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RNA polymerase molecules initiating transcription at tandem promoters can collide and cause premature transcription termination

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Using purified *E. coli* RNA polymerase we have studied the transcription in vitro of a series of DNA fragments carrying two tandemly arranged promoters, where the corresponding transcription start points were separated by 263, 138, 83 and 78 base pairs. In the case where the transcription start points are 83 base pairs apart, there is an interaction between RNA polymerase molecules transcribing from the two promoters. This interaction results in premature termination of the upstream transcript at a precise site. We propose that this is the result of RNA polymerase transcribing from the upstream promoter bumping into polymerase at the downstream promoter. The interaction between the two polymerase molecules is crucially dependent on the distance between the two promoters.

RNA polymerase; Transcription; Tandem promoter; Bumping; Transcript termination

1. INTRODUCTION

In vitro transcription assays are frequently used to study promoters in DNA sequences [1]. In such assays, purified RNA polymerase is incubated with a purified DNA fragment and, after the addition of nucleoside triphosphates, the transcripts are analysed. In cases where the DNA fragment contains several transcription start points, it is usually assumed that each promoter acts independently.

In several recent publications, we have described the properties of the P2 promoter from the *E. coli* galactose operon [2–5] using in vitro transcription assays on a variety of DNA fragments. In this paper we describe experiments on fragments carrying a second promoter upstream of *gal*P2: the direction of transcription from this promoter is the same as the direction of transcription from *gal*P2.

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We show that, under certain conditions, RNA polymerase bound at *gal*P2 causes premature termination of the transcript initiating at the upstream promoter.

2. MATERIALS AND METHODS

2.1. *DNA fragments*

The *Eco*RI-*Hin*dIII fragment carrying the *gal* operon promoter region has been described [6]. All the constructions described here carried the p14 promoter mutation such that *gal*P2 is the sole *gal* promoter that is active [3,4]. Insertions and deletions were made using standard recombinant DNA methods [7]. The 180 base pair insert in fragment A was a random *Hae*III fragment from pBdCI [8]. The creation of the deletions in fragments C, D and E has been described in [6,9]. The linker sequence used in fragment D was 5′-AATTCCC-3′ whereas in fragments C and E it was 5 bp longer, 5′-AATTCCCGGGGA-3′. DNA fragments were

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isolated from 500 ml chloramphenicol-amplified cultures by standard methods [7].

2.2. *Transcription experiments*

In the transcription experiments we used the 'run-off' protocol exactly as described by Spassky et al. [2]. Purified RNA polymerase (150 nM) was incubated with purified DNA fragments for 20 min at 37°C. The reaction was started by adding 0.2 mM ATP, CTP, GTP and 0.05 mM [α-^{32}P]UTP (Amersham). After 20 min the reaction was stopped by phenol extraction and the samples were run on a 10% polyacrylamide gel containing 7 M urea to analyse the size of transcripts due to polymerase running from promoters to the end of the DNA fragment. The gels were revealed by autoradiography. Individual transcripts were purified and mapped with ribonuclease T_1 (Boehringer) according to Simmons [10].

RNA polymerase was either home-made or obtained from NBL enzymes or BRL. According to SDS-PAGE analysis all the preparations were greater than 80% saturated with sigma factor. The results were substantially independent of the preparation of polymerase that was used.

