[Cell Cycle 5:4, 399-404, 16 February 2006]; ©2006 Landes Bioscience

Review

Is it Easy to Stop RNA Polymerase?

Irina Artsimovitch[1]

Dmitry G. Vassylyev[2,*]

[1]Department of Microbiology; The Ohio State University; Columbus, Ohio USA

[2]Department of Biochemistry and Molecular Genetics; University of Alabama at Birmingham; Schools of Medicine and Dentistry; Birmingham, Alabama USA

*Correspondence to: Dmitry G. Vassylyev; Department of Biochemistry and Molecular Genetics; University of Alabama at Birmingham; Schools of Medicine and Dentistry; 402B Kaul Genetics Building; 720 20th Street South; Birmingham, Alabama 35294 USA; Tel.: 205.975.8136; Email: dmitry@uab.edu

Received 12/29/05; Accepted 12/30/05

Previously published online as a Cell Cycle E-publication:
http://www.landesbioscience.com/journals/cc/abstract.php?id=2466

KEY WORDS

RNA polymerase, transcription, antibiotics, catalytic center, crystal structure, rifamycin, abortive initiation

ABSTRACT

Among transcription factors that bind to bacterial RNA polymerase (RNAP) and modulate its activity, a number of small molecules irreversibly inhibit RNAP thereby causing cell death. To be of clinical significance such inhibitors must (1) inhibit a broad range of bacterial RNAPs but not affect human cells, (2) penetrate bacterial cell walls and (3) circumvent bacterial resistance mechanisms. Rifamycins, the only class of RNAP inhibitors that have found their way into clinical practice, are widely used in the treatment of tuberculosis and leprosy. However, the practical value of this class of antibiotics is limited by a rapid rise in resistant bacterial isolates. In this review we focus on recent advances in studies of prokaryotic transcription that allow a detailed structural and functional characterization of a number of RNAP/rifamycins complexes, thereby opening new opportunities for the design of superior antibacterial agents.

INTRODUCTION

The mechanism of RNA synthesis from a DNA template is highly conserved in all living cells and can be roughly divided into three distinct phases: initiation, elongation, and termination. To initiate transcription in a promoter-specific fashion in bacteria, the RNAP core enzyme (a complex of five subunits; α_2, β, β' and ω) binds an additional subunit, initiation factor σ, to form a holoenzyme (molecular mass of ~450 kDa). Upon binding to the promoter, the holoenzyme unwinds ~13–15 base pairs (bp) of the DNA template immediately upstream of and including the start site, to form an open complex[1] and initiates RNA synthesis de novo using nucleoside triphosphates (NTPs) as both the primer and the substrate (in contrast, DNA polymerases extend a preexisting primer). The nucleotide addition reaction is thought to conform to a universal two-metal mechanism of catalysis,[2] wherein one of the catalytic Mg^{2+} ions remains tightly bound to RNAP while the other is delivered by the triphosphate moiety of each incoming NTP.

During initiation the transcription complex is unstable, and the RNAP is involved in "abortive cycling" in which it repeatedly synthesizes and releases short (3–12 nt) RNA products.[3] Once the transcript reaches the length of ~13–15 nt (of which 8–9 nt are base paired with the DNA template, forming an RNA/DNA hybrid within the RNAP catalytic cavity;[4,5] the complex undergoes a transition to a stable, highly processive elongation phase. This is accompanied by the loss of promoter contacts and (at least in some complexes) σ-factor release.[3,6] During elongation, RNAP carries out thousands of nucleotide addition cycles while maintaining stable contacts with the template and the nascent transcript. The formation of a nascent RNA hairpin or the helicase activity of the termination factor Rho triggers dissociation of the transcription complex,[7] demarcating transcription termination and allowing the released core RNAP to bind a σ specificity factor and initiate a new round of RNA synthesis.

As it is an essential enzyme, RNAP provides an attractive target for drug design, and considerable efforts have been expended to obtain and characterize the mechanism of action of bacterial RNAP inhibitors. Among these inhibitors, only rifamycins (Rifs), which display a broad spectrum of antibiotic activity against Gram-negative and Gram-positive bacteria, are widely used in clinical practice, where they retain the leading position in the treatment of tuberculosis. Rifs bind to RNAP with high affinity and block RNA synthesis beyond 3-4 nt during initiation,[8] but have no effect on elongation complexes. All Rifs contain an aromatic moiety bridged by an aliphatic chain (ansa ring) and differ from each other by the configuration of the "tails" attached to the ansa ring at either the C3 or C3/C4 positions (Fig. 1). Based on the type of the tail attachment Rifs can be divided

in the two classes, as the C3 and C3/C4 compounds demonstrate essentially distinct functional (Fig. 1A and see below) and structural (Fig. 1B) characteristics.

