Promoter clearance and escape in prokaryotes

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Abstract

Promoter escape is the last stage of transcription initiation when RNA polymerase, having initiated de novo phosphodiester bond synthesis, must begin to relinquish its hold on promoter DNA and advance to downstream regions (DSRs) of the template. In vitro, this process is marked by the release of high levels of abortive transcripts at most promoters, reflecting the high instability of initial transcribing complexes (ITCs) and indicative of the existence of barriers to the escape process. The high abortive initiation level is the result of the existence of unproductive ITCs that carry out repeated initiation and abortive release without escaping the promoter. The formation of unproductive ITCs is a widespread phenomenon, but it occurs to different extent on different promoters. Quantitative analysis of promoter mutations suggests that the extent and pattern of abortive initiation and promoter escape is determined by the sequence of promoter elements, both in the promoter recognition region (PRR) and the initial transcribed sequence (ITS). A general correlation has been found that the stronger the promoter DNA-polymerase interaction, the poorer the ability of RNA polymerase to escape the promoter. In gene regulation, promoter escape can be the rate-limiting step for transcription initiation. An increasing number of regulatory proteins are known to exert their control at this step. Examples are discussed with an emphasis on the diverse mechanisms involved. At the molecular level, the X-ray crystal structures of RNA polymerase and its various transcription complexes provide the framework for understanding the functional data on abortive initiation and promoter escape. Based on structural and biochemical evidence, a mechanism for abortive initiation and promoter escape is described.

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1. Overview

1.1. Scope and substance of this review

Promoter escape is the stage of transcription straddling the initiation and elongation phases. Depending on one’s research emphasis, promoter escape can be viewed as the last step of transcription initiation, or the ensuing tentative first step towards elongation. Promoter escape is linked intimately to the phenomenon of abortive initiation which, when first discovered, was considered enigmatic and puzzling. Yet its mechanism stands poised to be solved, due to substantial recent advances—quantitative and qualitative—in our knowledge of transcription. The diverse experimental approaches applied to studying the functional and structural interactions between RNA polymerase and nucleic acids are leading to the development of a mechanism of promoter escape. Thus, this is an exciting time to be in the area of promoter escape.

Two novel properties of RNA polymerase reported at the beginning of the 1990s turned the tide of the transcription field. These were the observations that RNA polymerase contains a transcript cleavage activity [1] and that at specific sequences, can undergo transcriptional arrest forming “dead-end” complexes [2,3]. These observations spurred the discovery of protein factors GreA, GreB, and TFIIS [4–6; also see Fish and Kane, this volume], which were shown subsequently to stimulate the intrinsic cleavage activity of
2.1. Definition and distinction of terms

Promoter clearance and promoter escape were both coined [36,37] to describe the phenomenon of abundant transcription and release of short template-specified RNAs (i.e. abortive initiation), delaying the movement of initiated RNA polymerase into the elongation phase [37–40]. “Escape” deals directly with issues affecting the downstream movement of a polymerase molecule. “Clearance,” on the other hand, implies a sufficient movement of the enzyme downstream to avert the core promoter elements for binding a second polymerase. Of the two terms, I will use promoter escape preferentially in this review.

2.2. Promoter escape: the process

Promoter escape refers to the last stage of transcription initiation spanning the de novo synthesis of the first phosphodiester bond to the formation of an elongation complex. On many promoters, this distance of 10–15 bp on the DNA template prescribes an arduous journey for the polymerase molecule. During this process, RNA polymerase at each promoter appears to synthesize and release a characteristic set of abortive RNAs. These short transcripts usually range 2–12 nucleotides (nt) in length, although abortive transcripts as long as 15–17 nt have been reported [16,41]. At most promoters where transcription initiation has been quantitatively examined, abortive initiation occurs at high molar excess to template concentration under single cycle conditions [37–40]. Abortive transcription is not affected by heparin (which binds free RNA polymerase) but is greatly diminished by rifampicin (which inhibits the formation of the second phosphodiester bond). These observations led to the conclusion that, during abortive initiation, RNA polymerase cycles repeatedly at the promoter without dissociating from the template but rather remains bound as open and initial transcribing complexes [37]. The picture of an ITC inferred from these observations is one of high instability: when the short transcripts are released, these complexes resume the open complex structure and can begin another round of chain initiation. Footprinting analyses confirm that ITCs are essentially indistinguishable from the open complexes [3,42].

2.3. Kinetic diagram of transcription initiation

To understand the promoter escape process and its contribution to promoter activity, it is necessary to place this process in the context of the multi-step program of transcription initiation (Fig. 1A). On most E. coli $^{70}$ promoters, RNA polymerase holoenzyme binds the promoter DNA to set up an active catalytic complex through two major conformational transitions. Upon first binding to the double-stranded promoter DNA, a complex lacking catalytic activity called the closed complex (RP$_0$) is formed. Subsequently, the enzyme melts apart 13–14 base pairs (bp) of template DNA from –11 to +2/+3 around the +1 start site of transcription, forming a catalytically competent open complex (RP$_3$). Kinetic investigations show that the closed complex formation reaction is a rapid equilibrium governed
by a binding constant $K_B$ (i.e. $k_1/k_{-1}$); the isomerization to form the open complex can be described by forward and reverse rate constants $k_2$ and $k_{-2}$. On several promoters, kinetically significant intermediates within each major transition have been demonstrated (reviewed in Refs. [43,44]). Here, we present a simplified view of open complex formation, assuming these steps are not rate-limiting for a promoter blocked at the escape stage.

On many promoters, the open complex is highly stable and its formation is essentially irreversible (i.e. $k_{-2}$ is negligible). For these promoters, the value $K_B \times k_2$ correlates with the frequency of chain initiation, as measured by formation of the first (few) phosphodiester bond(s) using limited nucleoside triphosphates (NTP) [45,46]. For a subset of promoters, however, this in vitro indicator of promoter strength is inadequate for predicting the level of promoter-specified gene product in vivo [47]. The discrepancy has been attributed to differences in promoter escape at different promoters [48].

In Fig. 1A, promoter escape is illustrated (for simplicity’s sake) as a six-step process spanning chain initiation by the open complex, to sigma release yielding an elongation complex. Each ITC has three rate constants governing its formation and disappearance, making the detailed kinetic analysis of this phase complicated and difficult. To study the kinetics of promoter escape, the rate of synthesis of productive RNA from pre-formed open complexes is measured to obtain a composite rate constant designated here as $k_E$ [49,50]. Relatively short run-off templates are used such that productive RNA synthesis is not complicated by downstream kinetic bottlenecks from the elongation phase.

In the $k_E$ measurement, even when the RNA chain initiation reaction is synchronized by adding NTP to pre-formed open complexes, the reaction soon yields a heterogeneous mixture of ITCs—due to their varied abortive release and reinitiation rates. The product of this mixed population of ITCs is a heterogeneous collection of abortive RNAs which, when fractionated on high percentage polyacrylamide gels according to their size and nucleotide composition, appear as a ladder of RNA bands (see Fig. 2).

