Kinetic study *in vitro* of *Escherichia coli* promoter closure during transcription initiation

Bernard SCHMITT and Claude REISS

Institut Jacques Monod, CNRS-Université Paris 7, Tour 43, 2 place Jussieu, 75251 Paris cedex 05, France

The rate of closure of two *Escherichia coli* promoters borne by plasmid pBR322, following transcription initiation from the open complex, was probed *in vitro* by the protection of unpaired thymines in the open complex against oxidation by KMnO_4 . Run-off transcription kinetics were also studied under identical conditions. Closure of the open promoter appears to be by far the rate-limiting step of transcription initiation and elongation

for the linearized β -lactamase gene, and is strongly dependent on template topology for the RNAI gene. It is suggested that the corresponding signals are deposited 30 bases at least downstream of transcription initiation and that promoter closure, and its clearance by elongating RNA polymerase, may occur almost simultaneously.

INTRODUCTION

Constitutive transcription initiation has been extensively studied in *Escherichia coli* and is to date best documented in this species (for reviews see [1–9]). *In vitro*, the major events of the process are successively the search for the promoter sequence by RNA polymerase (RNAP), a sequence of four steps at least leading to the transcriptionally competent RNAP promoter complex, and its entrance into abortive, then productive, transcription elongation. An initial, ‘closed’ complex and an intermediate complex are specified independently by a characteristic pattern of enzyme–DNA contact points and share the fact that promoter DNA is present in both complexes in its native (‘closed’) form. Formation of the intermediate complex at 37 °C is rather slow compared with the two following steps which yield ‘open’ complexes. The promoter DNA is locally unwound in these complexes. An initial open complex isomerizes, under stoichiometric control of Mg^{2+} ions, into a second form which has acquired the competence for initiating transcription [10], i.e. it starts transcription when nucleoside triphosphates (NTPs) are supplied. The process bifurcates following elongation of the transcript to less than about 10 nts. At the bifurcation point, transcription may abort, i.e. the nascent oligomer (from 2 to about 8 nts long) is released and the process restarts. Alternatively, transcription continues and normally ends at the transcription termination site, hundreds or thousands of bp downstream. Until the bifurcation point is reached the structure of the transcriptional complex appears to be conserved (the promoter is ‘open’, RNAP is in the holoenzyme state) and the RNAP footprint on the promoter is almost unchanged. Beyond the bifurcation point, the sigma factor of RNAP dissociates from the complex, the open promoter sequence closes and the productive elongation complex moves downstream.

The rate at which the promoter area is cleared by the elongating complex, and thereby becomes available for the next complexation process, is a measure of the promoter efficiency. This rate is a composite of the rate of closed, then open complex formation, the rate of elongation to the bifurcation point and the rate at which the latter can be overcome. Promoter-opening kinetics were studied by measuring the reaction rate of bases accessible to chemical attack only when in the unpaired, open, state [11]. The rate of transcription onset can be measured by the abortive

initiation assay in which only a limited set of the four NTPs is supplied so as to prevent transcription from proceeding beyond the bifurcation point. The dissociation constant of the first closed reversible complex and the isomerization constant to the open complex can then be derived. The less well-studied step is that of elongation passing the bifurcation point and is the subject of the present report.

Single-stranded or unwound promoter DNA can be detected using KMnO_4 , which oxidizes single-stranded pyrimidine, yielding pyrimidine-glycol residues (thymine is much more readily attacked than cytosine [12,13]). The modified pyrimidines can be located subsequently by the primer extension method, using T7 DNA polymerase for instance, as extension stops at modified Ts and Cs.

Employing this method, we have studied the rate of protection against oxidation of thymines in the open complex following initiation of transcription elongation by addition of the four NTPs to the complexes in the open state. This should amount to studying the closure of the unwound promoter sequence in the open complex under conditions enabling transcription to proceed irreversibly downstream of the bifurcation point.

Two promoters were studied, controlling transcription of β -lactamase and RNAI genes in plasmid pBR322. The results show that the rate of promoter ‘clearance’ as measured by this procedure (a step at which the nascent RNA would be about 20 nts long), is about ten times slower for the β -lactamase promoter than for that of RNAI.

We also measured for both genes the global elongation rates of RNAs over 100 nts long following addition of the four NTPs to the open complexes. The results show that, for the *bla* gene, the onset of transcription (about the first 20 nts) takes much longer than the subsequent elongation to 155 nts. Promoter clearance is by far the rate-limiting step of transcription elongation *in vitro*. This conclusion holds for the RNAI gene also, but promoter clearance and elongation rates are much closer.