Since their discovery in 1959 as fermentation products of actinomycetes and the introduction of rifamycin SV into clinical use in 1960's, Rifs have been a subject of numerous studies, and hundreds of synthetic and semi-synthetic derivatives were made in the pursuit of an "ideal antibiotic" (see ref. 9). Early on it was discovered that most alterations in the ansa ring result in the loss of Rif activity, and efforts were therefore diverted to the modification of the tail component.[10] The latter modifications improved the antibacterial properties but failed to address the major problem of resistance to Rifs, which arises from point mutations in *rpoB*, the gene that encodes the β subunit of RNAP.[11] Moreover, a wealth of information yielded by combinatorial chemistry failed to produce any robust rules for rifamycin design. The latest Rif compound that was hailed as a new "wonder drug," rifalazil,[12] exhibited severe side-effects in clinical trials and was abandoned.[13] Thus, in spite of numerous efforts, the classic combinatorial approach failed to produce the "perfect" Rif, underscoring the urgent need for unraveling the molecular mechanisms of Rif action and the bacterial resistance to these drugs at the atomic level.

Toward this goal, determination of the crystal structures of the bacterial RNAP core and holoenzymes complexed with three clinically important Rifs[14,15] pave the way for the structure-guided design of new Rifs that will be immune to the bacterial resistance mechanisms. These studies not only shed light on the molecular mechanisms of Rif action providing a basis for medical applications, they also suggest that the certain elements of the Rif inhibitory mechanism might be a common theme in transcription regulation. In this article we review the recent studies of the RNAP/Rifs complexes focusing on those mechanistic aspects that open up new opportunities for drug design and allow for better understanding of the general principles of transcription.

MECHANISM OF THE RIFAMYCINS' ACTION

The structure of the *Thermus aquaticus* RNAP core enzyme in complex with rifampicin (RFP) revealed that RFP binds in the path of the nascent RNA/DNA hybrid exclusively through the interactions of the ansa ring with the cluster of the β-subunit residues, whereas the tail moiety lacks contacts with the protein.[15] These structural considerations led to the proposal of an attractively simple, steric mechanism of action, in which the Rif backbone and tails clash with and intercalate into the upstream base pair of the 3–4 bp long RNA/DNA hybrid thereby facilitating its dissociation (Fig. 2). The steric model, however, did not account for several experimental observations, the most striking of which was the existence of β-subunit amino acid substitutions in and near the binding cavity for the ansa ring (common for all Rifs) and far away from the "variable" tail moieties that conferred resistance to RFP (belonging to the C3-class) but remained sensitive to the C3/C4 compounds (Wichelhaus et al., 2001; Williams et al., 1998) (Fig. 1A). Together with other experimental data,[16] these observations suggested that the actual mechanism of the Rif action might be more complex.

Indeed, further structural and functional studies of two other clinically important Rifs, rifapentin and rifabutin (representing C3 and C3/4 Rif classes; Fig. 1), bound to their natural target, a bacterial RNAP holoenzyme, suggested that an allosteric modulation of the RNAP active center constitutes an essential component of the Rif