3. RESULTS AND DISCUSSION

The *gal*P2 promoter was cloned on DNA

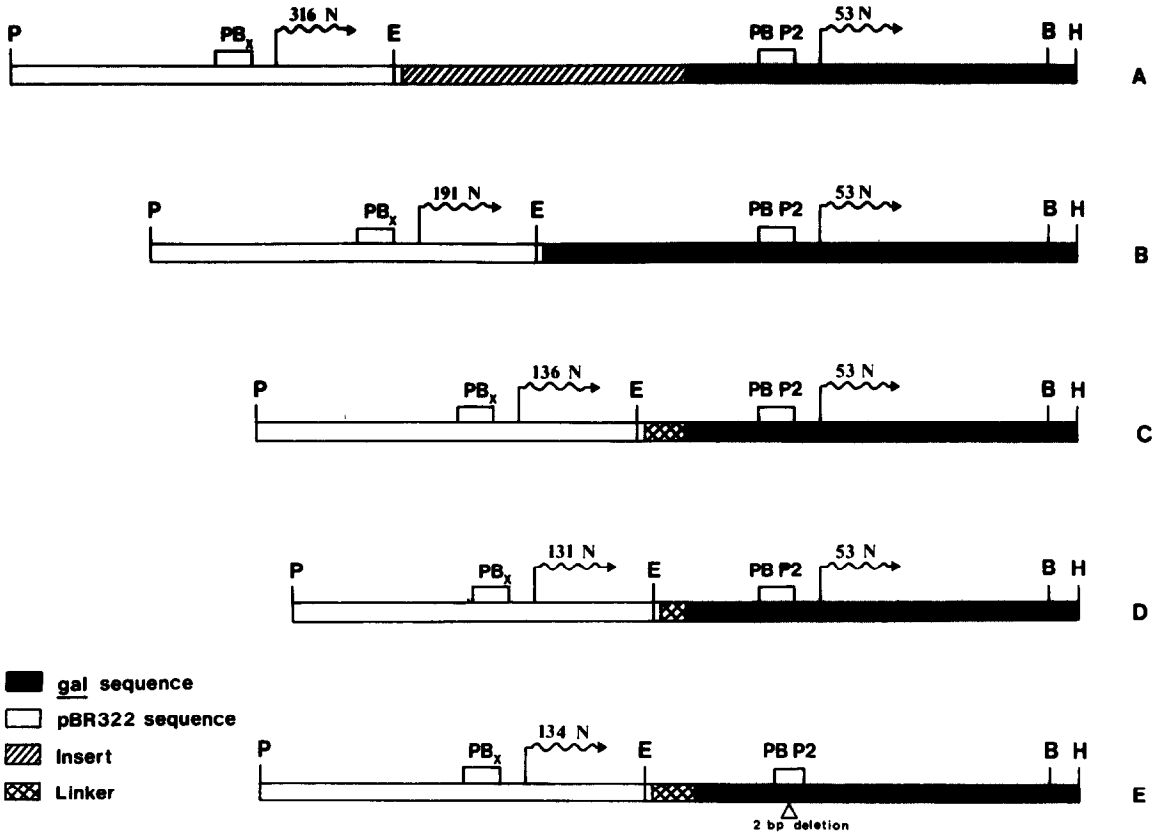

Fig.1. Diagram of DNA fragments used in this study. The lines represent the DNA sequence from a *Pst*I (P) to *Hin*dIII (H) site covering sequence from pBR322 to the *Eco*RI (E) site and *gal* promoter sequence. The positions of an insert is shown in fragment A and linker sequences in fragments C–E. The unique *Bst*EII site (B) is shown in each case. Above each line the positions of the Pribnow box sequences for the *gal* P2 and Px promoters are shown together with the location and orientation of the transcription start point and the length of the run-off transcript to the *Hin*dIII site. Note that the scale of each fragment has been deliberately distorted: in each case the *Pst*I–*Eco*RI distance is 750 bp whilst the *Eco*RI–*Hin*dIII distances are 269, 144, 89, 84 and 87 bp, respectively, in fragments A–E.