2.4. Promoter escape, the event, may or may not involve sigma release

To undergo promoter escape, an RNA polymerase molecule must relinquish its contacts to the core promoter elements. This event is best documented by changes in the DNase I footprinting analysis where the footprint of an open complex (from $-50$ to $+20$) shrinks to that of an elongation complex (from $-5$ to $+25$); this change was thought to coincide with the release of the sigma subunit [42,51,53]. On several promoter templates where sigma release has been specifically examined, this transition occurs when the nascent transcript reaches $\sim 9–10$ nt in length [51–54]. With the release of sigma factor, abortive initiation ceases, the initiation phase is deemed complete, and the elongation phase begins. Thus, achieving the elongation complex structure defines the endpoint of promoter escape.
Recently, the criterion of sigma release accompanying the initiation–elongation transition has become an issue [55,56]. One study found that under conditions of solid-state transcription, a fraction of RNA polymerase retains its $\sigma^{70}$ subunit throughout elongation on several promoters tested [55]. A second study, applying fluorescence resonance energy transfer (FRET) analysis on “in gel” transcribed complexes of lacUV5, found that 100% of the $\sigma^{70}$ subunit is retained on an enzyme positioned at +15 [56]. (Earlier studies showed that, on lacUV5, sigma was released when transcription had proceeded to +11 [51].) In earlier experiments, although sigma release was inferred rather than directly measured, the results were consistent with alteration and weakening in the association of sigma to core polymerase, such that additional physical manipulation resulted in release [51–53,57,58]. An experiment explicitly examining the status of sigma using sucrose gradient centrifugation of $^{35}$S-labeled P. putida RNA polymerase showed that, while sigma was not released from open complexes, it was released from complexes engaged in RNA synthesis [59]. Furthermore, binding of RNA polymerase to polynucleotides or tRNA led to almost quantitative release of sigma [59].

Given the ambiguous status of sigma release, I shall, in subsequent sections of this review, refer to promoter escape as an event culminating in sigma displacement—that is, the displacement of sigma from its binding to the $-10$ and $-35$ promoter elements. After displacement, sigma may be repositioned over another stretch of DNA or released, depending on template sequence [60]. Sigma displacement...
can account for two observations affiliated with the initiation–elongation transition: the shrunken DNase I footprints of elongation complexes, and the cessation of repetitive abortive initiation.

T7 RNA polymerase sets the precedence of utilizing the displacement of a crucial protein domain contact to mark the initiation–elongation transition. This single polypeptide enzyme carries out all steps of the transcription cycle without additional specificity subunits. Instead, the enzyme utilizes a protein feature called the “specificity loop” to contact promoter DNA during open complex formation. Later, this loop contacts the nascent RNA during the initiation–elongation transition [35].

3. Promoter escape can be a rate-limiting step in transcription initiation

The goal of research on promoter-specified transcription is twofold: one, to understand how a promoter achieves its strength; and two, to understand its regulation. Both require knowledge of the rate-limiting step for that promoter in the context of the sequential scheme of transcription initiation (Fig. 1A). Past research on E. coli EoT promoters has identified the rate-limiting step for the vast majority of them to be at either the $K_B$ or the $k_2$ step [36,43]. Only recently have a group of stringently controlled promoters been shown to be rate-limited at the $k_{-2}$ step; the open complexes formed from these promoters exhibit extremely short half-lives [61–64]. A few promoters were deemed to be rate-limited at the promoter escape step; they are lacUV5 [49], T5 N25 [48,65], and malT [50,66]. These promoters earned such a designation essentially by default; that is, determination of association constants as well as open complex half-life led to the conclusion that the slowest step in transcription initiation occurs after open complex formation.

Two features characterize promoters that are rate-limited at the promoter escape stage: one, $k_E$ governs the slowest step for these promoters; and two, they synthesize high levels of abortive RNA relative to the productive RNA. The composite constant $k_E$ measures the rate of productive initiation under typical in vitro conditions of single-cycle transcription from pre-formed open complexes. Accordingly, RNA polymerase was found to escape the lacUV5 promoter with a $t_{1/2}$ of productive synthesis of ~1 min [49], T5 N25, ~5–10 s [65; Hsu, unpublished results], and malT, ~10 min [50]. The knowledge of $k_E$ however, does not explain the pattern of abortive initiation displayed by these promoters. To gain insight into the pattern, a quantitative abortive initiation assay was devised [67]. This assay is performed under multi-cycle, fixed-time conditions, for the reason that abortive initiation is a reiterated process regardless of whether the reaction condition imposes single- or multi-cycle transcription. Under single-cycle conditions, the relative extent of productive initiation would actually be underestimated.

3.1. Quantitative assessment of abortive initiation

One of the first questions raised regarding abortive initiation is whether it reflects a substrate-binding limitation, thus displaying an apparent high $K_S$ value for a certain NTP. As illustrated in Fig. 1A, at each of the early template positions, RNA polymerase can either catalyze the incorporation of the next template-specified nucleotide, or release the nascent short RNA if it is held unstably. Presumably, the relative rate of these two reactions—incorporation versus release—determines the amount of abortive RNA released at each template position.

To address this issue, titration studies of NTP concentration were performed with T7 A1, T5 N25, and T5 N25$_{antiDSR}$ promoters. Interestingly, NTP concentrations as high as 800 μM did not abolish abortive initiation [68,69, and Hsu, unpublished results]. Instead, the level of abortive and productive initiation both rise with NTP concentration, due to increased frequency of chain initiation at higher substrate concentrations. The relative yield of productive to abortive RNA increases with NTP concentration to a point, but then reaches a plateau; different promoters appear to plateau at different NTP concentrations [68]. High $K_S$ barriers are seldom found on natural promoters (i.e. T7 A1 and T5 N25), but may arise in a mutant promoter with an altered initial transcribed sequence (ITS) (i.e. T5 N25$_{antiDSR}$). Regardless of the $K_S$ barriers, there is an intrinsic level of abortive initiation that is not overcome even at high substrate concentration.

The high degree of abortive initiation in vitro prompted an investigation of the abortive and productive initiation kinetics by pulse-labeling. This experimental approach uncovered the existence of a “moribund” fraction of initial complexes that are trapped in abortive transcription continuously and cannot undergo promoter escape [70]. There also exists a productive fraction of ITCs that can escape the promoter to yield full-length RNA, but only after going through the obligate sequence of abortive initiation steps [68]. Thus, the abortive RNAs derived from a promoter are made by both the productive and unproductive ITCs. However, the unproductive complexes contribute disproportionately to the abortive yield and their existence can greatly complicate the quantitative analysis of abortive initiation.

The partitioning of open complexes into productive and unproductive ITCs during in vitro transcription is widespread [68,71]. The fraction of unproductive ITCs varies depending on the promoter; however, it can be reduced to a minimum at higher NTP concentrations (i.e. 100 μM or above) [68]. Depending on the promoter, the unproductive ITCs may or may not become “moribund”—that is, become inactivated and undergo transcriptional arrest. The different classes of unproductive ITC can be distinguished by their differential susceptibility to Gre-factor mediated reactivation of the unproductive complexes [68,71,72].