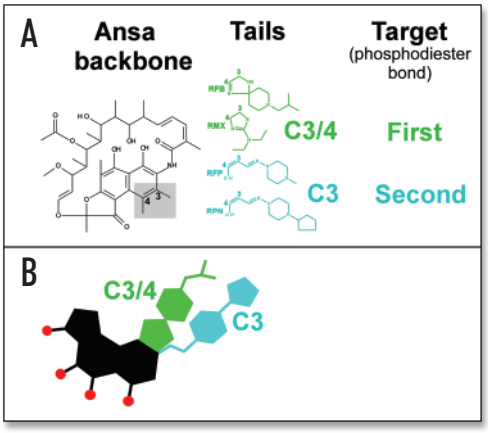

Figure 1. Two classes of rifamycins. (A) Rifs can be divided into two classes based on their structures and effects on transcription. The invariant ansa backbone (naphtohydroquinonic chromophore spanned by an aliphatic bridge, left) contains tails attached to either C3 (blue: RFP, rifampicin; RPN, rifapentin) or C3/C4 (green: RFB, rifabutin; RMX, rifamexyl) positions. The mode of tail attachment apparently determines the target of Rif action: the C3 Rifs inhibit the second phosphodiester bond formation, whereas the C3/4 compounds inhibit even the first bond formation. (B) The schematic representation of Rifs. The backbone is shown in black with four oxygen atoms (red circles) that make crucial contacts to β subunit residues, and tails attached to C3 and C3/4 positions.

inhibitory mechanism.[14] The results demonstrated that binding of Rif induces an allosteric signal that propagates over 19Å from the Rif-binding pocket to the enzyme's active site, where it decreases affinity of the RNAP to the major catalytic Mg^{2+} ion, thereby slowing down the catalytic reaction and subsequently facilitating the dissociation of short, unstable RNA/DNA hybrids. Two independent signal transduction pathways were identified (β and σ, Fig. 3). The β-pathway targets the second (if transcription is initiated in vitro from a di-nucleotide) phosphodiester bond formation and is likely activated by the interactions of the ansa-ring with β-subunit residues (Fig. 3A). These interactions are identical among all Rifs, and the β-pathway is independent of the tail configuration. By contrast, the σ-pathway affects the formation of the first phosphodiester bond and relies largely on the contacts between the Rif tail and the σ-subunit (more specifically with the tip of the σ hairpin loop, σHL), which are made only by C3/C4-substituted Rifs (Fig. 3B). The σ-pathway is thus a unique characteristic of the C3/C4 compounds and likely accounts for the functional differences between the two classes of the antibiotics. In particular, it explains the differential resistance of the β-subunit substitutions which, while disrupting the β-pathway and conferring resistance to C3 Rifs, leave the σ-pathway intact, thereby retaining sensitivity to C3/C4 Rifs. The two-pathway allosteric mechanism is in good agreement with the bulk of the experimental data accumulated over nearly half a century and thus represents a good starting point for the design of novel, more effective Rif-like antibiotics.

OVERCOMING BACTERIAL RESISTANCE TO Rifs

Based on structural, genetic and biochemical analyses, the numerous substitutions in RNAP that confer resistance to Rifs can be divided into three categories: (1) the "steric," bulky substitutions that reduce the space in the Rif binding site and preclude antibiotic binding through steric hindrance, (2) the "affinity" substitutions,

