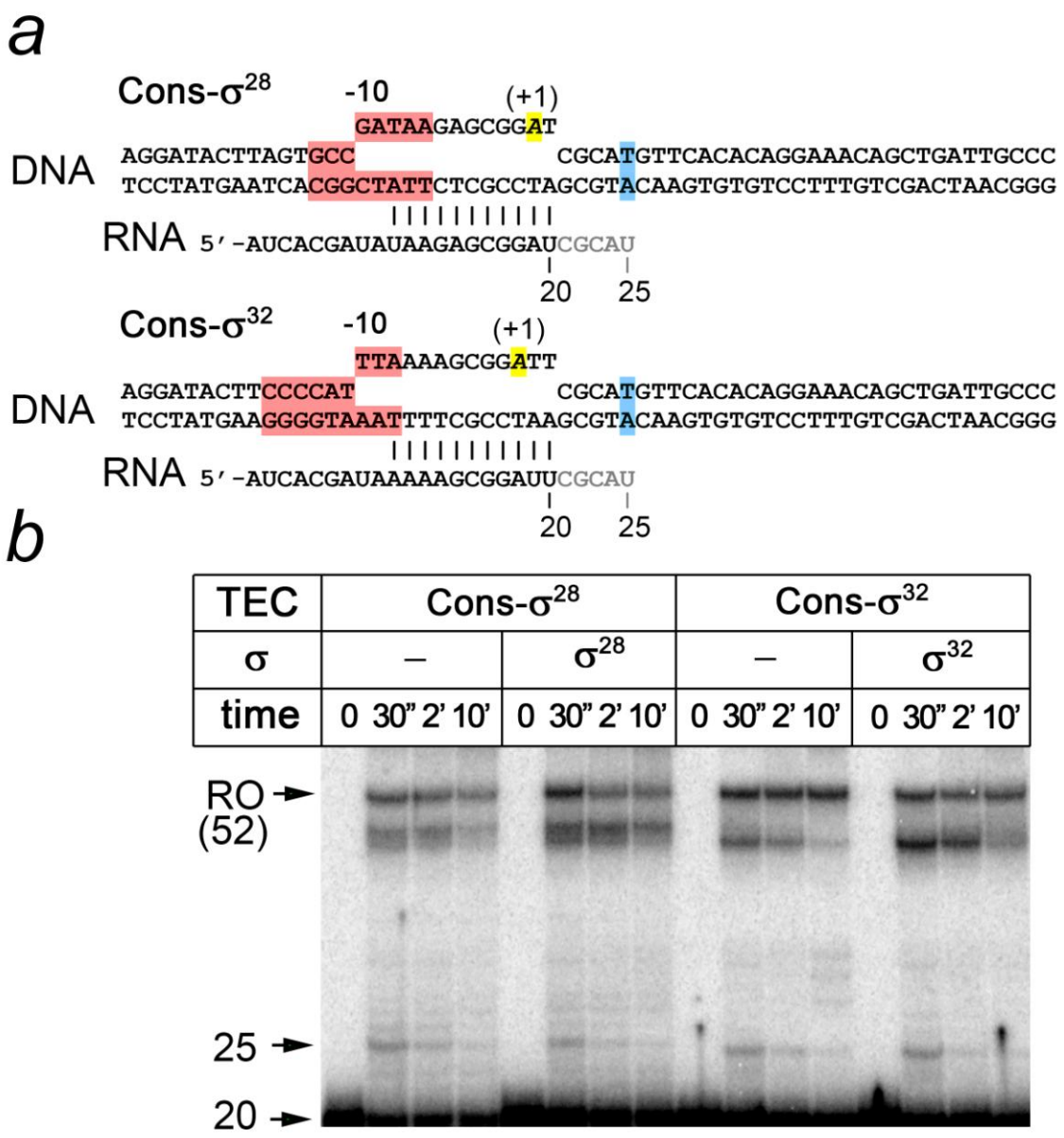


Fig. 1. Analysis of the formation of transcriptional pauses involving the σ^{32} and σ^{28} subunits. a) Structure of the TECs used in the experiments. The –10-like elements are shown in pink, the expected position of the transcription pause in blue, and the typical location of the TSS relative to –10 elements in promoters in yellow. Nucleotides added to the 3′-end of starting RNA during transcription are shown in gray. b) Analysis of transcription products synthesized in the TECs depending on the presence of the σ^{28} and σ^{32} subunits. An electrophoregram of RNA products separated by 15% PAGE under denaturing conditions is shown. Positions of the starting RNA (20 nt), paused RNA (25 nt), and the full-length product (RO, 52 nt) are indicated.

