Molecular mechanisms of RNA polymerase—the F/E (RPB4/7) complex is required for high processivity *in vitro*

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Received July 27, 2009; Revised September 22, 2009; Accepted October 8, 2009

ABSTRACT

Transcription elongation *in vitro* is affected by the interactions between RNA polymerase (RNAP) subunits and the nucleic acid scaffold of the ternary elongation complex (TEC, RNAP-DNA–RNA). We have investigated the role of the RNAP subunits F/E (homologous to eukaryotic RPB4/7) during transcription elongation and termination using a wholly recombinant archaeal RNAP and synthetic nucleic acid scaffolds. The F/E complex greatly stimulates the processivity of RNAP, it enhances the formation of full length products, reduces pausing, and increases transcription termination facilitated by weak termination signals. Mutant variants of F/E that are defective in RNA binding show that these activities correlate with the nucleic acid binding properties of F/E. However, a second RNA-binding independent component also contributes to the stimulatory activities of F/E. In summary, our results suggest that interactions between RNAP subunits F/E and the RNA transcript are pivotal to the molecular mechanisms of RNAP during transcription elongation and termination.

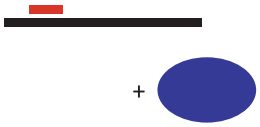
INTRODUCTION

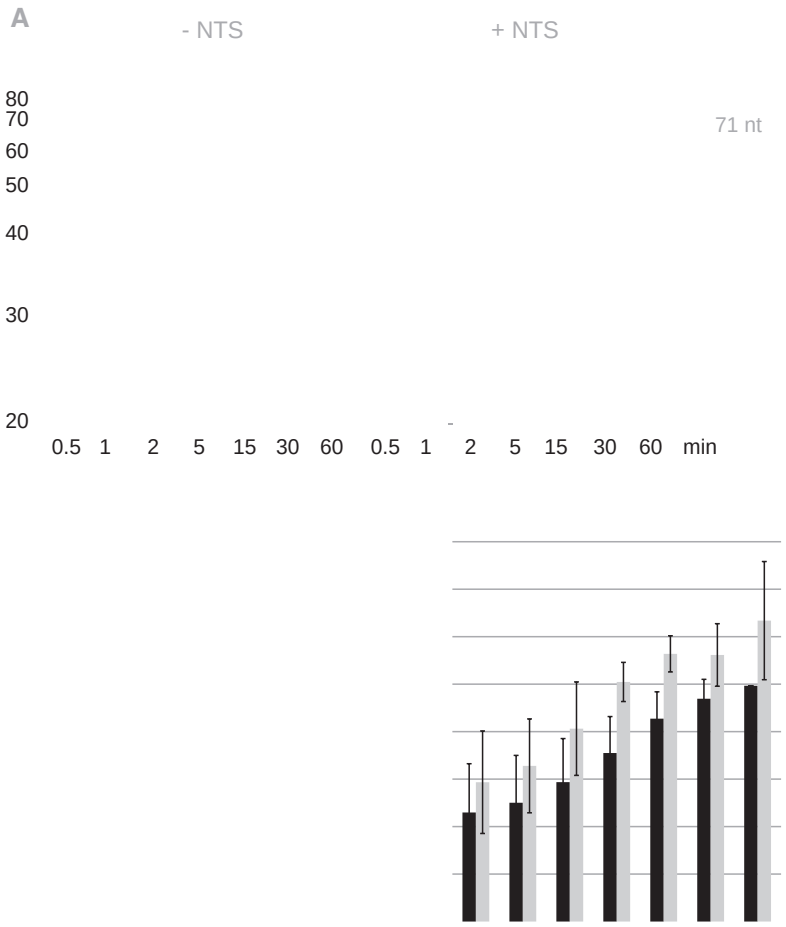
Evolutionary related multi-subunit RNA polymerases (RNAP) facilitate DNA-dependent RNA transcription in all three domains of life (1). Transcription is often likened to a cycle where RNAPs initiates at the promoter, elongate through the gene, terminate at its 3′-end, and reinitiate at the promoter again. During the transcription cycle RNAPs are undergoing substantial conformational changes and distinct interactions are made between RNAP subunits and the promoter DNA during transcription initiation, and the DNA…RNA scaffold during transcription elongation. The overall structural layout of all multi-subunit RNAPs is conserved. However, the archaeal RNAP and eukaryotic RNAPII share a subset of subunits that are not present in the bacterial enzyme (2). The most prominent structural feature that discriminates between all bacterial and archaeal/eukaryotic enzymes is a stalk-like protrusion formed by a heterodimeric complex of RNAP subunits F/E (RPB4/7 in RNAPII). The F/E complex is stably associated with the RNAP core and binds RNA in vitro and in vivo (3…5). During transcription initiation F/E, in conjunction with the basal transcription factor TFE, is involved in DNA melting (6,7). Much research has been devoted to the understanding of the mechanisms that regulate transcription initiation, which traditionally was