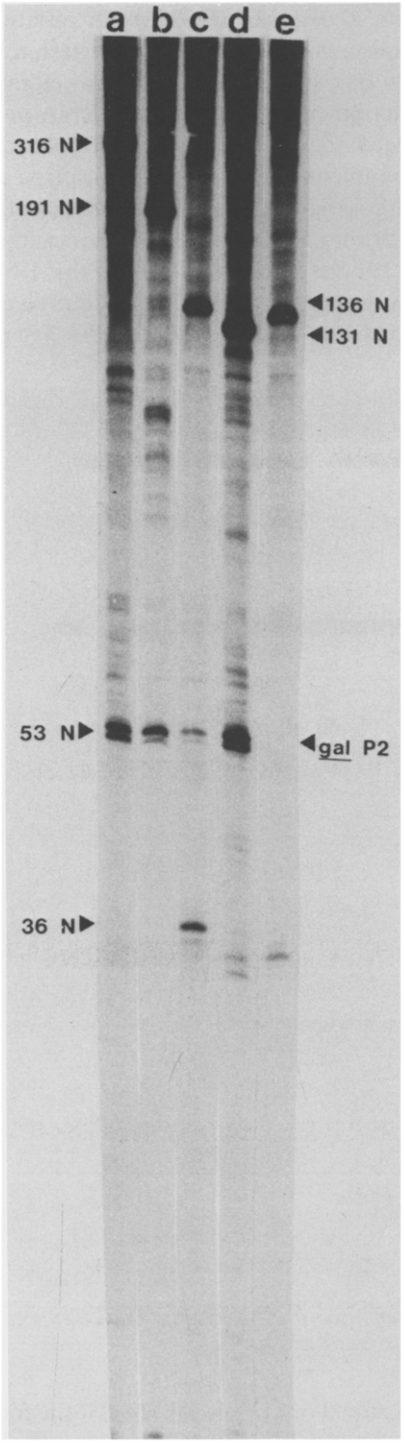

Fig.2. Transcription of fragments A–E. (Lanes a–e) Autoradiogram of a gel run to analyse the transcripts made by fragments A–E (see fig.1). The calibrations refer to the lengths of RNA molecules that run to that point on the gel, deduced from size markers.

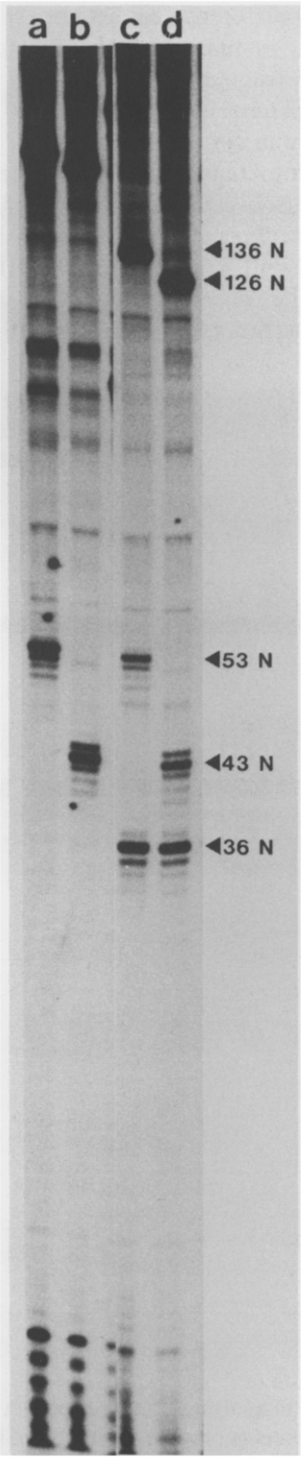

Fig.3. Autoradiogram of a gel where the transcripts made by fragment B (lanes a,b) and C (lanes c,d) were analysed. In cases b,d the fragment was cut with *Bst*EII prior to the experiments.

fragments of different sizes between the *Eco*RI and *Hin*dIII sites of plasmid pBR322. Here, we used *Pst*I-*Hin*dIII fragments isolated from the resultant plasmids. These fragments are illustrated schematically in fig.1.

The starting fragment (B) contained 144 bp of *gal* sequence between the *Eco*RI and *Hin*dIII sites. The *gal*P2 transcript starts 53 bp upstream of the *Hin*dIII site and runs to the *Hin*dIII end of the fragment. The other fragments that we used contained an insertion (A) or deletions of *gal* sequence from the *Eco*RI site (C,D,E). In addition, fragment E also carries a 2 base pair deletion in the P2 Pribnow box sequence. Fig.2 shows a gel analysis of transcripts made in vitro when RNA polymerase was added to each of the five DNA fragments. With fragment B a 53 base transcript is clearly seen, that corresponds to initiation at the *gal*P2 transcription start point (lane b). Another major band is 191 bases long: analysis of this RNA shows that this transcript starts 47 base pairs to the left of the *Eco*RI site and runs across the *gal* promoter