EXPERIMENTAL

Materials

RNAP was purchased from Pharmacia. It was used without further purification and stored in a buffer containing 10 mM

Figure 1 Restriction enzyme sites on pBR322 and oligomers used to probe the β -lactamase and RAI genes

Numbering is according to the revised sequence [30]. (a) The 19-mer primer A1 (see Materials section) hybridizes to the non-template strand of the *bla* gene from address 4125–4143. Extension from this primer is towards the transcription initiation site, address 4187, and the *bla* promoter. Extension runs opposite to transcription. A, *Apa*LI; E57, *Eco*57I; H, *Hha*I; E, *Eco*RI. (b) The 20-mer primer A2 (see Materials section) hybridizes to the template strand from address 2909–2928. Extension from this primer is towards the transcription-initiation site, address 2975, and the *RAI* promoter. Extension runs as transcription. The transcription terminator is at 3082. E57, *Eco*57I; B, *Bst*YI.

Tris/HCl (pH 7.9), 100 mM NaCl, 0.1 mM EDTA and 0.1 mM dithiothreitol (DTT) in 50 % glycerol. The content in sigma factor of this RNAP preparation was not checked. Because of the large RNAP:promoter ratios used in our experiments (about 10), and of the addition of heparin following the formation of open complexes, which dissociates non-specific core enzyme–DNA complexes, the content of RNAP in sigma factor is irrelevant for the present study. NTPs and *Eco*RI were from Boehringer, deoxynucleoside phosphates (dNTPs) from Pharmacia and *Apa*LI, *Hha*I, *Eco*57I and *Bst*YI from New England Biolabs.

DNA and oligomers

pBR322 DNA was either prepared in the laboratory and purified by a caesium chloride centrifugation step, or purchased from Boehringer. The oligomer 5'-OHGGGCGACACGGAAT-GTTG-3'-OH (A1 in Figure 1a) hybridizes to the non-template strand of the *bla* promoter [4125 to 4143, or +63 to +45 with respect to the start of *bla* transcription, +1, at G(4187)]. The oligomer 5'-OHGCGAGGTATGTAGGCGGTGC-3'-OH (A2 in Figure 1b) hybridizes to the template strand of the *RAI* promoter [2909 to 2928, or –66 to –47 with respect to the start of transcription, +1, at A(2975)]. The primers were synthesized in an Applied Biosystems SAM1 DNA synthesizer and purified by acrylamide gel electrophoresis. The oligomers (100 pmol) were 5'-labelled with [γ - 32 P]ATP (ICN) and polynucleotide kinase (10 units, Gibco BRL) using the One Phor All buffer (Pharmacia). They were extracted using phenol–chloroform, washed with ether (three times), precipitated with ethanol (with 35 μ g glycogen), rinsed, dried and dissolved in 100 μ l of distilled water.

Methods

Treatment with KMnO_4

An RNAP:DNA molar ratio of around 10 was chosen which was high enough to warrant the formation of a large proportion of specific complexes, but still avoided the binding of a second RNAP upstream of the *bla* promoter, known to inhibit transcription from this promoter [14]. The plasmid pBR322

(4 nM) and RNAP (43 nM) were incubated at 37 °C for 45 min in 30 mM Tris/HCl (pH 8)/100 mM KCl/10 mM MgCl_2 /200 μ g/ml BSA/0.2 mM DTT. Then 10 μ l of the solution was mixed with 1 μ l of heparin at 100 μ g/ml; 20 s later 1 μ l of NTPs was added at final concentrations of 160 μ M (except UTP, 16 μ M). Incubation was continued for selected times and then 1 μ l of 80 mM KMnO_4 was added for 1 min and the reaction was stopped with 2.5 μ l of mercaptoethanol–EDTA solution (final concentrations of 1.1 M and 20 mM respectively). The solution volume was increased to 250 μ l, 7 ng (2.5 fmol) of a reference DNA and 50 μ l of phenol/chloroform (1:1) were added. After centrifugation, 200 μ l of the supernatant was collected. The phenol/chloroform was extracted three times with ether and 35 μ g of glycogen was added to the aqueous phase which was precipitated with 3.3 μ l of saturated NaCl solution and 5 volumes of ethanol (1 h at –80 °C). The precipitate was rinsed with 70–95 % ethanol and after drying was dissolved in 15 μ l of distilled water and kept at –30 °C or processed immediately.