The above observations suggest the importance of optimizing the concentration of NTP in transcription assays
designed to measure the abortive and productive activity of a promoter. The existence of multiple conformations of ITCs demands a revision of the sequential transcription initiation scheme to include branched pathways (see Fig. 1B), where only the productive branch contributes to productive RNA synthesis, while both branches contribute to abortive RNA synthesis [68].

An interesting feature of abortive initiation is the different abortive pattern displayed at each promoter [68; also see Fig. 2]. Abortive pattern refers to the unique collection of abundant and scarce abortive products obtained at each promoter. Although abortive release from each ITC occurs stochastically, the abortive RNA profile—qualitative and quantitative—obtained at each promoter is reproducible. This led to the definition of a quantitative parameter called abortive probability—a calculation of the probability an ITC positioned at a given template nucleotide would release its RNA [67]. The prominent abortive RNAs give rise to high abortive probability values, suggesting the ITCs at these template positions are highly unstable. The overall abortive pattern, therefore, conveys a telltale description of the promoter escape process at a given promoter, identifying the multiple high-barrier steps, and suggesting where promoter escape might be complete.

The maximum length of the abortive transcripts provides another gauge of promoter escape. The abortive RNAs forming a ladder are detected because of their high abundance from reiterative cycles of initiation and release. Where the abortive ladder ceases, a fundamental change in the transcription complex has occurred: either it can no longer reinitiate or the complex has become highly stabilized. The maximum length of the abortive RNA was shown to correlate with the position of sigma release on several promoters [51,53,54]. On most E. coli promoters with wild-type ITS (roughly defined as positions 1 to +20), the abortive ladders span 2–12 nt [Hsu, unpublished observations]. However, abortive ladders longer than 12 nt have been reported, usually from promoters with altered ITS, 14 nt for λ Pr, AL [70] and 15 nt for T5 N25antiDSR [16], suggesting that the ITS plays a role in the abortive initiation—promoter escape program. The wild-type ITS of λ Pr prescribes a long abortive or paused RNA ladder of +16/+17. In this case, the ITS is crucial in mediating the loading of the antiterminator protein, Q [41].

3.2. Factors that influence abortive initiation and promoter escape

In the above, I have alluded to various factors that can affect abortive initiation and promoter escape. These factors can be grouped into intrinsic and extrinsic elements. Among the intrinsic elements are the core promoter recognition region (PRR), ITS, and the conformational state of the template DNA. The extrinsic elements include activator and repressor proteins, transcript cleavage stimulatory factors, as well as reaction solution conditions, such as ionic strength, pH, etc.

Of the intrinsic elements, the PRR functions primarily to recruit RNA polymerase through specific binding interactions. RNA polymerase binds first at the −35 and −10 hexamers via sigma contacts, and secondarily via α-CTD contacts either to an upstream (UP) DNA element [73] or transcription factors [74] to set up an open complex with a distinct level of RNA chain initiation capability. E. coli promoters can differ in chain initiation frequency over three to four orders of magnitude [46]. The ITS, first shown to play a role in promoter clearance from the T5 N25 and T5 N25antiDSR promoters [47,48], does so by prescribing distinctly different escape programs for each [16]. Although the PRR and the ITS appear to exert control over separate aspects of the transcription initiation process, they do not function independently [53,68]. For example, the downstream ITS sequence can affect the open complex stability, start site selection and initiation frequency of a promoter [75]. In turn, a promoter element can alter the abortive probability at different downstream positions, lengthen or shorten the maximum length of abortive transcripts [68].

A comprehensive analysis of the contribution of each PRR element from −60 to −1, including the UP element, the −35 hexamer, the spacer DNA, the −10 hexamer, and the discriminator (sequence between the −10 hexamer and the +1 site) on promoter escape has been performed [68]. The results overwhelmingly support an inverse correlation: starting with a consensus promoter, any PRR sequence change that weakens the RNA polymerase–promoter interaction invariably facilitates promoter escape. These results extend the previous observation with an UP element: its presence on a consensus promoter increased promoter-proximal stalling in vivo [76] and delayed promoter clearance in vitro [77]. Thus, PRR interactions that enhance RNA polymerase binding and subsequent melting isomerization raise the barrier for promoter escape, presumably because later these same interactions must be broken for the enzyme to move away from the promoter region. Remarkably, Carposis et al. [78] noted this dichotomy regarding the dual functional roles of sigma during transcription initiation as early as 1982.

That the RNA polymerase–promoter DNA interaction can affect promoter escape predicates the existence of RNA polymerase mutations with altered properties of abortive initiation. A number of such mutations have been identified. Some of the rifR mutations mapped to the β subunit were found to be defective in promoter escape due to damaged catalytic (site) activity [79–81]. Others were defective in abortive initiation versus slippage synthesis because a high $K_S$ requirement for UTP was created [82,83]. Several mutations in $\sigma^{70}$ revealed more interesting phenotypes in abortive initiation and promoter escape. RNA polymerase holoenzyme with mutations in $\sigma^{70}$ region 3.1 gave rise to reduced abortive transcription on T7 A1, T5 N25, T5 N25antiDSR, and λ Pr promoters [84,85]. On λ Pr, the
The promoter escape process.

sigma interacts with the rest of the polymerase can impact varied by changes in the sigma subunit, suggesting that how yield and the maximum length of abortive RNA, can be implicated in the binding of sigma to core RNA polymerase [85,86]. Thus, the abortive initiation properties, the abortive yield and the maximum length of abortive RNA, can be varied by changes in the sigma subunit, suggesting that how sigma interacts with the rest of the polymerase can impact the promoter escape process.

Vo [68] also examined the effect of mutating the N25 antisense ITS sequence placed downstream of a consensus PRM promoter by replacing different sequence blocks within +1 to +20. A variety of sequence-dependent results were obtained. Some changes affect the total rate of initiation by the promoter, others alter the ratio of abortive to productive initiation, and still others bring about a reduction in the maximum length of abortive transcripts. Although the results are complex, they show that the ITS plays an important role in promoter escape.

An intriguing example of the effect of the ITS on abortive initiation and promoter escape is shown in Fig. 2 [Hsu, unpublished results]. When the ITS of T5 N25 promoter was randomly mutagenized from +3 to +19, the “mutant” promoters invariably underwent abortive initiation to a greater distance on the template DNA—generally to +15/+16, compared to +11 for the “wild-type” T5 N25 sequence, although abortive initiation to +19/+20 has also been observed—while giving a 30-fold fluctuation in the productive yield (i.e. 150% to 5% of T5 N25). One can draw the following conclusions from this analysis. One, the ITS can greatly influence the extent of productive synthesis. Two, there is no correlation between promoter escape efficiency and the length of the abortive initiation program. Three, the natural ITS sequence of T5 N25 prescribes the shortest abortive initiation program (+11, see lane 4 in Fig. 2), suggesting this promoter has evolved an “optimal” ITS to bring about the most efficient promoter escape in a promoter that is rate-limited at this stage. Ongoing investigations of this type will provide further insights towards understanding the sequence-prescribed idiosyncrasies of transcription initiation.