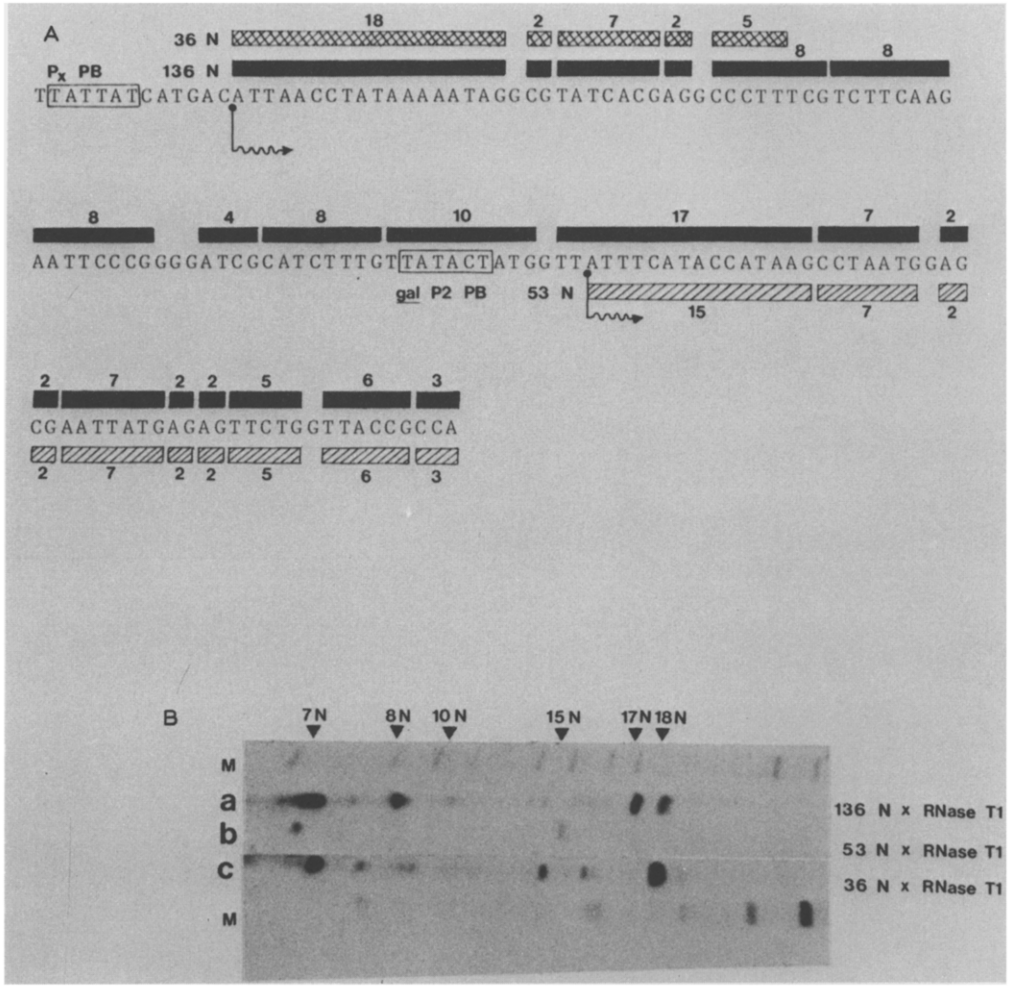

Fig.4. RNase T_1 analysis of transcripts. (A) DNA sequence of the upper strand from Px to the *Hin*dIII site for fragment C. Oligonucleotides produced by RNase T_1 digestion of the 136 N transcript (■), 36 N transcript (▩) and 53 N transcript (▨) are shown. The transcription start points (↝) and Pribnow boxes (PB) are also indicated. (B) Autoradiogram of RNase T_1 digests of the different transcripts from fragment C. Lanes: a, 136 base transcript from Px; b, 53 base transcript from P2; c, 36 base transcript. The gel was calibrated with markers (M) and the sizes of the main T_1 oligonucleotides are shown. The location of each band can be checked in A.

region. Sequence analysis suggests the presence of a promoter in this zone but as we do not know its function in vivo, it is labelled Px. The only other transcript runs to the *Pst*I end of the fragment from the promoter of the β-lactamase gene which is located well to the left of Px [11]. As this transcript is over 500 bases in length, it hardly migrates in this gel system and remains near the top of the gel.

The transcription experiment was repeated with fragments A and D, which contain an insertion and a deletion at the *Eco*RI site, respectively. Fig.2