∼20…50 bases downstream of the transcription start site is a regulatory mechanism that allows rapid induction of gene expression because it does not depend on recruitment cascades of transcription factors and RNAPs. This phenomenon was initially discovered at Drosophila heat shock promoters but has subsequently been proven to be a widespread mode of regulation from yeast to man (16…18). The sequence dependent pausing of all types of RNAPs is facilitated by interactions between the RNAP and DNA template and the RNA transcript. Structural information obtained from RNAPII elongation complexes has illuminated the interactions between the downstream DNA duplex and the RNAP jaw domains, and the interactions between the DNA…RNA hybrid and the RNAP hybrid compartment (19…21). However, it has not been possible to solve the structures of the RNA transcript and the nontemplate strand (NTS) in the context of the TEC. Both the NTS and the RNA transcript are components of the protein-nucleic acid interaction network of the elongation complex, and thereby have the potential to in”uence transcription elongation and termination (22).

We are using a wholly recombinant hyperthermophilic archaeal in vitro transcription system to investigate the molecular mechanisms of transcription (7,23,24). This system is a genuine model system for eukaryotic RNAPII and allows us to study the functional contributions of RNAP subunits such as the F/E complex (RPB4/7) (2,3). The tractability of the RNAPII system is limited by its biochemical properties, thus it has not been possible to produce recombinant RNAPII. In addition, archaeal transcription systems allow us to uncover basic RNAP functions that are often masked by the complexity of the eukaryotic system (6,7,25).

Here we characterize the molecular mechanisms of archaeal RNAP downstream of the initiation phase, during elongation and termination of transcription. We have investigated the role of the NTS during the elongation and termination phases of transcription and characterized the functional contribution of RNAP subunits F/E during these two processes. We have discovered that transcript binding to F/E enhances the processivity and decreases transcriptional pausing, but also that the F/E complex improves the e ciency of transcription termination on weak termination signals. The modus operandi of F/E is comprised of two components, one of which depends on the RNA-binding activity of F/E. The second mechanism is likely to involve conformational changes within RNAP such as the