Primer extension analysis

5'-Labelled oligomer (4 μ l) was added to 15 μ l of the KMnO_4 -treated DNA and the solution was incubated at 95 °C for 5 min, 47 °C for 15 min and finally chilled in ice. dNTPs (each 250 μ M) and buffer were added and then 1 unit of T7 DNA polymerase (Pharmacia). Incubation was performed at 37 °C for 15 min. The reaction was stopped with 125 μ l of ethanol. The precipitate was kept for 1 h at –80 °C, centrifuged, rinsed, dried and dissolved in 10 μ l of 90 % formamide/25 mM EDTA/0.05 % xylene cyanol. Sequencing gels (0.4 mm thick) containing 8 % acrylamide-bisacrylamide (39:1) urea (50 % by weight) in TBE [90 mM Tris-borate/1 mM EDTA (pH 8)] buffer were used for electrophoresis of 5 μ l samples. Samples from dideoxy chain reactions were electrophoresed simultaneously in preliminary experiments for the identification of thymines attacked by KMnO_4 . Electrophoresis at 52 °C (Macrophor, Pharmacia) was continued until the labelled oligomer went out of the gel (40 cm long gel, 2 h 30 min at 1500 V). After drying, the gel was exposed to a Phosphorimager screen (Molecular Dynamics) for a few hours to several days and then scanned, each band was quantified and normalized with the reference DNA band in the same lane. The gel was also exposed to an X-ray film for autoradiography and archiving.

Transcription

The procedure was the same as above (see Treatment with KMnO_4 section) before the KMnO_4 step, except that the NTPs included radioactive [α - 32 P]UTP. The transcription reaction was stopped with ethanol (5 volumes), nucleic acids precipitated (1 h at –80 °C) and then rinsed with 70–95 % ethanol and dissolved in 10 μ l of formamide. Electrophoresis and quantification were performed as above, but omitting the normalization step.

Analysis of the data

Addition of the four NTPs to the open promoter (RPO) yields transcripts (RNA) > 10–20 nts (see Discussion section) and brings the promoter back to the closed state (PC). This is written as a single reaction:

then

$$d(\text{PC})/dt = d(\text{RNA})/dt = k(\text{RPO})(\text{NTPs})$$

Since NTPs are in large excess, $d(\text{RPO})/dt = k'(\text{RPO})$, where

k' is a pseudo-first-order rate constant. The data from the thymine-protection kinetics were fitted to an exponential $A = A_0 \exp(-k't) + B$, using a non-linear regression program [15]. A_0 and B were selected in order to minimize the overall error. For the full transcription data, B was omitted.

RESULTS

Rate of β -lactamase promoter clearance

Open complexes, formed at the *bla* promoters borne by pBR322 templates linearized by *Eco*RI and *Apa*LI, were allowed to initiate transcription by addition of NTPs (160 μ M, except UTP, 16 μ M). At selected times after the addition of NTPs, aliquots were removed, KMnO_4 was added for 1 min and then the reaction was stopped by mercaptoethanol. KMnO_4 reacts mainly with unpaired or distorted thymines [12,13]. In order to localize the positions of the oxidized thymines, a 5'-labelled primer (A1 in Figure 1a), hybridized to the non-template strand of the *bla* gene from +63 to +45 with respect to the transcription start, was extended using T7 DNA polymerase. Extension is arrested at adenines facing oxidized thymines [16]. An identical amount of *Hha*I-digested pBR322 is added to each aliquot following the mercaptoethanol treatment. This allows the signal in each lane of the autoradiogram to be normalized and the effects which may occur during subsequent steps of the experiment to be monitored.

Figure 2 shows the PAGE autoradiogram of the primer extension products on the non-template strand. Thymine at position +3 with respect to the *bla* transcription start (at G 4187), (T+3) hereafter, appears to be strongly attacked, as does (T-11) on the template strand of the same promoter (revealed by another oligomer, not shown). These thymines should be inside, or near the frontiers of, the bubble formed by the unwound promoter sequence. The bubble therefore extends over 14 bases at least, close to the size reported by Suh et al. [10] for the λ P_R promoter.