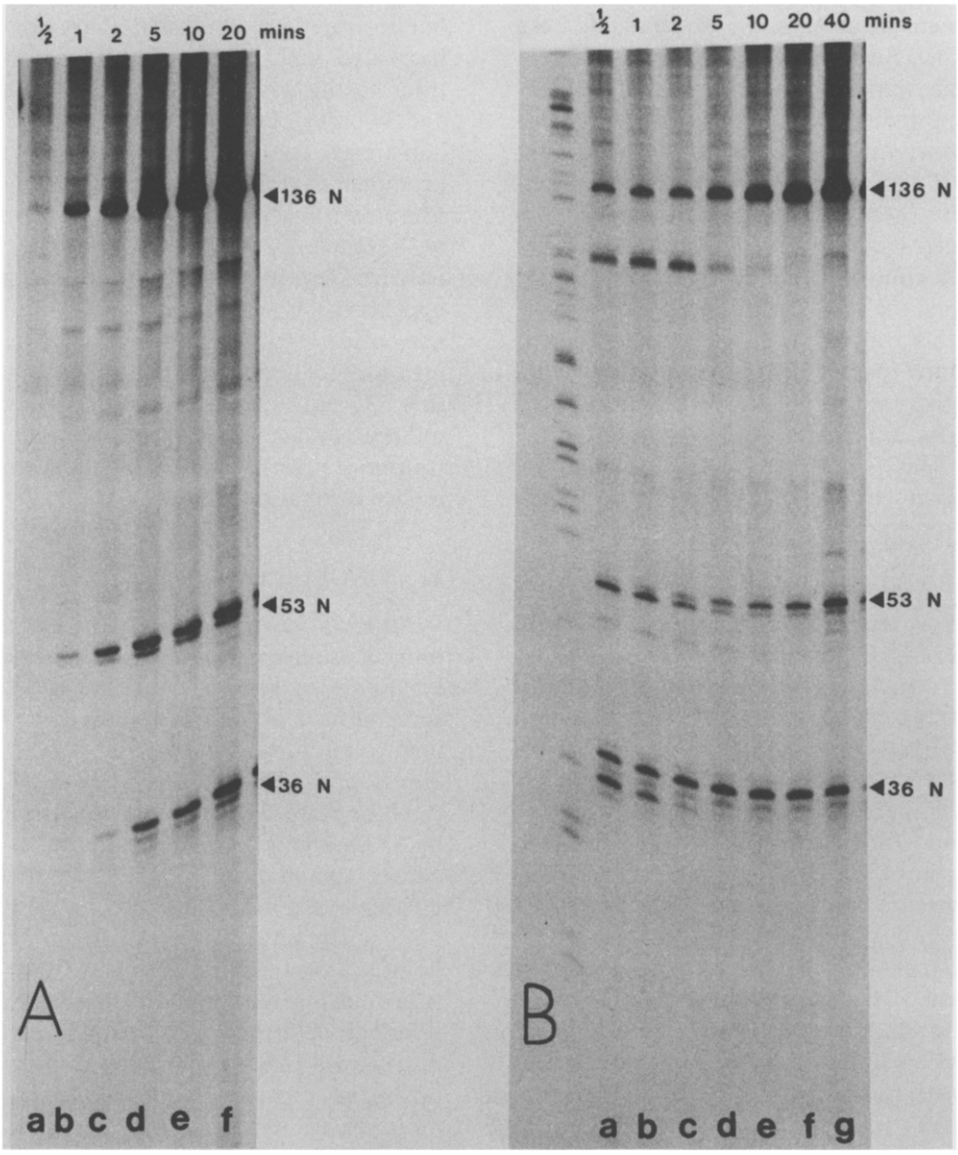

Fig.5. (A) Run-off transcripts produced when fragment C was incubated for different times with RNA polymerase prior to the addition of nucleoside triphosphates and heparin. The incubation times are given. (B) Run-off transcripts produced when fragment C was incubated with RNA polymerase and then nucleoside triphosphates and heparin were added for the different times given. The lengths of the Px (136 N), *gal*P2 (53 N) and short (36 N) transcript are indicated in both panels.

(lanes a,d) shows that whilst the 53 base transcript from the *gal*P2 promoter is unaltered by insertion or deletion, the length of the transcript originating at Px changes. As expected, with the insertion, the transcript is lengthened to 316 bases, whereas with the deletion it is shortened to 131 bases.

The experiment was repeated with fragment C: although this contains the same 60 base pair deletion as fragment D, an extra 5 base pairs had been inserted at the *Eco*RI site by using a different linker sequence during the construction of the deletion. As expected, the transcript from P2 is 53 bases long whereas the Px transcript length is increased to 136 bases (fig.2, lane c). However, surprisingly, the intensity of the P2 transcript is greatly reduced and a new band, 36 bases in length, appears. As a control we modified fragment C by deleting 2 bp in the *gal*P2 Pribnow box. Transcript analysis of this fragment, E, shows that whereas the Px promoter gives a 134 base transcript, *gal*P2 promoter activity is lost and the 36 base band does not appear (fig.2, lane e).