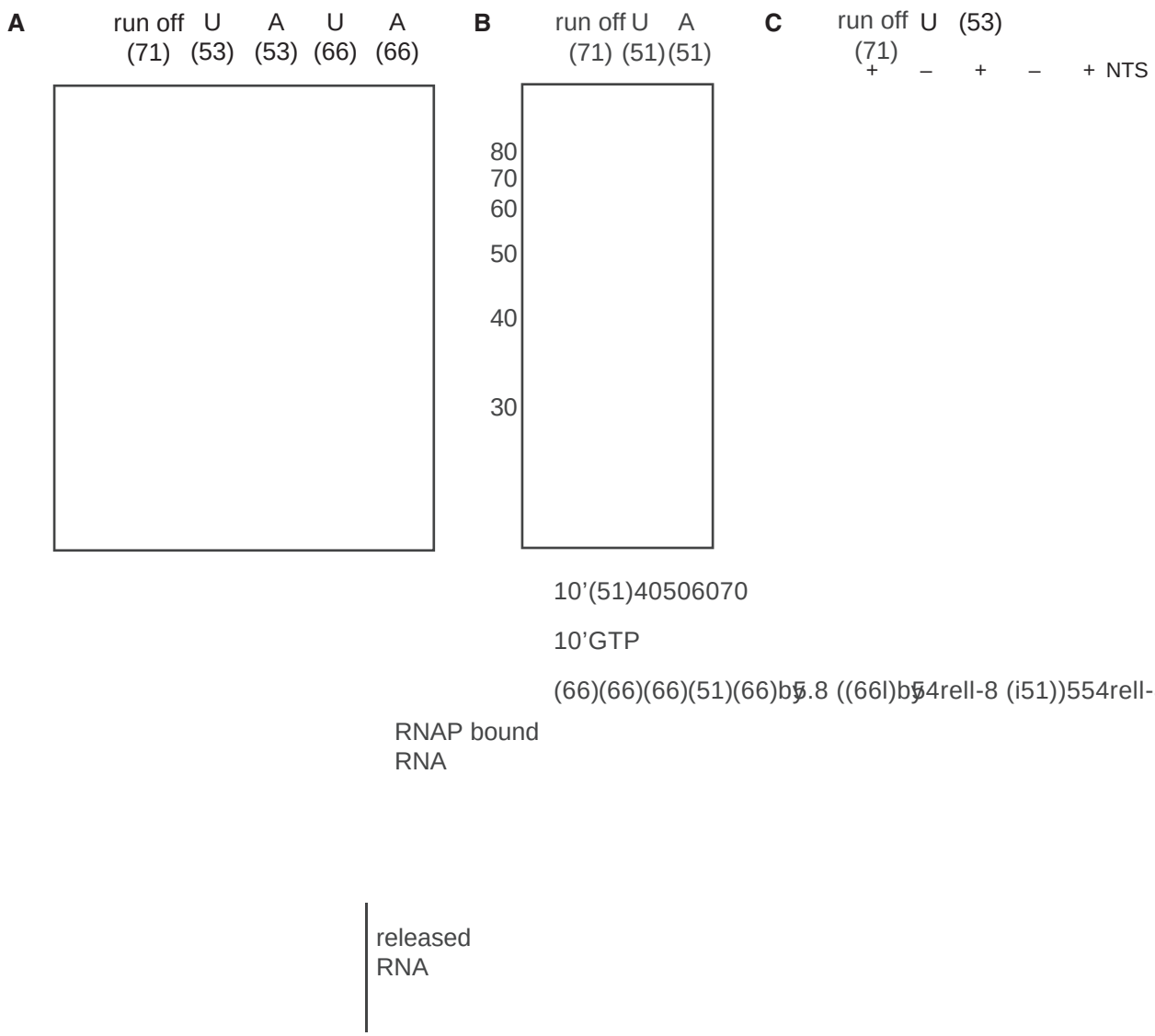
presence of the NTS. In the “rst experiment RNAP utilizes a template strand (TS) DNA:RNA primer sca old (Figure 2A, left) and in the second experiment a TS/NTS DNA:RNA sca old (Figure 2A, right). The recombinant archaeal RNAP can utilize synthetic elongation sca olds independently of the NTS, similar to bacteriophage T7 RNAP, bacterial RNAP and eukaryotic RNAPII (Figure 2) (27,28). Under the chosen assay conditions the NTS a ects transcription elongation both quantitatively and qualitatively. Wild type RNAP synthesizes fewer partial- and more runo transcripts (71 nt), and the runo transcript is produced earlier in the presence of the NTS (Figure 2B and C, 2 min instead of 5 min). This demonstrates that the processivity is improved by the presence of the NTS, which is likely due to an increased stability of the TEC, i.e. improved •traction• of RNAP on the template. Under our experimental conditions the earliest full-length runo transcript appears after 2 min suggesting an in vitro elongation rate of approximately 35 nt/min. This is in good agreement with the in vitro transcription elongation rate of eukaryotic RNAPII, but it is also signi“cantly slower than elongation rates estimated in vivo (∼10…25 nt/s) (29,30). In the absence of the NTS the “rst runo transcript is generated after 5 min, which suggests that the elongation rate is about two fold slower without the NTS (Figure 2A…C). However, under these conditions RNAP is also paused for a longer time period (Figure 2A, ∼23 and 33 nt) and it is therefore problematic to clearly distinguish an increased transcription elongation rate from improved processivity (Figure 2A). The total amount of radio-labelled transcripts synthesized is only marginally increased by the NTS (Figure 2C).

