Transcription initiation is the first, and the most highly regulated, process in gene expression. In the first steps of transcription initiation, RNA polymerase (RNAP) binds to promoter DNA and unwinds ∼14 base pairs (bp) surrounding the transcription start site to yield a catalytically competent RNAP-promoter open complex (RPo) (1–3). In subsequent steps of transcription initiation, RNAP enters into initial synthesis of RNA as an RNAP-promoter initial transcribing complex (RPitc), typically engaging in abortive transcription initiation complexes, we show that initial transcription proceeds as an RNAP-DNA elongation complex (RPo) (1–3). We show further that putative abortively initiating transcription initiation complexes, we show that initial transcription proceeds and unwinds ~14 base pairs (bp) surrounding the transcription start site to yield a catalytically competent RNAP-promoter open complex (RPo) (1–3). In subsequent steps of transcription initiation, RNAP enters into initial synthesis of RNA as an RNAP-promoter initial transcribing complex (RPitc), typically engaging in abortive transcription initiation complexes, we show that initial transcription proceeds and unwinds ~14 base pairs (bp) surrounding the transcription start site to yield a catalytically competent RNAP-promoter open complex (RPo) (1–3). We show further that putative abortively initiating transcription initiation complexes, we show that initial transcription proceeds and unwinds ~14 base pairs (bp) surrounding the transcription start site to yield a catalytically competent RNAP-promoter open complex (RPo) (1–3). We show further that putative abortively initiating transcription initiation complexes, we show that initial transcription proceeds and unwinds ~14 base pairs (bp) surrounding the transcription start site to yield a catalytically competent RNAP-promoter open complex (RPo) (1–3).
within the unwound region; on release of the abortive RNA, RNAP extrudes the accumulated DNA, which regenerates the initial state.

The three models are not necessarily mutually exclusive; in principle, combinations of mechanisms may be used, or different mechanisms may be used at different stages of initial synthesis (e.g., one for synthesis of short RNA products, and another for synthesis of longer RNA products).

In this work, we have directly tested the predictions of the three models in Fig. 1A by monitoring distances within single molecules of RPo and RPin (17). We used fluorescence resonance energy transfer (FRET) (18) to monitor distances between fluorescent donors and acceptors incorporated at specific sites within RNAP and DNA. We used confocal optical microscopy with alternating-laser excitation (ALEX) (19–21) to detect and to quantify fluorescence of single molecules in solution transiting a femtoliter-scale observation volume (Fig. 1B, top left). For each such single molecule, we extracted the donor-acceptor stoichiometry parameter, S, and the observed efficiency of donor-acceptor energy transfer, E* (Fig. 1B, top right). We analyzed Escherichia coli RNAP holoenzyme (RNAP core in complex with the initiation factor σ70) (1–3) and a consensus E. coli promoter (lacCONS) (22) (fig. S1).

We performed four sets of experiments to assess the following: (i) movement of the RNAP leading edge relative to DNA; (ii) movement of the RNAP trailing edge relative to DNA; (iii) expansion and contraction of RNAP; and (iv) expansion and contraction of DNA. In each case, we performed measurements with RPo containing the initiating dinucleotide ApA [RPo + ApA, referred to hereafter as RPo (Fig. 1B, bottom)] and with RPin engaged in iterative abortive synthesis of RNA products up to 7 nt in length [RPin<7; prepared by addition of UTP and GTP to RPo (Fig. 1B, bottom)].

To assess possible movement of the RNAP leading edge relative to downstream DNA in initial transcription, we monitored FRET between a fluorescent donor incorporated at the RNAP leading edge (σ70 residue 366, located in σR2, the σ70 domain responsible for recognition of the promoter –10 element) and a fluorescent acceptor incorporated at a site in downstream DNA (position +20) (Fig. 2A). The results indicated that, on transition from RPo to RPin<7, the mean observed efficiency E* significantly increases, which implies that the mean donor-acceptor distance, R, significantly decreases (Fig. 2A, right). The quantitative increase in mean E* corresponds to a decrease in mean R of ~7 Å (Fig. 2A, right; table S1). Parallel experiments performed using a donor incorporated at a different site at the RNAP leading edge (σ70 residue 396, also located in σR2), or using an acceptor incorporated at a different site in downstream DNA (position +15 or position +25), also showed decreases in mean donor-acceptor distance [decreases of ~5 to ~16 Å (fig. S2)]. Control experiments performed in the presence of rifampicin, an inhibitor that blocks synthesis of RNA products >2 nt in length (23), showed that the observed decreases in mean donor-acceptor distance required synthesis of RNA products >2 nt in length (fig. S3). We infer that the RNAP leading edge translates relative to downstream DNA in initial transcription. Furthermore, we infer that, during initial transcription with these reagents and reaction conditions, complexes predominantly occupy states in which the RNAP leading edge is translocated relative to downstream DNA, not states in which the RNAP leading edge is positioned as in RPo. This implies that the rate-limiting step in initial transcription with these reagents and reaction conditions is abortive-product release and RNAP active-center reverse-translocation [see also (24)]. The finding that the RNAP leading edge translates relative to downstream DNA is consistent with the predictions of all three models for initial transcription (Figs. 1A and 2A). The finding that the RNAP primarily occupies states in which the RNAP leading edge is translocated is incompatible with, or at least problematic for, the transient-excursion model, which invokes translocated states that are short in duration and infrequent in occurrence.

