Role of the σ^{54} Activator Interacting Domain in Bacterial Transcription Initiation

Alexander R. Siegel[1] **and David E. Wemmer**[1,2]

*1 - **Biophysics Graduate Group,** University of California, Berkeley, CA 94720, USA*
*2 - **Department of Chemistry,** University of California, Berkeley, CA 94720, USA*

***Correspondence to David E. Wemmer:** 508C Stanley Hall #3220, 1 Hearst Mining Circle, University of California, Berkeley, CA 94720-3220, USA. dewemmer@berkeley.edu*
http://dx.doi.org/10.1016/j.jmb.2016.10.007
Edited by M.F. Summers

Abstract

Bacterial sigma factors are subunits of RNA polymerase that direct the holoenzyme to specific sets of promoters in the genome and are a central element of regulating transcription. Most polymerase holoenzymes open the promoter and initiate transcription rapidly after binding. However, polymerase containing the members of the σ^{54} family must be acted on by a transcriptional activator before DNA opening and initiation occur. A key domain in these transcriptional activators forms a hexameric AAA+ ATPase that acts through conformational changes brought on by ATP hydrolysis. Contacts between the transcriptional activator and σ^{54} are primarily made through an N-terminal σ^{54} activator interacting domain (AID). To better understand this mechanism of bacterial transcription initiation, we characterized the σ^{54} AID by NMR spectroscopy and other biophysical methods and show that it is an intrinsically disordered domain in σ^{54} alone. We identified a minimal construct of the *Aquifex aeolicus* σ^{54} AID that consists of two predicted helices and retains native-like binding affinity for the transcriptional activator NtrC1. Using the NtrC1 ATPase domain, bound with the non-hydrolyzable ATP analog ADP-beryllium fluoride, we studied the NtrC1–σ^{54} AID complex using NMR spectroscopy. We show that the σ^{54} AID becomes structured after associating with the core loops of the transcriptional activators in their ATP state and that the primary site of the interaction is the first predicted helix. Understanding this complex, formed as the first step toward initiation, will help unravel the mechanism of σ^{54} bacterial transcription initiation.

Introduction

The five subunits of the bacterial "core" RNA polymerase (RNAP), α, α, β, β′, and ω, are sufficient for transcribing mRNA once the promoter has been opened. However, in order to recognize promoter sequences, "melt" the promoter DNA, and initiate transcription, the core RNAP requires an additional, modular subunit, the sigma factor [1]. The sigma factors bind to the core RNAP, forming the RNAP holoenzyme, and bind the sequence specifically to DNA in the promoter region, with different sigma factors targeting different subsets of genes to accomplish differential transcriptional regulation [2]. For many sigma factors, the regulation occurs by controlling the formation of the promoter–holoenzyme complex, either through anti-sigma proteins that compete with polymerase for a particular sigma factor [3] or through repressors that block the promoter [4]. Once the RNAP holoenzyme–promoter complex forms, the sigma factors help open DNA and initiate transcription. After initiation, the sigma factor can disassociate from the complex and the core RNAP can continue to transcribe mRNA using the single-stranded DNA template [5,6] (Fig. 1).

Sigma factors fall into two broad families that share no sequence homology: the more common σ^{70} family and the rarer σ^{54} family [7,8]. All sigma factors serve the same purpose in directing RNAP to specific promoters, but they differ in their mechanism of action and regulation. All sigma factors bind to core RNAP to form a holoenzyme, and all bind the promoter regions slightly upstream from the transcription start site. σ^{70} RNAP holoenzyme is capable of opening the promoter