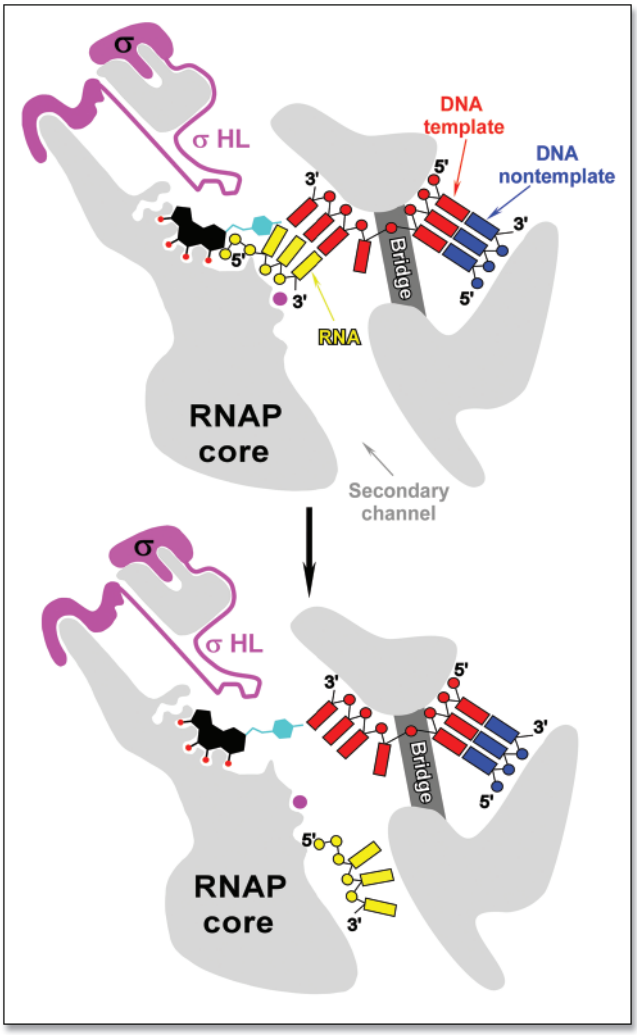

Figure 2. The steric model for Rif action. The transcription initiation complex is composed of core RNAP (light gray, with the catalytic bridge helix shown in dark gray), initiation factor σ (magenta, with the flexible hairpin loop, σHL that extends toward the RNA/DNA hybrid), promoter DNA (with the template strand in red and the nontemplate strand in dark blue), and the nascent RNA (yellow, paired to the template DNA to form the RNA/DNA hybrid) with the triphosphate at the 5' end and the 3' OH positioned in the enzyme's active site (marked with the high-affinity catalytic Mg^{2+} ion, magenta circle). Upon binding, Rif clashes with the upstream base pair of the 3-bp long RNA/DNA hybrid thereby facilitating its dissociation[15] through the RNAP secondary channel. The catalytic Mg^{2+} ion remains bound in the active site.

which in many cases create more space in the binding cavity but lead to a loss of the crucial polar and van der Waals interactions with the Rif backbone, thereby dramatically decreasing its binding affinity; and (iii) the "allosteric" substitutions, which do not significantly reduce Rif binding, but likely disrupt transmission of the allosteric signal[14] (Fig. 4). Single substitutions of three residues (βSer456, βHis451, and βAsp441) that together account for more than 85% of the Rif-resistant clinical isolates of *Mycobacterium tuberculosis* represent all three categories, underscoring the importance of each resistance mechanism for the in vivo response of bacteria to antibiotic treatment. Improved, more efficient Rif analogs should therefore possess structural and chemical properties that would allow them to neutralize the effects of all types of resistant substitutions.

Most of the "steric" RNAP substitutions target the rigid portion of the Rif ansa backbone which consists of the three consecutive planar rings (Fig. 4A, left). Any bulky substitution in ansa ring binding site would clash with this highly inflexible Rif substructure and dramatically reduce overall Rif affinity, since this group contains several important binding determinants. One obvious possibility to weaken Rif resistance of the "steric" variants would be the design of a "rubber" compound through the replacement of the planar rings with a more flexible backbone, which would allow conformational alterations in Rif to circumvent the bulky RNAP substitutions without substantial repositioning of the other Rif binding determinants (Fig. 4A, right). Indeed, the structural and biochemical analysis of sorangicin, which interacts with RNAP similarly to Rif but is missing the three planar rings in its backbone, demonstrated that clinically relevant "steric" Rif-resistant RNAP variants remained sensitive to sorangicin due to the higher flexibility of its backbone.[17] However, some other "steric," as well as nearly all of the "affinity and "allosteric" RNAP mutants (including substitutions of the βHis451 and βAsp441 residues), remained resistant to both antibiotics, indicating that more flexibility in the Rif-like inhibitors is required to overcome the effect of the "steric" mutations on one hand, and that the increased flexibility alone would not suffice to overcome Rif resistance in bacterial populations on the other.

Rifs are tightly bound to RNAP through an extensive network of polar and van der Waals interactions. The "affinity" substitutions in RNAP not only directly disrupt the crucial contacts with the respec-