Figure 2 Kinetics of the attack by KMnO_4 of (T+3) on the non-template strand of the *bla* promoter during transcription start (see text)

Lanes 1–10: 0.5, 0.75, 1, 2, 3, 5, 10, 20, 40 and 60 min after addition of the NTPs to the open complex respectively. 'Fragment end' corresponds to the primer extension to *Eco*RI site of pBR322. 'Reference DNA' is the *Hha*I digest of pBR322 used as an internal standard. The autoradiograph is overexposed. Quantification was done with the help of a Phosphorimager (Molecular Dynamics) and the associated software.

Figure 3 Kinetics of thymine protection against KMnO_4 oxidation and run-off transcription

(a) β -Lactamase promoter, linearized. Kinetics of protection against KMnO_4 oxidation of (T+3) on the non-template strand (■) and run-off transcription kinetics under identical conditions (▲). The curves are best fits obtained with the help of a non-linear regression program [15]. (b) *RNAI* promoter, linear or supercoiled. As above, but for the (T-11) on the template strand of the *RNAI* promoter (■) and 108 nts full-length transcription (▲).

In each lane of the autoradiogram, we took the intensity of the band corresponding to (T+3) as proportional to the number of complexes for which the promoter is under KMnO_4 attack. The (T+3) band intensities were normalized with the help of the reference band in each lane. The time dependence of the (T+3) band intensities fitted a single exponential rather satisfactorily (Figure 3a), computed using a non-linear regression program [15]. The derived, pseudo-first-order rate constant of (T+3) protection against KMnO_4 attack is $0.17 \pm 0.03 \text{ min}^{-1}$ (result from three independent experiments).

The bands labelled 'fragment end' (235 nts) on Figure 2 correspond to primers extended to the *Eco*RI site of pBR322. The sum of the intensities of the bands corresponding to (T+3) and 'end', relative to that of the reference band, is almost constant in all lanes (contributions of the other bands seen on Figure 2 are minor and can be neglected). This confirms that primer extension by T7 DNA polymerase is processive and indicates that pyrimidine oxidation does not occur significantly on the non-template strand between (T+3) and the *Eco*RI site upstream.

Rate of *bla* promoter clearance probed by run-off transcription

How does the rate of protection of (T+3) against attack by KMnO_4 , which probably reflects the rate of promoter clearance (see Discussion section), compared with the rate of elongation downstream of the promoter? The 325 bp DNA fragment, resulting from the digestion of pBR322 by *Eco*RI and *Apa*LI (Figure 1a), is expected to yield a *bla* run-off transcript of 155 nts. Following addition of 160 μ M NTP (except UTP, 16 μ M) to the open complexes, the kinetics of the 155 nts run-off transcription was studied by removing aliquots at selected time intervals (Figure 4). The kinetic data (Figure 3a) were fitted to a

Table 1 Rate constants (min^{-1}) of run-off transcriptions following the addition of NTPs to open complexes for two NTP concentrations

Number of independent experiments given in parentheses; the data were fitted to a single exponential by a non-linear program [15].

	Promoter	
	<i>bla</i>	RNAI
160 μM NTP (except UTP, 16 μM)	0.15 ± 0.05 (6)	0.6 ± 0.3 (6)
160 μM NTP	0.36 ± 0.13 (3)	1.5 ± 0.9 (3)

single exponential yielding a pseudo-first-order rate constant of $0.15 \pm 0.5 \text{ min}^{-1}$ (average from six independent experiments, see Table 1). This rate is not significantly different from that found for the protection of (T+3) against attack by KMnO_4 .

Thus, for the fragment of the *bla* gene investigated and under the present experimental conditions, the rate of run-off transcription is close to that of promoter clearance, starting from the open complex in both cases. Elongation of mRNA, from the point at which (T+3) enters pairing, to the 155 nts run-off appears to be much more rapid than *bla* promoter clearance. Extensive pausing of the elongation complex, observed at bases 66, 79, 92 and 96 at NTP concentrations about ten times lower [17], remains undetected under the present conditions.

The rate constant increases to $0.36 \pm 0.13 \text{ min}^{-1}$ if all four NTPs are kept at 160 μM (see Table 1).

Rate of RNAI promoter clearance

We took advantage of the presence of the RNAI promoter on the same template to study its kinetic characteristics and compare then with those obtained for *bla* promoter under identical experimental conditions. We observe (result not shown) that base (T-11) of the template strand (2964) is subjected to heavy attack by KMnO_4 when in the open complex state. The kinetics of protection of (T-11) against this attack, following transcription elongation upon addition of 160 μM NTP (except UTP, 16 μM) to the open complex, was monitored by extension of a primer hybridized to the template strand, address -66 to -47 relative to the transcription start (A 2975). pBR322 linearized by *Eco*57I was used as an internal standard.