An intrinsic element not yet systematically examined for its effect on promoter escape is the state of the DNA template. To the extent that DNA in vivo is negatively supercoiled, and supercoiling can bend or melt DNA, position or strengthen polymerase contacts, DNA topology is potentially an important determinant of promoter escape [87]. For a promoter optimized at recruiting the polymerase, rate limitation at the promoter escape stage manifests itself in vivo as stalling of the transcription bubble at +6 to +12 [88]. Whether stalling in vivo is accompanied by the production of abortive products is unclear.

Several extrinsic factors are now known to affect promoter escape. Transcript cleavage stimulatory factors GreA and GreB can prevent or rescue arrested complexes [4,5] or correct misincorporation [89], by inducing polymerase active center-mediated transcript cleavage [see Fish and Kane, this volume]. That GreA and GreB act on short RNA-bearing ITTs to stimulate promoter escape in vitro and in vivo suggests these complexes may become arrested prior to releasing the transcripts [15–17]. Another means by which Gre factors can stimulate promoter escape is to facilitate the conversion of the moribund complex into an active escape-competent complex [72].

A number of transcriptional activator and repressor proteins have been shown to exert their regulatory effect at the promoter escape step. These include cAMP receptor protein (CRP), Lac repressor (LacI), bacteriophage P22 Arc protein, and protein p4 of B. subtilis phage φ29. These examples are discussed in detail in the next section.

3.3. Promoters regulated at the promoter escape step

Extensive investigation of bacterial promoter activity has led to the view that promoters are individually optimized to achieve in vivo strength consonant with their physiological requirement [65,90]. Regulation of a promoter, whether by activation or repression, is most effectively exerted at the slowest step [91]. Several promoters known to be regulated at the promoter escape stage also fit this scheme.

Regulation during promoter escape is implemented in various ways. For example, lacUV5 promoter is repressed by the LacI protein. Unlike other repressors that regulate transcription by occluding the binding of polymerase or interfering with isomerization, LacI and RNA polymerase can bind simultaneously at the promoter. When both proteins are bound, only abortive RNA is synthesized [92]. The lacUV5 promoter has multiple lac repressor binding sites; of these, LacI binding at the operator sequence which overlaps the initial transcribed region (ITR) created a high $K_S$ block at the +6/+7 junction, limiting abortive initiation to +6 and preventing escape which requires transcription past +9 [93].

The case of the malT promoter presents an interesting paradox. This weak promoter is rate-limited at the $k_E$ step and requires activation through the upstream binding of CRP. In the absence of CRP, the malT promoter produces mainly abortive RNA, but the addition of CRP greatly stimulates productive RNA synthesis [50]. CRP binding, however, was found not to affect the rate of isomerization in open complex formation, nor change the rate of promoter escape (i.e. $k_E$). Rather, CRP was shown to facilitate promoter escape by two means: one, by raising the affinity for the nucleotide substrate UTP [50]; and two, by forming a more stable open complex [66].

These conclusions seem incongruent with the expectation that a regulatory molecule would act at the rate-limiting step. However, with the knowledge of the existence of productive and unproductive ITTs—the former facilitates promoter escape and the latter doomed to abortive cycling.
[68,70]—an alternative explanation for CRP action can be proposed. That is, there are two open complex conformations in the absence of CRP: the active one, a precursor of the productive ITC; and the inactive one, a precursor to an unproductive ITC [68,70]. CRP binding favors the equilibrium distribution towards the active precursor open complex. This interpretation is consistent with the recent finding that CRP acts transiently at a diverse set of promoters to stimulate the formation of productive open complexes [94]. Viewed in this light, CRP activates promoter escape by favoring the “productive” branch of ITC formation. The increased affinity for UTP may be an indirect consequence of promoter escape from this productive ITC.

Another example of a regulatory protein acting at the promoter escape stage was reported for the bacteriophage P22 Arc protein which represses the E\textsuperscript{70} promoter during late lytic growth [95]. Normally, Arc binds as a dimer at tandem operator sites (designated as −35 proximal or −10 proximal) in the spacer region of the P\textsubscript{ant} promoter. It was found that the binding of a cooperativity-defective Arc-SL35 dimer at the −35 proximal site was sufficient to bring about regulation. However, the regulatory consequence was different depending on the nature of the variant P\textsubscript{ant} promoter used; the non-consensus (NC) variant differs from the consensus (C) variant in harboring a single AT transversion at −8. Arc-SL35 binding decreased the rate of open complex formation at both promoters, and caused repression of the NC promoter but activation of the C promoter. What is the basis of this paradox?

Interestingly, the answer resides with the rate-limiting step involved. The non-consensus promoter is rate-limited at the open complex formation step. Since Arc-SL35 binding further depresses this rate, repression results. For the consensus variant, the AT transversion created a consensus promoter, greatly elevating its intrinsic rate of open complex formation such that it was no longer the slowest step. As a result, the C variant is now rate-limited at promoter escape. Arc-SL35 binding to C decreases the rate of open complex formation, but to a degree insufficient to cause a reversion in the rate-limiting step; instead, it accelerates the rate of promoter escape to yield the productive RNA. It is not clear how Arc binding at the spacer region facilitates promoter escape, but its activity serves to confirm the principle of regulation at the rate-limiting step.

The regulatory scenario presented by the B. subtilis phage φ29 p4 protein is not unlike that of Arc [96]. Protein p4 binds upstream of two promoters—late A3 and early A2c, activating the former but repressing the latter. Activation or repression is not a consequence of the different positioning of p4, resulting in different interactions with RNA polymerase via α-CTD contacts. Rather, the mode of regulation depends on the presence or absence of a −35 element in the target promoter. Protein p4 represses the activity of the A2c promoter, which contains a −35 element, presumably by raising the overall polymerase–DNA binding affinity above a threshold level such that the added affinity keeping the polymerase at the promoter could not be overcome to achieve escape. Repression in this case was detected as increased abortive initiation [96]. Thus, analogous to Arc, p4 binding may have altered the rate-limiting step for the A2c promoter. The activity of p4 on the A2c promoter confirms the dichotomy between tight promoter binding and poor promoter escape; interactions favoring one may be detrimental to the other.

A well-known example of in vivo regulation of transcription exerted at the promoter escape stage involves slippage synthesis. In vitro, slippage synthesis occurs in response to template sequence with repeated nucleotide triplets or longer. RNA polymerase “stutters” at the repeat sequence to make complementary homopolymers, some of extraordinary length. This reaction is particularly prominent in the initial region and can affect gene expression by competing with normal template-specified transcription [97]. A group of pyrimidine biosynthetic operon promoters in E. coli has evolved a mechanism to sense the intracellular UTP level as a means of regulating promoter escape in vitro and in vivo [98–101]. High UTP concentration favors nonproductive slippage synthesis, while low UTP concentration stimulates the “normal” process of abortive initiation and promoter escape.