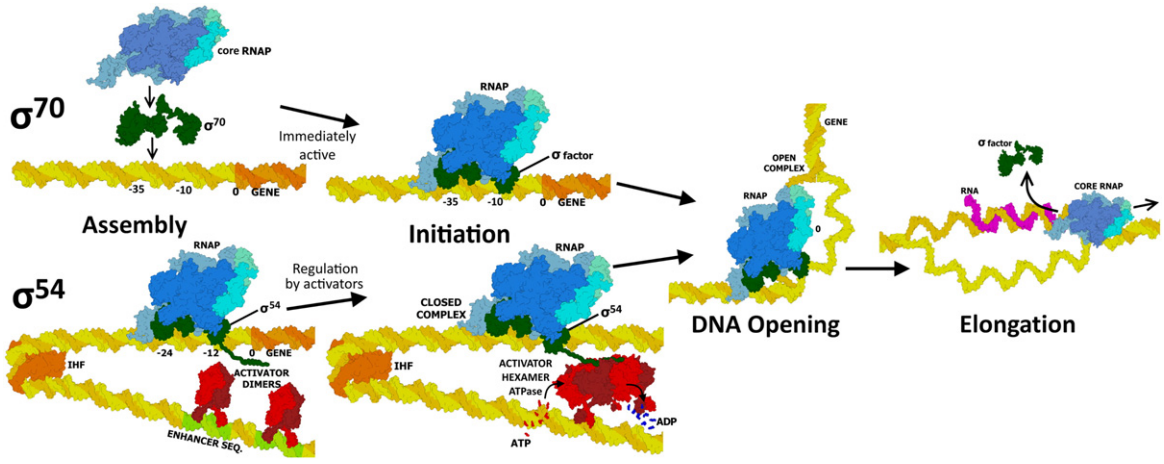

Fig. 1. Diagram of transcription initiation mediated by the two classes of bacterial sigma factors. (1) Assembly: sigma factor with RNAP binds upstream of the start site. (2) Initiation: σ^{70} is immediately able to initiate DNA opening, while σ^{54} requires an activation event from one of the transcriptional activators. (3) DNA opening: the RNAP holoenzyme melts DNA. (4) Elongation: the sigma factor can dissociate and the core RNAP continues to transcribe RNA using the single-stranded DNA template.

DNA and initiating transcription immediately after binding the promoter [9]. However, σ^{54} polymerase requires an additional activation step, a conformational change that is driven by a transcriptional activator, before it can open the promoter [7]. The σ^{54}–RNAP holoenzyme recognizes conserved sequences −24 and −12 bp upstream of the transcription start site [10], where it binds and awaits activation by a transcriptional activator that assembles further upstream [11]. The transcriptional activators themselves must be triggered, often in response to an environmental stimulus [12], after which they act on the σ^{54}–RNAP holoenzyme, which then transcribes the DNA for the encoded protein initiating gene expression [13]. The additional activation requirement affords genes under the control of σ^{54} an extra layer of control that both reduces background levels of transcription and gives a rapid cellular response when conditions are right. Consistent with this behavior, genes regulated by σ^{54} include those necessary for response to starvation and heat shock, among others [14]. The detailed mechanism by which these transcriptional activators reconfigure σ^{54} and the RNAP holoenzyme into a form capable of opening the DNA is not known.

The σ^{54} transcriptional activators typically have three functional domains: (1) an N-terminal regulatory domain that receives a signal and promotes the assembly of the active, hexameric form of the activator, (2) a central AAA+ ATPase domain that binds σ^{54} and hydrolyzes ATP, and (3) a C-terminal DNA binding domain (DBD) that binds to enhancer sequences well upstream of the site of DNA melting [15]. Regulatory domains are quite diverse [16,17,18], responding to different kinds of signals including phosphorylation of a receiver domain [19] or ligand binding by a GAF domain [20]. Regulation can be positive, for example, the phosphorylation of the receiver domain promoting the formation of the active hexamer ATPase, or negative, where the domain inhibits the formation of the hexamer until the signal is received. The central domain of the negatively regulated activators may oligomerize into an active conformation when expressed without its regulatory and DBDs, as is the case with the NtrC1 central domain construct ($NtrC1^{C}$) used in the experiments reported here [21].

Activated transcriptional activators assemble into hexameric rings with six ATP-binding sites, each at the cleft between subunits [19]. A highly conserved loop at the top of the central pore, with the sequence GAFTGA, has been shown to be involved in the interaction between the activator and σ^{54} [22,23]. Crystal structures show that the GAFTGA loop extends upward on subunits bound to ATP (or a non-hydrolyzable ATP analog) but retracts inward for subunits bound to ADP [19,24,25].

σ^{54} has several functional domains, two of which had structures determined as individual domains [26,27] (Fig. 2). The focus of the present work is the N-terminal ≈50 aa of σ^{54}, which are responsible for interacting with the assembled ATPase of the activator that we term the activator interacting domain (AID) and has also been called Region I [28]. This is followed by a variable length, low conservation linker and then the core binding domain (CBD), which consists of two subdomains, a four-helix and three-helix bundle, which dock together [27]. The CBD is a primary region of interaction with core RNAP subunits, making important contacts to the core polymerase to form the holoenzyme. The next domain, which we term the −12 DBD, interacts with DNA in the −12 region of the promoter where DNA opening occurs