Experiments were performed with the pBR322 template, either linearized by *Eco*RI and *Apa*LI or in supercoiled form. The pseudo-first-order rate constant of (T-11) protection was found to be about 2.0 min^{-1} (Figure 3b) irrespective of the template topology. The precision of the data is rather poor, due to the fast rate of the reaction, with regard to the time required for experimental handling. Nevertheless, it can be stated that this rate is one order of magnitude larger than that measured for (T+3) of the *bla* promoter.

Transcription kinetics of the RNAI gene

The RNAI gene has a strong natural, *rho* independent terminator on pBR322, yielding a 108 nts transcript [18]. The rate of full-length transcription was measured by monitoring the production of 108 nts transcripts at times after the addition of NTPs (160 μM ; except UTP, 16 μM) to open promoter complexes (Figure 4). The pseudo-first-order rate constant is $0.6 \pm 0.3 \text{ min}^{-1}$ (six independent experiments, see Table 1). With all four NTPs

Figure 4 Kinetics of *bla* run-off transcription and full-length RNAI transcription on *Eco*RI-*Apa*LI-linearized pBR322 in the presence of 160 μM NTP (except UTP, 16 μM)

Lanes 1-10: 0.5, 0.75, 1, 2, 3, 5, 10, 20, 40 and 60 min after NTPs addition to the open complex. Autoradiogram is overexposed in order to demonstrate the large excess of the full-size transcripts over the shorter species.

at 160 μM , the rate constant increases to $1.5 \pm 0.9 \text{ min}^{-1}$ (three independent experiments).

The rate of clearance of the RNAI promoter, as monitored by the protection of (T-11), exceeds by about a factor of 3 the rate of the full-length transcription.

DISCUSSION

The main aim of the present study was to probe whether the selective access of KMnO_4 to unpaired pyrimidines can be useful for studying the kinetics of the promoter closure. The method was applied to the study of closure kinetics of the open promoter sequence upon clearance by elongation complex, an important step in transcription which has received little or no attention so far.

Our results show that the method as developed is valid for both linear and supercoiled templates, can address a particular promoter on a template which supports several, and works in comparatively high concentrations of NTPs. It could be used as well to study the kinetics of other steps of transcription, like bp disruption during the open complex formation process, and the rate of exposure (or protection) of thymines in halted elongation complexes, either stalling at extensive pause sites or artificially arrested due to the absence of the NTP needed for the transcription of the next base. In addition, the method can also work *in vivo*, as shown by Sasse-Dwight and Gralla [19].

At several places in the Results section, we assume that the rate of protection against KMnO_4 attack of the thymines studied reflects the rate of closure of the corresponding promoters. This assumption implies that, when exposed to the attack, the thymine under investigation is indeed in the unpaired state, and that the closure of corresponding A-T pair is representative of, and coincides with, that of the other bases present in the open promoter sequence. KMnO_4 was shown to preferentially oxidize unpaired, but also distorted, pyrimidines in DNA, hence it cannot be ruled out that already paired (T+3) or (T-11), but distorted due to the presence of RNAP in the process of entering productive transcription for instance ('stressed' [20] or 'bent' [21]) complexes, could remain exposed to the attack. We observe that the other Ts unpaired in the open promoters also react with KMnO_4 , but less vigorously than the Ts selected for the study.

Obviously, the accessibility of the latter is much easier, due to either reduced protection by the enzyme or their more favourable exposure to the chemical (in general, it is observed that the Ts located at the ends of the open sequence are more reactive towards KMnO_4). Although the kinetics of protection of the other Ts in the open complex against attack by KMnO_4 follow, qualitatively, trends similar to those of the selected Ts, the precision of the corresponding data does not allow any meaningful quantitative comparison.