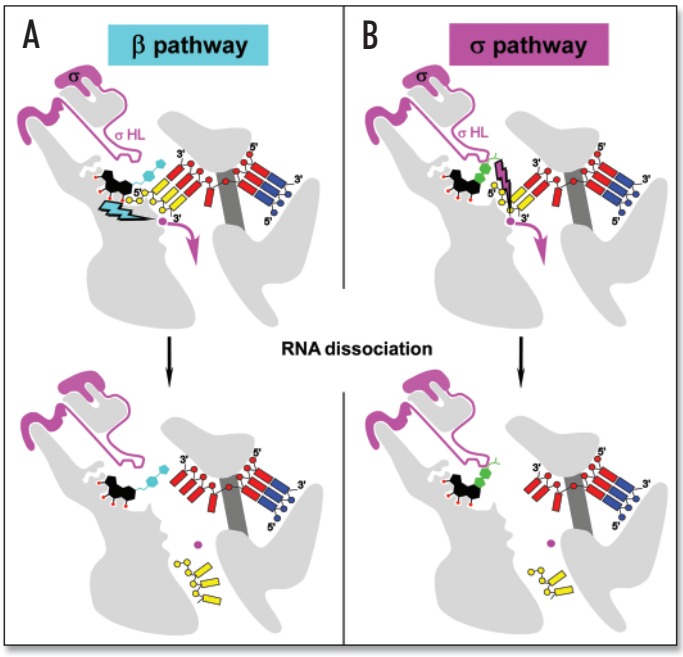

Figure 3. Two-pathway mechanism of Rif action. (A) The β pathway (blue) is activated by interactions between the ansa ring and the β subunit residues. An allosteric signal (blue lightning bolt) generated through this pathway is transmitted through the β subunit to the active site, where it inhibits the second phosphodiester bond formation. (B) The σ pathway (magenta lightning bolt), which is activated through contacts between the C3/4 tail and the tip of the σHL, acts at an earlier step inhibiting the formation of the first phosphodiester bond. Both pathways trigger release of the catalytic Mg^{2+} ion (bent magenta arrow) and dissociation of the short, unstably bound RNAs from the initiation complex.

tive Rif-binding determinants, but may also alter the shape of the binding cavity and conformations of other Rif-interacting side chains, thereby disturbing the entire network of RNAP/Rif contacts and weakening antibiotic binding (Fig. 4B, left). To overcome the effects of these substitutions, new Rif derivatives equipped with additional functional groups that will establish new contacts with RNAP in place of the lost ones need to be designed (Fig. 4B, right). An analysis of the high-resolution RNAP/Rifs complex structures suggests several possible Rif modifications involving the addition of groups targeting the RNAP grooves adjacent to the Rif binding site lined with either the main chain atoms or hydrophobic side chains. These interactions would be largely independent of the side chain configurations, thereby circumventing the potential "affinity" RNAP substitutions. The Rif binding cavity constitutes a portion of the binding site for the RNA/DNA hybrid, a critical determinant for elongation complex stability and processivity,[18,19] and substitutions in this region have been shown to confer various transcriptional defects.[20-23] Moreover, substitutions of the critical residues would likely be lethal; introducing interaction counterparts of these determinants into the Rif structure would minimize the emergence of the viable Rif-resistant mutants.

At a first glance, resistance due to "allosteric" substitutions, which do not significantly decrease Rif binding affinity but rather disrupt transmission of an allosteric signal, should prove difficult to ameliorate through modifications of the Rif structure (Fig. 4C, left). However, identification of the two independent (β and σ) signal transduction pathways and the subsequent analysis of their effects on transcription suggest a few promising possibilities. The β-pathway encompasses many residues in the β-subunit core that extensively interact with each other and with the Rif backbone. Therefore a substitution that even slightly disturbs the optimal interaction network may obstruct the propagation of the allosteric signal. Such mutations are practically impossible to predict and to avoid (Fig. 4C, left). In contrast, the σ-pathway is likely activated in response to only a few, not highly specific contacts of the Rif tails with the σHL, which itself has no interactions with the rest of the protein. Assuming that the activation of the σ-pathway does not rely heavily on the sequence composition of the σHL, and given that there are several distinct indispensable σ-subunits in each bacteria (in which Rif-resistant mutations are unlikely to emerge simultaneously), the σ-mediated allosteric signal appears well protected against "allosteric" RNAP substitutions. In excellent agreement with this conclusion, no Rif resistant mutations have so far been isolated in the genes that encode σ-subunits. In contrast, all isolated "allosteric" mutations in RNAP cluster in the β-subunit interface and target exclusively the β-pathway, as revealed by their differential sensitivity to the C3 and C3/C4 inhibitors. Thus, Rif analogs with tails attached to the C3/C4 positions of the ansa-ring that would strengthen the contacts with the σHL may not only increase the antibiotic binding but might also afford better protection against resistance mechanisms (Fig. 4C, right).