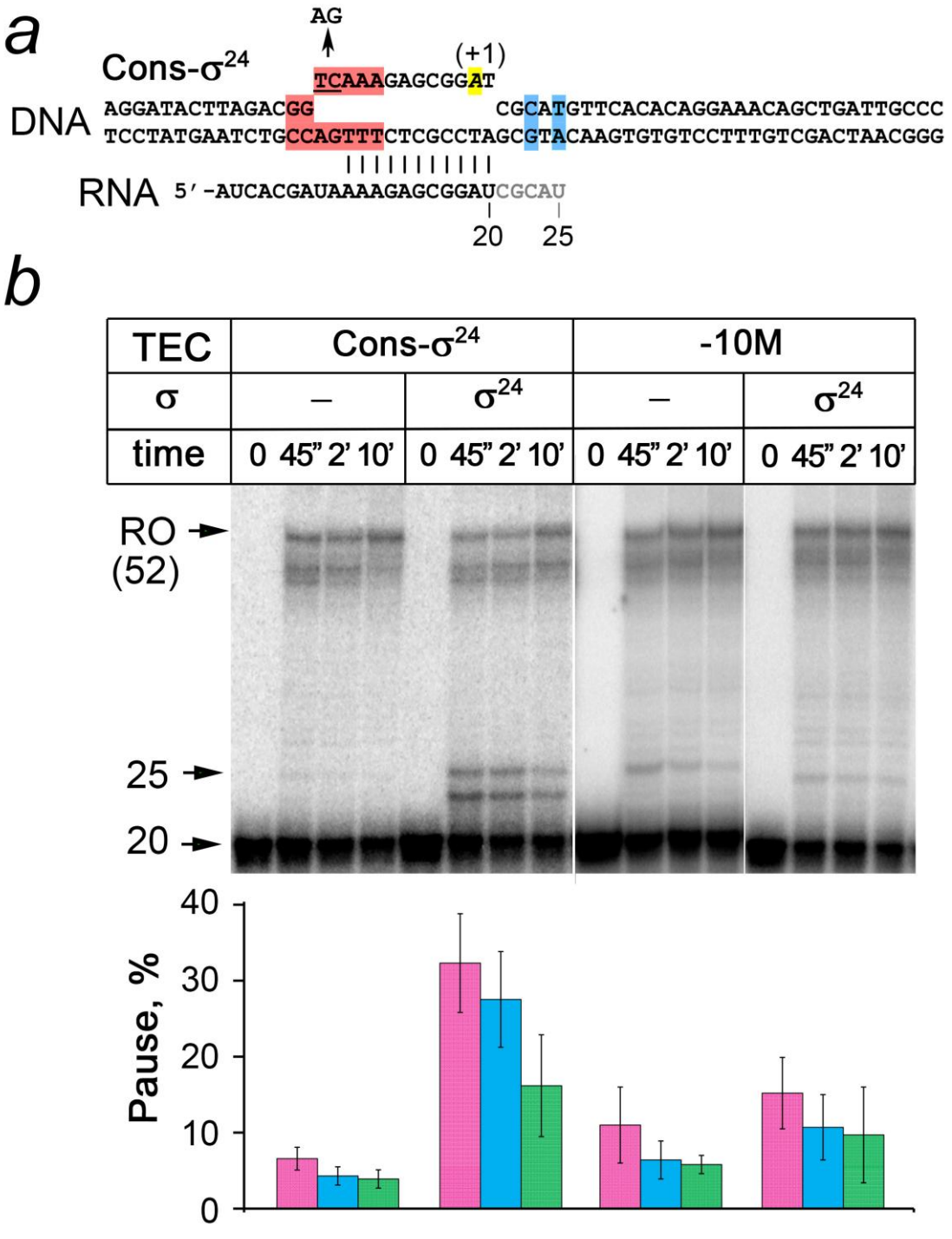


Fig. 2. Analysis of σ^{24}-dependent transcriptional pausing. a) The TEC structure. b) Analysis of transcription products in the TEC containing the consensus –10-element for σ^{24} or –10-element with the substitution of the conserved TC dinucleotide with AG (–10M). Electrophoregram of RNA products separated by 15% PAGE is