A final example of regulating gene expression at the promoter escape stage can be found at λ P\textsubscript{R} promoter. P\textsubscript{R} ensures the transcriptional expression of λ late genes by Q-mediated antitermination. Q protein is brought onto the transcription complex paused at +16, subsequently facilitating promoter escape and the conversion of the elongating RNA polymerase into the anti-terminating conformation. How Q facilitates promoter escape is not known [102]. All of the examples presented here indicate that the cell has taken full advantage of promoter escape for regulatory options.

4. Abortive initiation and promoter escape in the context of RNA polymerase structure

In recent years, the study of transcription has received a huge boost from the resolution of X-ray crystal structures of RNA polymerase. The breakthrough started with T7 RNA polymerase and its various complexes [22–25], followed by the multi-subunit RNA polymerases from Thermus aquaticus and yeast [26,27]. With the bacterial polymerases, it was possible to model the vast amount of functional data obtained with E. coli E\textsuperscript{70} onto the Taq core enzyme structure [103–105]. These modeling efforts are valid because the multi-subunit polymerases all show a high degree of sequence homology and structural and functional conservation of the catalytic core of the proteins, suggesting that they share a common catalytic mechanism [106–109].

It is pertinent to point out that structural information obtained by modeling offers great advantages, but with a caveat. This is illustrated by efforts to obtain the structure of
elongation complexes. Having solved the Taq core enzyme structure, Korzheva et al. [103] modeled the nucleic acid component of an elongation intermediate based on cross-linking data (containing 40 bp of DNA and 14 nt of nascent RNA) onto the core structure and obtained the first picture of an elongation complex. Later, a crystal structure of a yeast RNA polymerase II (yeast Pol II) elongation complex containing a 9-nt RNA made on a tailed template was solved [28]. While the two structures show remarkable agreement, the comparison highlights the omission by modeling of protein conformational changes between the core enzyme and the elongation complex. However, by modeling, the path of the nucleic acid component was augmented, not only confirming the existence of an 8-bp RNA–DNA heteroduplex in the elongation complex, but also identifying an RNA exit channel for the nascent transcript.

Another advantage of the modeled bacterial elongation complex structure came recently. Its coordinates were merged with those of a Taq holoenzyme/fork-junction DNA structure to achieve a first open complex structure of Taq RNA polymerase [30].

T7 RNA polymerase is structurally distinct from that of the multi-subunit enzymes reflecting RNA polymerase’s divergent evolutionary history. However, by all evidence, T7 RNA polymerase seems to have evolved a functional mechanism highly similar to that of the multi-subunit enzymes [110–112]. Thus, mechanistic insights gained from the investigation of T7 RNA polymerase activity can be applied towards solving a common mechanism of transcription.

The present cast of RNA polymerase and transcription complex structures shows us that RNA polymerase undergoes a well-orchestrated sequence of conformational changes at each step of transcription, brought about by protein–protein and protein–nucleic acid interactions. Abortive initiation–promoter escape is an activity of the ITCs, the structures of which have been difficult to probe due to their high degree of heterogeneity and instability [3,42]. In the following section, I shall describe the structures of RNA polymerase molecules in conformations bracketing the initiation–elongation transition to identify the molecular features and strategies important for achieving promoter escape. I will also consider the structures (crystal and biochemical) of initiation complexes to elucidate the relevant features of the intermediate states. The goal is to achieve a descriptive mechanism of abortive initiation and promoter escape.

4.1. Structures of open and elongation complexes

4.1.1. Core enzyme [26,27]

Both Taq and yeast Pol II core enzymes fold into a “crab-claw” structure, with β (Rpb2) and β′ (Rpb1) subunits each contributing a pincer, forming a central channel with a diameter of 27 Å that can accommodate double-stranded nucleic acids. The channel on both sides is lined by β and β′ protein mass, and the active site Mg$^{2+}$ is located centrally on the back wall. Behind the active site Mg$^{2+}$, a secondary channel opens out to the rear surface of the enzyme. Through this channel, NTP substrates can reach the active site, and released short transcripts can diffuse out. While RNA polymerase is overall a highly acidic molecule, there is an uneven distribution of the charged residues, with the basic residues congregating along the active site channel.

4.1.2. Holoenzyme [29,104,105]

Holoenzyme is formed from the joining of sigma and core enzyme. Taq σ$^A$ is a member of the σ$^{70}$ family proteins that show a high degree of sequence and functional homology in four regions and their sub-regions [29,113–115]. A Taq σ$^A$ molecule lacking sub-region 1.1 folds into three independent domains (designated σ$_2$, σ$_3$, and σ$_4$) joined by a flexible loop (eight residues between σ$_2$ and σ$_3$) and a linker (33 residues between σ$_3$ and σ$_4$) (see Fig. 3). In promoter DNA recognition, σ$_2$ mediates −10 element binding and melting, σ$_3$, binding to the extended −10 nucleotides, and σ$_4$, binding to the −35 element. Sigma binding to the core enzyme leads to large conformational changes in domains of β and β′ (including the β flap and the β′ clamp) that result in closing of the active site channel to 15 Å, a distance too narrow to accommodate the double-stranded DNA. To form an open complex, it is proposed that the holoenzyme must “open” first to accept the DNA before closing again to yield the open complex structure, with the opening and closing mediated by protein conformational changes.

On the holoenzyme, the three sigma domains are splayed out on the surface across the upstream opening of the active site channel, σ$_2$ binding to the β′ clamp and σ$_3$ binding to the β$_3$ domain across the channel (see Fig. 3). The σ$_4$ domain is anchored at the tip helix of β flap, separated by a surface distance of 45 Å from σ$_3$. The connection between these two domains is made through the interior of the polymerase active site crevice by a long polypeptide linker (σR3.2) that first descends from σ$_3$ towards the active site, then turns and wanders underneath the β flap out toward σ$_4$. Two surprising features of this linker are noted. One, the path of the σR3.2 linker occupies the entire length of the RNA exit channel through which a nascent transcript longer than 8 nt must also traverse. Two, this polypeptide is highly acidic, and therefore, binds to neutralize the basic nature of the upstream portion of the active site channel. The persistent path of the σR3.2 polypeptide led to the suggestion that nascent RNA transcripts would encounter steric hindrance during the initial steps of nucleotide incorporation, and therefore, undergo abortive release [29].

In the Taq holoenzyme structure, σR1.1 is absent, but its orientation can be inferred from the direction of the polypeptide backbone, pointing it towards the downstream binding site (DBS) for double-stranded DNA. This disposition of σR1.1 is confirmed by FRET analysis of the Eo$^{70}$ holoenzyme and open complex—σR1.1 occupies the DBS.
in the holoenzyme, but is displaced about 51 Å out of the channel to interact with the βp pincer domain. The large conformational switch of σR1.1 is consistent with the finding that it plays a role in open complex formation [116,117]. Interestingly, σR1.1 is also highly acidic and, when bound to the active site channel, can complement its positive charges. Thus, in the holoenzyme, sigma can be viewed as a “molecular plug,” filling the channel (with σR1.1 and σR3.2) and “clasping” it closed at the top through the α2–α3 binding (see Fig. 3).