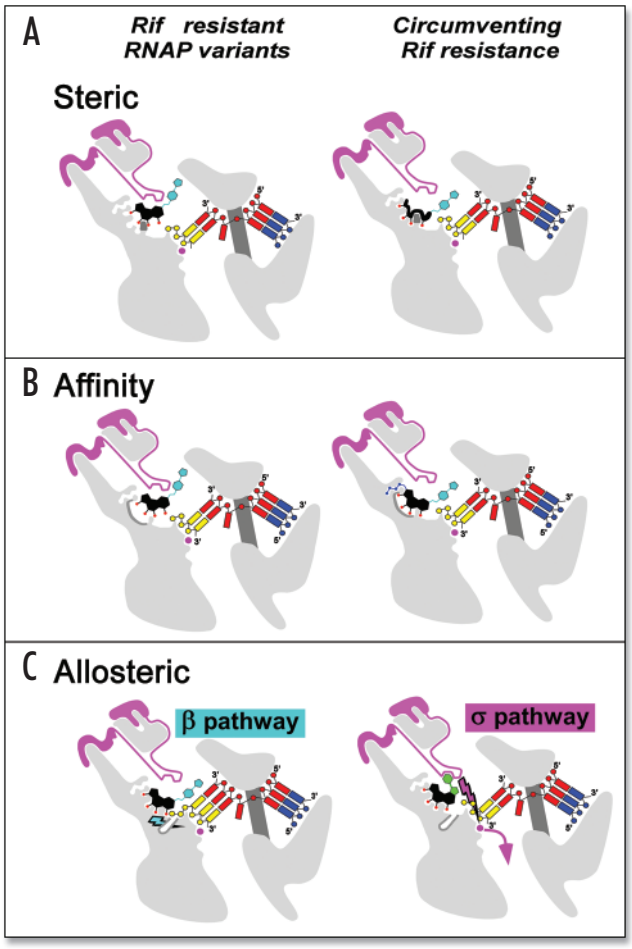

Figure 4. Approaches to circumvent resistance to Rifs through alterations of the antibiotic structure. (A) Left, *steric* substitutions (dark gray) hinder binding of the rigid Rif backbone to the core RNAP. Right, a modified "rubber" backbone allows for an elastic deformation of the Rif-RNAP contact network and restores the antibiotic affinity for the enzyme. (B) Left, *affinity* substitutions (dark gray) remove the crucial contacts between RNAP and Rif. Right, addition of a new functional group to the Rif backbone (in dark blue) would not only establish a set of new, side-chain independent contacts but also will strengthen the interactions weakened by substitutions. (C) Left, *allosteric* substitutions (dark gray) interrupt the β pathway. Right, a tail (in green) attached to the C3/C4 positions of the ansa-ring strengthens the contacts with the σHL thereby increasing Rif binding and possibly augmenting the σ pathway, while getting around the β pathway.

RIFAMYCINS AND ABORTIVE CYCLING

The abortive multi-round RNA synthesis during which the DNA template slides back and forth on RNAP that repeatedly synthesizes and releases the short RNA fragments remains one of the biggest mysteries of transcription. It is not immediately clear why RNAP spends time and wastes substrates on this seemingly futile process. Rifs stimulate abortive cycling, locking the enzyme in this initial phase of transcription suggesting that the mechanisms of Rif action and those of the abortive cycling may have a common origin.

Whereas the mechanism of abortive transcription does not appear to have anything in common with the previously proposed "steric" model of Rif action, a parallel could be drawn with the "allosteric" model: Rifs may simply amplify the inherent allosteric signals that occur during initiation in response to the interactions of the nascent RNA transcript with the corresponding structural RNAP elements, thereby slowing down catalysis and resulting in a spontaneous release of short abortive products.

An analysis of the patterns of abortive transcription obtained on different promoters reveals existence of three major classes of abortive products (namely 3, 6–8 and 13–15 nt long).[24-26] The 13–15 nt long abortive transcripts are detected at a subset of promoters, where they likely arise from specific interactions of the 5'-terminus of RNA with RNAP and/or promoter DNA elements,[24]