The prima facie interpretation of this unusual result with fragment C is that when P2 and Px are juxtaposed at a particular distance, an interaction occurs which leads to the production of a 36 base transcript. To investigate the nature of this we repeated the transcription experiment with fragments B and C that had been shortened by 10 bp, by restriction with *Bst*EII (see fig.1). Shortening fragments B and C leads to a reduction in the size of both the *gal*P2 and Px transcripts (fig.3). However, with fragment C, the size of the 36 base transcript is unaltered (lanes c,d), showing that this band is not due to a new transcript running to the end of the fragment but is most likely due to premature termination of the Px or P2 transcripts.

To locate the origin of the 36 base transcript from fragment C the transcript was purified by gel electrophoresis and digested with ribonuclease T_1. Fig.4B shows a calibrated gel on which T_1 digests of the 36 base transcript, the 53 base transcript from *gal*P2 and the 136 base transcript from Px were analysed. Fig.4A shows the sequence of the upper strand of the relevant part of fragment C running from the Px Pribnow box to the *Hin*dIII end. The startpoints of the 53 base and 136 base transcripts are shown together with the expected pattern of oligonucleotides generated by T_1 digestion. From fig.4B it is clear that the 36 base transcript originates from Px: the major band is 18 bases long and corresponds exactly to the oligonucleotide generated from the 5′-end of the 136 base Px transcript. The 36 base transcript is thus due to premature termination of the Px transcript.

Fig.5 shows a kinetic analysis of the transcripts due to fragment C. Firstly, RNA polymerase was incubated with the fragment for different times prior to the addition of nucleoside triphosphates and heparin. The result (fig.5A) shows that the half-times for the formation of polymerase-promoter complexes that generate the 36, 53 and 136 base transcripts are around 2–3 min. Secondly, complexes were formed by incubating polymerase with fragment C for 20 min and the appearance of transcripts after the addition of nucleoside triphosphates and heparin was measured. The result (fig.5B) shows that whereas the 53 and 136 base transcripts take several minutes to accumulate, the amount of 36 base transcript present after 30 s does not increase with longer incubation times.

4. CONCLUSIONS

Although over a dozen cases of tandemly arranged promoters have been reported in *E. coli*, very little is known about interactions between such pairs of promoters [1]. Here we have studied in vitro initiation from a pair of transcription start points separated by 263 (A), 138 (B), 83 (C) and 78 (D) base pairs. When the start points are separated by 83 base pairs, an interaction takes place which causes termination of the transcript from the upstream promoter at a precise position 47 base pairs upstream of the downstream promoter start point.

We have several reasons for believing that this termination is due to bumping between RNA polymerase that has initiated transcription at the upstream promoter and a second polymerase molecule at the downstream promoter. Firstly, the termination of the upstream transcript requires polymerase binding to the downstream promoter: when the downstream promoter is destroyed by a 2 base pair deletion no termination of the upstream transcript occurs (fig.2, lane e). Secondly, during a time course, the short transcript due to termination

accumulates only in the first moments after the addition of nucleoside triphosphates: as polymerase moves away from the downstream *gal*P2 promoter no more bumping occurs. Thirdly, RNA polymerase bound to *gal*P2 covers about 40 bp upstream of the transcription start point [3] and thus it is reasonable that if a bump is to occur, it will be after the upstream polymerase has progressed 30–40 bases. Finally, the amount of 53 base *gal*P2 transcript made with fragment C is greatly reduced compared to fragment D: the interaction between the two polymerase molecules hinders formation of the downstream transcript.

The fact that this interaction is absent when the distance between the two transcription start points is reduced by 5 base pairs (cf. fig.2c,d) argues strongly that the precise juxtaposition between two polymerase molecules as they collide determines the result. Recall that 5 base pairs is roughly equivalent to half a turn of the DNA helix. Thus with fragment D, any collision between two polymerase molecules has little effect and transcription continues to the end of the fragment. In contrast, with fragment C, the juxtaposition of the two molecules must be such that, on bumping, the polymerases can interact together strongly, causing premature termination of the upstream transcript.

At present we cannot say whether this observation is any more than a curious in vitro artefact. However the specific and precise nature of this interaction suggests to us that a study of possible interactions between similarly spaced tandem promoters in vivo would be worthwhile.

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