4.1.3. Open complex [30,104,105]

Holoenzyme, as described above, is perfectly configured to bind to the upstream double-stranded promoter DNA
through the surface exposed domains of sigma by major groove contacts, forming a closed complex \((\text{R}P_{C1}; \text{see Ref. [44]})\). The downstream DNA is then “brought” into the DBS channel, displacing \(oR1.1\) and forming \(\text{RP}_{C2}\) \([44]\). As bound, \(\alpha_2\) is perfectly positioned to nucleate the opening of the transcription bubble, from upstream at \(-12/-11\) to downstream at \(+3/+4\) \([30]\).

The formation of the open complex again involves substantial conformational changes leading to a complete closure of the active site channel. Within this enclosed structure, the two strands of DNA in the transcription bubble are buried in separate tunnels formed by a \(\beta'\) protein mass that pokes through the two strands to interact with the \(\beta\) protein mass. Of the protein mass that separates the two tunnels is the highly conserved structural feature called the \(\beta'\) rudder; its position implicates a role in open complex formation \([118]\). One can view the open complex as a “solid” protein unit with five interior tunnels—the DBS tunnel, the RNA exit tunnel, the template strand (T) tunnel, the non-template strand (NT) tunnel, and the secondary tunnel through which NTP and small RNA diffuse to and from the active site (see Fig. 3). The separately buried nature of the template and non-template strands suggests DNA must be threaded through the enzyme during the progression of transcription.

### 4.1.4. Elongation complex \([28,103]\)

The structures of the elongation complex reveal most clearly the path of the DNA and RNA (strands) inside the enzyme. DNA inside the enzyme protein is kinked sharply (about \(90^\circ\)) near the catalytic site, where a chelated Mg\(^{2+}\) ion is positioned between the \(i\) and \(i+1\) nucleotide substrate binding sites. (The \(i\) and \(i+1\) sites correspond to transcribed positions \(+1\) and \(+2\) in the open complex. In steps after the first phosphodiester bond, the \(i\) site is occupied by the 3'-OH nucleotide of the nascent transcript, while the \(i+1\) site accommodates the successive incoming NTP.) Downstream of the active site, the bubble is open for 2–3 nt before annealing to form double-stranded DNA securely held by the DBS. Upstream of the catalytic center, the template and non-template strands of the bubble rise up towards the edge of the active site crevice. The RNA transcript is bound to the template strand as an 8-bp RNA–DNA heteroduplex. In the modeled elongation complex, RNA longer than 8 nt would be peeled away from the template strand and directed into the RNA exit channel (see Fig. 3).

### 4.2. Structural insights of ITCs

#### 4.2.1. Initiated complex \([24,25,119]\)

The only available structure of an initiated complex is that of T7 RNA polymerase containing a 3-nucleotide base-paired to the template DNA in the form of a perfect A-helix. Comparison of this structure to that of a T7 RNA polymerase open complex reveals that during translocation, template DNA is “scrunched” into the enzyme interior volume. Scrunching not only involves threading of the DNA strands, but also the generation of a strain that manifests as distortion in the DNA backbone. In the structure of the T7 initiated complex, this strain is localized to the immediate upstream (5') edge of the RNA–DNA heteroduplex, and two nucleotides are scrunched, corresponding to the two steps of translocation performed to synthesize the 3-nucleotide RNA.

Using fluorescence quenching to monitor the collapse of the open complex bubble, Liu and Martin \([112]\) found that for T7 RNA polymerase, the upstream edge of the initial open complex bubble does not rewind until the nascent RNA has reached \(+9\), giving a maximum bubble size of 13 melted base pairs. This evidence also indicates that T7 RNA polymerase can scrunch in as many as 8 bp of DNA during the early steps of transcription without causing upstream rewinding. Furthermore, the 5' end of the nascent RNA does not peel off the template strand until past \(+10\), suggesting an RNA–DNA heteroduplex of at least 10 bp.

It is not clear how many basepairs of DNA can be scrunched into the interior volume of \(E. coli\) RNA polymerase, but if its catalytic mechanism is identical to that of T7 RNA polymerase, then at least 8 bp. There is a caveat in extrapolating these measurements for the \(E. coli\) enzyme, however. Owing to the different promoter architecture of \(E. coli\) and T7 RNA polymerase and the much smaller size of the T7 enzyme, the above dimensions should be regarded as minimum estimates.

Structurally, a complex perched on the brink of the initiation–elongation transition was obtained by FeBABE footprinting of the \(\lambda\) \(P_{R} + 16\) complex \([60]\). In the open complex, probes in \(\alpha_{2}\) and \(\alpha_{4}\) mapped to the \(-10\) and \(-35\) elements of the promoter DNA, respectively, as expected. When the polymerase paused at \(+16\), \(\alpha_{2}\) and \(\alpha_{4}\) have been repositioned to the \(+1\) to \(+6\) region on the non-template DNA and the \(-25\) region of the spacer DNA, respectively. That the contacts of sigma domains to the promoter DNA have been displaced classifies the \(+16\) complex to be a paused elongation structure, perhaps one that has just undergone the initiation–elongation transition.

A more revealing look was obtained with a probe in \(oR3.2\) located near the N terminus of the polypeptide linker that occupies the RNA exit channel. In the open complex, it mapped in the vicinity of the catalytic center. Upon transcription initiation, this region moved in a “retrograde” fashion, eventually contacting the upstream surface DNA in the \(+16\) complex. This “disjunctive” movement of the separate sigma domains was puzzling at first, but now can be reconciled with the positions of the sigma domains on the holoenzyme \([29,30,60]\). The data suggest that between the open complex and the paused elongation conformations, \(\alpha_{2}\) and \(\alpha_{4}\) stay anchored on the surface of the enzyme, making contacts with the threaded DNA that has translocated towards the upstream, while \(oR3.2\) has “moved” outward (from the enzyme interior)—probably because of displacement by the advancing RNA, converging onto the vicinity of \(\alpha_{4}\). In the \(\lambda\) \(P_{R} + 16\)
structure, the interaction of the sigma subunit with the core enzyme has been altered.

4.3. Key issues affecting abortive initiation and promoter escape

The discussion above has identified several factors critical for understanding abortive initiation and promoter escape. In the open complex, the location of the oR3.2 linker appears to pose a formidable physical block for the advancing RNA. Steric clash would be encountered by a transcript 6 nt or longer, but maybe even as short as 2 nt, leading to abortive initiation [29]. Given this barrier, can transcription ever proceed past the abortive initiation stage? In the elongation complex structure, an RNA–DNA heteroduplex of 8 bp is observed [28]. Mechanistically, what role does this heteroduplex play during the early steps of transcription to bring about the initiation–elongation transition?

Regarding the oR3.2 block, this barrier appears formidable but is not insurmountable. On most E. coli promoters under routine transcription conditions, some fraction of the RNA polymerase can escape the promoter to form productive RNA, accompanied by varying levels of abortive initiation. Thus, this linker can be displaced during transcription, and the FeBABE footprinting experiment described above offers supporting evidence [60]. It is a challenge, therefore, to understand how the efficiency of displacement of this linker might vary from promoter to promoter.