The RNAP subunits F/E enhance the processivity

Transcription elongation complexes are characterized by an astounding processivity in vivo, e.g. during the transcription of human muscle dystrophin transcription unit

RNAPII elongates through 2.4 MB of coding sequence during which it remains associated with the DNA template for ~16 h (31). The high stability of the elongation complex is re”ected in a complex interaction network between RNAP subunits, the DNA template, the RNA-DNA hybrid and RNA transcript. We have recently solved the structure of the*M. jannaschii* RNAP subunits F/E and characterized its RNA-binding properties (4,32). We tested the functional contribution of F/E during transcription elongation by comparing wild type enzymes with recombinant RNAP variants lacking subunits F/E (RNAP ΔF/E). The elongation properties of RNAP ΔF/E are severely compromised (Figure 3A). The RNAP ΔF/E is not capable of synthesizing the run o transcript in the absence of the NTS, even after 60 min of reaction time, and all RNAPs are paused on the DNA template at approximately position +33 (Figure 3A and C). The nature of this pause site is unclear and was not dependent on the CTP tracer nucleotide (data not shown). In comparison, the wild-type RNAP has already synthesized the runo transcript at the earliest time point of 5 min (Figure 3A and C). In the presence of the NTS, the RNAP ΔF/E is capable of forming runo transcripts but the processivity of the wild-type enzyme is substantially greater (Figure 3B and D). In order to ascertain that the stimulatory activity of F/E on transcription elongation was dependent on the incorporation of F/E into the RNAP, and not due to •free• F/E in solution we made use of the fact that the F/E complex can be incorporated into RNAP ΔF/E prior to the reaction and that the incorporation of the F/E complex into RNAP is strictly dependent on subunit K (3). When we added F/E complex to either RNAP ΔF/E or RNAP ΔK/F/E only the former responded by synthesizing the runo transcript (Figure 3E). This e ect could be observed both in the absence and presence of the NTS (Figure 3E). This result demonstrates that the stimulatory properties of the F/E complex strictly depend on its association with the RNAP. We have previously generated F/E variants that are defective in RNA binding in vitro but do not a ect their stability and incorporation into RNAP [(4) and data not shown]. We compared the activities of three mutant variants of F/E, F/E^{K33E}, F/E^{R37E} and F/E^{loop} (a subunit E triple mutant E$^{R155A, K156S, R157A}$) in RNA binding and transcription elongation assays. Both assays we02ect*T c-264.se

stimulating the formation of the runo transcript ~5-fold at its highest concentration (Figure 4D). These results demonstrate that the molecular mechanisms by RNAP subunits F/E contribute to the processivity have two components. The main component is dependent on the



termination, we examined the elongation pro“les of templates harbouring stretches of seven A instead of T residues in the NTS, resulting in rA:dT RNA…DNA hybrids. The A_7-signals at +53 and +66 results in 90% and 100% read-through, respectively (Figure 5A). The results demonstrate that a weakening of the RNA…DNA hybrid by inserting an A_7-signal is not su cient to facilitate robust transcription termination, even though it is important to keep in mind that the interactions between rU:dA basepairs are less stable than rA:dU basepairs (33).

E ciency of transcription termination correlates with the number of U-residues

In order to test whether the number of U-residues in the terminator signal correlated with termination e ciency and read through, we reduced the number of T residues

not able to detect a stimulatory e ect of the homologous

respectively (6,7,25). Here we demonstrate that the F/E complex, and by inference RPB4/7, is involved in the molecular mechanisms of RNAP during transcription elongation and termination. We attribute the main function of F/E to its ability to interact with the RNA transcript, but also propose that the interaction between F/E and the RNAP core leads to conformational changes within latter that modulate its DNA binding and possibly catalytic properties. F/E-like complexes are characteristic for all eukaryotic classes of RNAP (I…V) and archaeal enzymes, but they have no homologues in bacterial RNAP. What insights can we gain into overall di erences or similarities between the molecular mechanisms of bacterial and archaeo-eukaryotic RNAP, the way their respective genes are transcribed and how this process is regulated? In the context of transcription elongation it is tempting to speculate that F/E-like complexes facilitate a higher processivity and thereby enable RNAPs to transcribe longer genes, even though that is only relevant for eukaryotic, and not archaeal genes. It is noteworthy that the properties of bacteriophage antitermination factors, such as lambda Q, are reminiscent of the F/E complex. Even though Q cannot directly bind RNA, Q interacts with the beta-”ap region of RNAP, which is in close proximity to the RNA-exit channel (47). Association of Q with RNAP increases its processivity and alters transcription termination patterns with the overall outcome of facilitating and thereby regulating the expression of the late genes of phage lambda (48). The RNAP subunits F/E and RPB4/7 function on a global scale, whereas Q is an operon-speci“c transcription factor. However, both modulate their cognate RNAP to achieve an increased processivity.

SUPPLEMENTARY DATA

Supplementary Data arTJ Toctor.cnof RNAPthei-497.5manu(F/E)-essivity.FUNDINGNTARY DATA