shown. Positions of the starting RNA (20 nt), paused RNA (25 nt), and the full-length product (RO, 52 nt) are indicated. The histogram shows the efficiency of pause formation (the ratio of paused RNA products to the sum of paused and read-through RNAs in percent) for each gel lane.

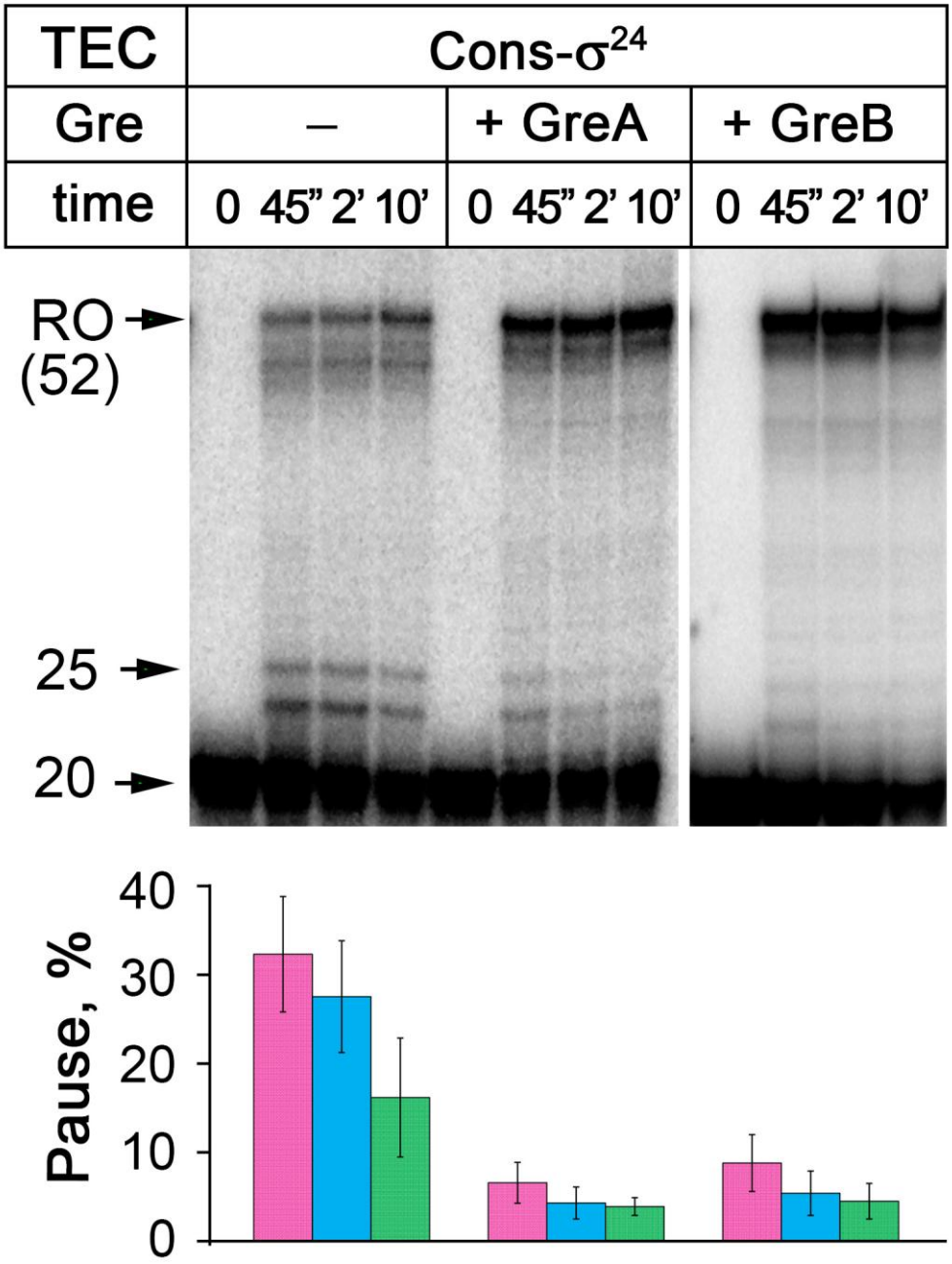
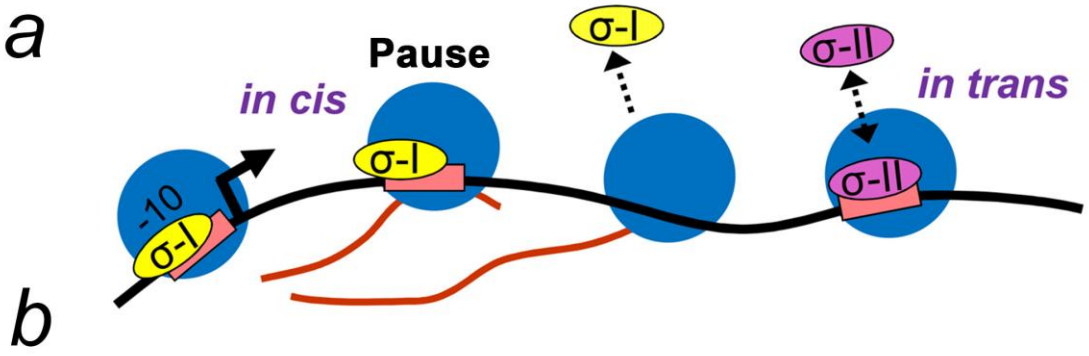