The role of the RNA–DNA heteroduplex in a transcription complex has been more controversial (reviewed in Refs. [20,120]). Many groups have demonstrated the existence of an 8–9 bp RNA–DNA heteroduplex in elongation complexes [12,121,122]. However, claims that the RNA–DNA hybrid is the central stability determinant for an elongation complex were soon faced with the observation that the length and/or GC content of an RNA shows no correlation with its abortive potential [51,68,86, and Hsu, unpublished results]. The question remains: if not stability, then what role does the heteroduplex play in bringing about promoter escape?

In the following section, I shall describe a mechanism to account for abortive initiation and promoter escape by making two assumptions. One, E. coli RNA polymerase performs translocation by scrunching DNA into the enzyme interior [119]. Two, the RNA–DNA heteroduplex serves a role in guiding the development of strain in the ITC from steps of scrunching–translocation. The growing length of the RNA–DNA heteroduplex increasingly places the strain locus closer to the upstream edge of the transcription bubble, eventually causing the collapse of the upstream edge of the transcription bubble to achieve promoter escape.

4.4. A descriptive mechanism of promoter escape

The following descriptive mechanism is aimed at accounting for the distinct pattern of abortive initiation seen at different promoters; an example of the widely different patterns is shown in Fig. 2. Starting with an open complex, the transcription bubble is buried inside the protein tunnels and the DNA single strands emanating to the surface become reannealed upstream and are held in place by α2, α3, and α4 domains that, in turn, are anchored to the polymerase. In addition, the oR3.2 linker occupies the tunnel for the nascent RNA (see Fig. 3). The transcription bubble spans 13–14 bp (from –11 to +2/+3) and represents the most stable (i.e. relaxed) conformation at the initiation stage.

At de novo initiation, NTP is brought in from the secondary channel, positioned in the i and i+1 sites, and phosphodiester bond formation occurs. A translocation event must follow rapidly to reposition the next template base before the catalysis of the next phosphodiester bond can occur. Translocation involves flipping out the next template base, scrunching 1 bp of template and non-template DNA into the enzyme interior volume to position the next template base at the new i+1 site.

As scrunching takes place, the size of the transcription bubble increases, and strain develops. This strain is manifested as a distortion in the DNA backbone and is at first localized to the immediate upstream border of the RNA–DNA heteroduplex along the template strand [119]. The strain elicits stress and the ITC now becomes a "stressed
must be disrupted. Consequently, incorporation of the next strain energy, since more of the heteroduplex interaction segment should discourage the downstream propagation of the scrunching strain locus closer to the mid section of the sequence context of the position.

Correlation with the length of the RNA, but is dependent on the abortive probability at any position is distinct, shows no release from that position. This hypothesis can explain how complexes is set up, leading to a different level of abortive upstream facilitates incorporation. Two, at the next template release; the fraction that distributes its strain energy downstream would disrupt the RNA–DNA heteroduplex, releasing the RNA. Propagation of the strain energy along the DNA heteroduplex should also stabilize the structural conformation at the catalytic site, enabling the incorporation of the next nucleotide. By contrast, propagation of the strain energy downstream would disrupt the RNA–DNA heteroduplex, leading to release of the short RNA. (In the above scheme, the non-template strand must also accommodate the scrunching-induced strain. However, without the constraint of an RNA–DNA heteroduplex, the subsequent steps to relieve the stress can take a random course.)

After the incorporation of the second nucleotide, scrunching–translocation follows, and the strain generation-stress relief cycle repeats. Strain is developed and localized to the upstream border of the heteroduplex, which is now 3-bp long. Propagation of the strain energy along the downstream DNA backbone disrupts the RNA–DNA heteroduplex, releasing the RNA. Propagation of the strain energy towards the upstream stabilizes the new intermediate, leading to the incorporation of the next nucleotide (see illustration in Fig. 4, panel A).

This scheme satisfactorily explains two features of abortive initiation. One, abortive release at a given position is a “population” phenomenon—that is, some complexes release their transcripts, while others can proceed to incorporate the next nucleotide. In this scheme, the fraction that propagates the strain downstream gives rise to abortive release; the fraction that distributes its strain energy upstream facilitates incorporation. Two, at the next template position, a new “equilibrium” of abortive and incorporating complexes is set up, leading to a different level of abortive release from that position. This hypothesis can explain how the abortive probability at any position is distinct, shows no correlation with the length of the RNA, but is dependent on the sequence context of the position.

As the nascent transcript grows (for example, to +6), the longer RNA–DNA heteroduplex increasingly places the scrunching strain locus closer to the mid section of the transcription bubble. The longer length of the heteroduplex segment should discourage the downstream propagation of strain energy, since more of the heteroduplex interaction must be disrupted. Consequently, incorporation of the next nucleotide should be favored. However, an additional factor comes into play after a few incorporation/translocation cycles—that is, the scrunching strain from the earlier steps has been accumulating, because the collapse of the upstream edge of the transcription bubble does not occur until at least +9 [112]. The cumulative strain located upstream of the heteroduplex segment can “backfire” by favoring downstream propagation and now, with more force, can disrupt the 6-bp heteroduplex. Cumulative strain, therefore, can affect the equilibrium distribution of abortive versus incorporating complexes at the early steps in an unpredictable manner, favoring abortive release unexpectedly at position +6 and beyond (see illustration in Fig. 4, panel B).

Presumably, when RNA–DNA heteroduplex reaches 8–9 bp in length, the cumulative strain energy becomes sufficiently large to force the collapse of the transcription bubble upstream. This leads to rewinding of the −10 promoter DNA, dislodging it from the original (open complex) interactions with α2 and α3, allowing the DNA to “translocate” in the upstream direction and simultaneously dislodging the −35 promoter DNA contacts to α2. (Here, I wish to propose the term “outward translocation” to refer to the upstream movement of the DNA away from the enzyme, to distinguish it from the step-wise “inward translocation” that occurs after each nucleotide incorporation step.) This first major outward translocation of the promoter DNA would encompass a distance of 8–9 bp, equal to the number of nucleotides scrunched inside the enzyme interior, and is reminiscent of an enzymatic step in the “discontinuous” movement model of transcription elongation [19]. Coincidentally, the protein contacts to the −10 and −35 promoter sequences have been displaced. This loss of interaction is sufficient to give the reduced DNase I footprints observed in complexes that have just undergone the promoter escape transition [3,42]. Thus, upon the first major outward translocation, the σ–promoter DNA contacts are lost, reiterative initiation should cease, and promoter escape is effectively accomplished.