To what step of the transcription initiation process does the closure of the open *bla* complex, thought to coincide with the closure of the bp involving (T+3), correspond? For the T7A1 promoter, which behaves almost the same as that of *bla* [22], the elongating complex produces 10- to 11-mer transcripts as it crosses the abortive/productive bifurcation checkpoint [23]. This is about the size of the nascent messenger RNA, found heteroduplexed to the template strand in the transcription 'bubble' of certain elongation complexes, which involves the disruption of 16–17 bps (2). By analogy, it could be surmised that at the bifurcation point, the complex would still retain the promoter in its open form. Furthermore, it has been shown by Metzger et al. [24] that a prominent structural transition in the ternary complex occurs when the length of the nascent RNA is 18–20 nts long. Assuming again that the bubble extends over 16–17 bases, we can speculate that closure of thymine (T+3) occurs short of, or during that transition. It is therefore plausible that: (1) promoter closure (or clearance) would take place when the nascent RNA is 10–20 nts long; and (2) the sequence elements controlling the closure kinetics of the open promoter are deposited 30 nts downstream (the downstream footprint limit of the ternary complex as it crosses the bifurcation check-point). Further investigations are on schedule to substantiate these speculations, which are consistent with results obtained *in vitro* [25,26] and *in vivo* [27].

The data obtained for the thymine-protection kinetics, at a large excess of NTPs, fit rather well with a single exponential, and hence could reasonably suggest a pseudo-first-order reaction scheme. The complex series of events leading to an elongation complex, following the addition of NTPs to the open complex, should therefore include a dominant, rate-limiting step. The latter may occur upon formation of the stressed [20] or bent [21] complexes mentioned earlier, or the prominent structural change described by Metzger et al. [24].

How do the kinetics of promoter closure, as investigated in the present work, compare with those of other steps during transcription initiation? Using the abortive-initiation assay, the rate of open complex formation of the *bla* and RNAI promoters was studied in this laboratory [28], under conditions close to those applied in the present study. For the *bla* promoter on a supercoiled (linear) template, the apparent binding constant for the formation of the closed complex is $15 \times 10^6 \text{ M}^{-1}$ ($99 \times 10^6 \text{ M}^{-1}$), the closed-to-open complex isomerization rate constant is 1.1 min^{-1} (0.5 min^{-1}); the corresponding data for the RNAI promoter are $4.6 \times 10^6 \text{ M}^{-1}$ ($56 \times 10^6 \text{ M}^{-1}$) and 10 min^{-1} (0.6 min^{-1}).

If we assume that the rate of protection of the thymines against oxidation is a measure of the rate of closure of the promoter they belong to, then, for the linearized *bla* promoter, the rate of opening would exceed by a factor of 3 at least that of its closure, i.e. the rate-limiting step in transcription initiation would be its closure. The *bla* promoter appears clearly as a 'slow starter' in the linearized form. As for the RNAI promoter, in the supercoiled form opening would be 5 times faster than closure but would be about 3 times slower in the linear form. Here, the rate-limiting step of initiation would depend on the template topology, being

the promoter closure on a supercoiled template and its opening on the relaxed one.

Overall, these data suggest that the RNAI promoter is strong mainly because it is a fast starter (specially in the supercoiled form), whereas the *bla* promoter in the linear form is weak because it has only slow start capabilities. Obviously, the start-rate performances of a promoter are important in setting its strength. As suggested above, the late promoter instructions located downstream +30, probably controlling, in part at least, the rate of passage from abortive to productive elongation, may play a major role in specifying the start rate and hence the promoter strength.

Although the precision of some of our data is rather poor, we can estimate the average transcription time per nt during the promoter clearance step and the subsequent elongation step, starting in both cases from the open complex. With standard NTP concentrations, we estimate for the *bla* sequence an average elongation time of 18 s/nt during promoter clearance (the first 20 nts) and only 0.35 s/nt downstream (135 nts). The corresponding data for the RNAI sequence are 1.5 s/nt (clearance) and 0.8 s/nt (89 nt downstream). The average elongation time per nt after promoter clearance is in both cases smaller than during that step, hence the former is (by far for the *bla* sequence) the rate-limiting step in transcription elongation for the two gene fragments studied. This is consistent with the observation that run-off (*bla*) or full length (RNAI) transcription kinetics following the addition of NTPs to open complexes fit single exponentials reasonably well and follow pseudo-first-order kinetics. For the experiment with the four NTPs at 160 μM , the average, overall elongation time is 1 s/nt for the *bla* sequence and 0.37 s/nt for the RNAI sequence, 2–3 times shorter than the corresponding values at standard NTP concentrations.

The method used in this investigation appears well suited for obtaining structural and kinetic data of the transcription-initiation and elongation steps. These will help to support and foster theoretical modelling efforts [29] aimed at a comprehensive understanding of the rather complex transcription mechanisms.

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