To assess possible movement of the RNAP trailing edge relative to upstream DNA in initial transcription, we monitored FRET between a fluorescent donor incorporated at the RNAP trailing edge (σ70 residue 569, located in σR4, the σ70 domain responsible for recognition of the
promoter –35 element) and a fluorescent acceptor incorporated at a site in upstream DNA (position –39) (Fig. 2B). In this case, the results indicated that, on transition from RPo to RPitc, mean $E^*$ remains unchanged (Fig. 2B, top right), which implies that the mean donor-acceptor distance remains unchanged. Parallel experiments performed using a donor incorporated at a different site at the RNAP trailing edge ($\sigma^{70}$ residue 596, also located in $\sigma$R4) also imply that the mean donor-acceptor distance remains unchanged (fig. S4). Control experiments showed that our probe sites are well positioned to detect a translocation-dependent change in mean donor-acceptor distance and would detect a change if it occurred (fig. S5). We infer that the RNAP trailing edge does not translocate relative to upstream DNA in initial transcription. Specifically, we infer that the $\sigma^{70}$ domain that interacts with promoter –35 element does not alter its interactions with DNA in initial transcription. This is true even for reaction conditions where it can be shown that the RNAP leading edge translocates relative to downstream DNA (Fig. 2A). These findings are inconsistent with the fundamental prediction of the transient-excursions model; that is, any molecule having the RNAP leading edge translocated relative to DNA also must have the RNAP trailing edge translocated relative to DNA (Figs. 1A and 2B). We conclude that initial transcription does not involve transient excursions.

To assess possible expansion and contraction of RNAP in initial transcription, we first monitored FRET between a fluorescent donor incorporated at the RNAP leading edge ($\sigma^{70}$ residue 366, located in $\sigma$R2, and shown in Fig. 2A to translocate relative to downstream DNA) and a fluorescent acceptor incorporated at a site in –10/–35 spacer DNA (position –20).

**Fig. 3.** Initial transcription does not involve inchworming. (A) Experiment documenting absence of movement of the RNAP leading edge relative to –10/–35 spacer DNA [tetramethylrhodamine as donor at $\sigma^{70}$ residue 366 (located in $\sigma$R2, the $\sigma^{70}$ domain responsible for recognition of the promoter –10 element); Alexa647 as acceptor at DNA position –20]. Subpanels as in Fig. 2A. (B) Experiment documenting absence of movement of the RNAP trailing edge relative to –10/–35 spacer DNA [tetramethylrhodamine as donor at $\sigma^{70}$ residue 569 (located in $\sigma$R4, the $\sigma^{70}$ domain responsible for recognition of the promoter –35 element); Alexa647 as acceptor at DNA position –20]. Subpanels as in Fig. 2A.

**Fig. 4.** Initial transcription involves scrunching. (A) Experiment documenting contraction of DNA between positions –15 and +15 [Cy3B as donor at DNA position –15; Alexa647 as acceptor at DNA position +15]. Subpanels as in Fig. 2A. [The two donor-acceptor species in the $E^*$ histograms comprise free DNA (lower-$E^*$ species) and RPo or RPitc (higher-$E^*$ species; higher FRET attributable to RNAP-induced DNA bending)]. Free DNA is present in all experiments, arising from dissociation of nonspecific complexes after heparin challenge during preparation of RPo, but is detected only in this experiment, because DNA contains both donor and acceptor only in this experiment. (B) Summary of results. Structural model of RPo (28) showing all donor-acceptor distances monitored in this work (Figs. 2 to 4A and figs. S2 to S8). Distances that remain unchanged on transition from RPo to RPitc are indicated with thin blue lines. Distances that decrease on transition from RPo to RPitc are indicated with thick blue lines. The red and pink arrows show the proposed positions at which scrunched template-strand DNA and scrunched nontemplate-strand DNA, respectively, emerge from RNAP (i.e., near template-strand positions –9 to –10 and near nontemplate-strand positions –5 to –6).
does not involve inchworming. We conclude that initial transcription involves scrunching.

We note that all measured distances between RNAP and upstream DNA or –10–35 spacer DNA remain unchanged on transition from RPo to RPinc57. Thus, we postulate that the accumulated DNA-scrunching stress in the stressed intermediate provides the driving force for abortive initiation; or (ii) by retaining the RNA product, breaking interactions with promoter DNA, breaking interactions with initiation factors, translocating the RNAP trailing edge, and forming RDc (promoter escape and productive initiation).

References and Notes
17. Materials and methods are available as supporting online material on Science Online.
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References
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