In a complex that has just come through the promoter escape transition, the transcription bubble would be restored to a stable size, an 8–9 bp RNA–DNA heteroduplex would be in place at the active site, and the RNA 5′ end would be directed into the RNA exit channel. During the subsequent steps of nucleotide incorporation, the scrunching strain would be placed immediately near the mid to upper segment of the transcription bubble, facilitating the distribution of strain energy in the upstream direction to effect the rewinding of the upstream edge of the bubble. If the strain development–stress relief cycle becomes monotonic, that is, one basepair of inward translocation followed by one basepair of outward translocation, the transcription bubble would be maintained at a constant size and the progress of elongation would proceed at an even pace—one template step at a time [124]. However, monotonic translocation appears not to be the default mode of movement for the

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Fig. 4. Schematic representation of the strain generation/stress relief cycle during translocation involving scrunching. (For further detail, see text.) Only the nucleic acid component of an ITC is shown: blue, template strand; green, non-template strand; and purple, nascent RNA with a dot marking its 5' end. The gold star denotes the catalytic center which performs catalysis from below the template strand. During the abortive initiation-promoter escape phase, inward translocation occurs without outward translocation; this movement of the DNA is gauged by the black and red rectangles, each marking a particular upstream and downstream base pair, respectively. Scrunching generates strain shown as distortion in the DNA backbone. The strain is at first localized at the 5' edge of the RNA–DNA heteroduplex (structures a and d) and subsequently delocalized by propagating the backbone distortion upstream (indicated by “~ up”) or downstream (“~ dn”). Downstream propagation disrupts the RNA–DNA heteroduplex, leading to abortive release of the short RNA and rewinding of the bubble downstream, re-establishing the open complex structure (see a→b and d→b transitions). Upstream propagation temporarily stabilizes the conformation around the active site (structures c and e), enabling the incorporation of the next nucleotide (see c→d transition). In this scheme, upstream propagation of the strain is reversible, while downstream propagation is irreversible. Panel A shows a strain generation/stress relief cycle undergone by an ITC bearing a 3-nt RNA (ITC₃; structure a). This ITC has scrunched in 2 bp of DNA. Panel B shows an analogous stress relief cycle in ITC₆ that harbors cumulative strain from scrunching in 5 bp of DNA (see structure f). When propagated upstream, the cumulative stress can eventually lead to the collapse of the bubble, leading to the first major outward translocation (here shown as occurring at +10; see black rectangle in structure b), yielding a ternary elongation complex (TEC₁₀).
polymerase during elongation. Many studies have observed the asymmetric expansion and contraction in the size of the transcription bubble and/or in the elongation complex footprint for complexes elongating over a contiguous stretch of 10–20 bp, suggesting that RNA polymerase translocates in a discontinuous fashion \([121,125,126]\). Specifically, the expansion of the bubble and footprint is continuous in the downstream boundary in sync with the growth of the RNA, while the upstream boundary remains fixed until a “jump” occurs \([125,126]\). These observations support a translocation mechanism that involves many steps of inward translocation for each step of outward translocation.

Thus, the scrunching–translocation mechanism appears to function throughout transcription. During transcription, each complex must become a stressed intermediate for a time, and individual complexes can differ in the amount of internal stress depending on the cumulative strain each has harbored. Stress relief, especially by distributing the stress in the downstream direction, would bring about different consequences for the initiation versus elongation complexes. In the ITCs, downstream distribution of the stress disrupts the short RNA–DNA heteroduplex, causing the RNA to be abortively released. During elongation, an occasional complex might have accumulated a level of strain that is insufficient to cause bubble rewinding upstream (to lead to outward translocation) but, when propagated downstream, is sufficient to disrupt the 8-bp RNA–DNA heteroduplex. In such a complex, the RNA has grown too long to be released. Instead, the RNA is caught in a partly released configuration, with its 3′ tail portion exuded into the secondary channel and an upstream stretch—recently retracted from the RNA exit channel—now tied up in an RNA–DNA heteroduplex, resulting in the formation of an arrested complex \([10–13]\).

Note that up to the point of the first major outward translocation, sigma release has not been accounted for, although sigma displacement has occurred. At the first major outward translocation step, it is unclear whether the dislodging of promoter DNA–σ2 interactions might also loosen the σ2–core enzyme interaction. However, as the nascent transcript becomes longer than 8 nt, it gains additional stabilization from entry and binding in the RNA exit channel, displacing the αR3.2 linker. By 14 nt, the αR3.2 linker should be completely displaced. This would loosen the binding of σ4 to the flap tip helix located at the exit of the RNA channel. With multiple sigma domain–core interactions broken, sigma release might eventually occur \([127]\).

The above description can account for promoter escape occurring around the +10 position. However, many promoters undergo abortive initiation till a greater distance on the template, for example, to +16. Since reiterative initiation requires the maintenance of the promoter DNA–sigma domain contacts, by the above scheme, longer abortive RNA can result only if scrunching continues past +10 to accumulate even more strain energy, leading to the first major outward translocation at +16. This begs the question about how many bases of the melted DNA can be scrunched into the *E. coli* RNA polymerase.

To summarize, a model for abortive initiation and promoter escape suggests the formation of “stressed intermediates” during initial transcription \([51]\). Stress is induced by the scrunching of DNA during inward translocation \([25]\). The length of the RNA–DNA heteroduplex serves to place the strain locus to different segments of the transcription bubble and can influence the subsequent stress relief steps. Cumulative strain from many steps of scrunching is required to cause the collapse of the open complex bubble and lead to promoter escape.

5. Concluding remarks and future prospects

In this review, I have described a stepwise mechanism of abortive initiation and promoter escape to account for the unique pattern of abortive products seen at different promoters. The scheme proposes that abortive release results from conformational rearrangements occurring along the template DNA strand of the transcription bubble. However, the RNA polymerase protein clearly plays the central role in catalysis; thus, the next major challenge is to elucidate the polymerase–DNA interactions and the stepwise conformational readjustments in both the DNA and the enzyme protein during transcription. The proposed scheme makes several assumptions and borrows heavily the mechanistic insights from T7 RNA polymerase. For example, a major assumption is that translocation proceeds by scrunching. This hypothesis has been tested for the T7 RNA polymerase \([128,129]\) but has yet to be examined for *E. coli* RNA polymerase. Equally important for the *E. coli* enzyme is information regarding the dimension of the transcription bubble and the extent of cumulative scrunching during initiation. The above scheme cannot account for sigma release at the first major outward translocation step. The issue of sigma release, whether it occurs at the promoter escape transition, or if it occurs at all during the transcription cycle, awaits clarification. Biochemical characterization has shown the widespread existence of the unproductive ITCs during abortive initiation. These complexes carry out abortive initiation continuously and are prevented from undergoing promoter escape. With a structure-based mechanism of abortive initiation and promoter escape, one can begin to uncover the structural basis for the unproductive conformation. Finally, a major question is concerned with how the promoter DNA sequence can set capabilities. In this context, the role of the ITS on promoter escape is especially intriguing. For example, how is the sequence information in this region transmitted to the enzyme to yield ITCs of widely different stability? Is the information relayed through the template or the non-template strand? How might this sequence influence the posi-
tion of sigma displacement as reflected in the different points where abortive initiation ceases? The wealth of biochemical and biophysical knowledge now available places us in a good position to solve the mechanism of transcription.

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