Fig. 3. Effects of Gre factors on σ^{24} pausing. All designations correspond to Fig. 2.

a



b

σ	Promoter	-35	-10	+1	-10-like element
σ^{24}	consensus	GGAACTT	TCAAA		
	*yfeKyfe*Sp	GAAACTTTACCTGATTCTGGCAGTCAAATCGGCT**A**TCACA			
	*yie*Ep	CGAACTTTTAGCCGCTTTAGTCTGTCCATCATTCC**A**GTAAATGATTACTCTTGTATTCATAA			
	*rpo*Ep	GGAACTTTACAAAAACGAGACACTCTAACCCTTTG**C**TTGCTCAAATTCGAT			
σ^{28}	consensus	TAAAG	GCCGATAA		
	*Ves*p	TAACGTAAATCACCCGAGTTGCCGATAACCATCC**A**CGGTTACCTGAACGATAACAATGA			
	*flg*Mp	TAAAGATTACCCGTCCCTTGCCGATAAATAAGC**A**ACACATGATAAAAGCGCCCTCAAT			
	*yjd*Fp2	TAAAGATTACCCGTCCCTTGCCGATAAATAAGC**A**ACACATGATAAAAGCGCCCTCAAT			
σ^{32}	consensus	CTTGAAT	CCCCATAT		
	*dna*Kp2	GTTGAAACCAGACGTTTCGCCCCTATTACAGACTC**A**CAACCACATGATGACCGA			
	*yad*F2	GTTGAAACCAGACGTTTCGCCCCTATTACAGACTC**A**CAACCACATGATGACCGA			
	*yga*Dp	GTAAAATTGCAACGCCAACACCATCTTCCTGAC**G**AAAGTGCTATCTTGTCCGGCATAAATTT			

Fig. 4. Possible mechanisms for the formation of transcriptional pauses with the participation of alternative σ subunits. a) Formation of pauses *in cis*, when the pause signal is recognized in promoter-proximal region by the same σ subunit that participated in transcription initiation, and *in trans*, when another σ subunit binds free TEC. b) Examples of promoters containing potential σ-dependent pause signals in the initially transcribed regions. For each σ subunit, consensus sequences of –35 (turquoise) and –10 (pink) elements are shown; the starting point of transcription is shown in bold.