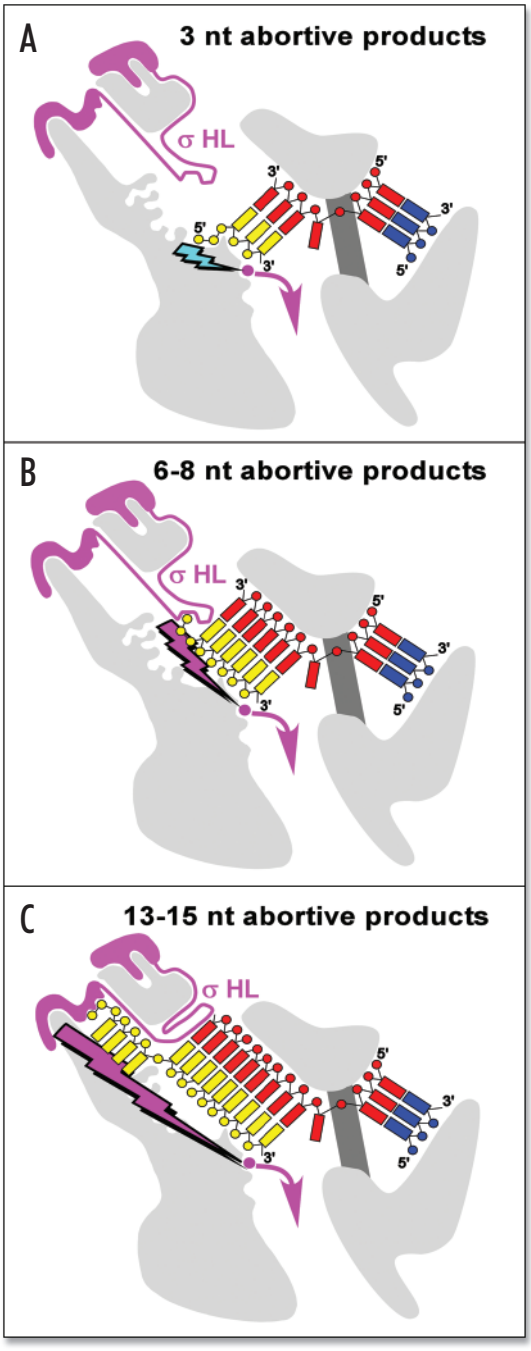

Figure 5. Hypothetical pathways of abortive initiation. (A) Interactions of the phosphates of the 5'-terminal RNA nt in the Rif binding pocket "switch on" the β-pathway even in the absence of Rif, leading to abortive synthesis of 3 nt products. (B) Once the transcript reaches 6-8 nt in length, the RNA/DNA interacts with the σHL similarly to the C3/C4 Rif tails, thereby activating the σ-mediated allosteric signal and favoring release of the RNA products. (C) Particular promoter sequences may favor interactions between the 5' terminus of the 13–15 nt long RNA and the DNA or the enzyme, triggering an allosteric signal (such as a σ-mediated pathway shown or an alternative pathway) that ultimately leads to release of the abortive RNAs.

far away from the Rif binding site. The excessive production of the other, 3 and 6-8 nt long, products are in excellent agreement with activation of the β- and σ-mediated signal transduction pathways proposed on the basis of Rif studies. Indeed, the putative interactions of the phosphates of the 5'-terminal RNA nt in the Rif binding pocket that likely occur prior to formation of the second phosphodiester bond (3 nt long RNA), were proposed to "switch on" the β-pathway;[14] to a lesser extent, the same effect might be achieved in the absence of the inhibitor, producing abundant 3 nt products (Fig. 5A). On the other hand, molecular modeling shows that at a length of ~6–8 nt (but not shorter) the RNA/DNA hybrid would interact with the σHL, thereby mimicking the contacts of the C3/C4 Rif tails and presumably activating the σ-mediated allosteric signal (Fig. 5B). Following this line of argument, one might anticipate that the 5' terminus of the 13–15 nt long RNA transcript interacting with the enzyme and/or upstream promoter DNA may close the circuit of a third, yet unknown allosteric signal (Fig. 5C).

What is the functional role of the abortive RNA synthesis? RNAP readily dissociates from the nucleic acids in the transcription complexes only during initiation (open complexes) or in response to a termination signals culminating the transcription cycle, whereas the elongation complexes, once formed, are remarkably stable. However, the high stability of the elongation complexes is advantageous only if coupled with high processivity, allowing for rapid completion of the RNA synthesis; stable but stalled elongation complexes would block the progression of other macromolecular machines (such as DNA polymerases) along the DNA and thus kill the cell. For example, transcription-coupled DNA repair depends on the accessory proteins that destabilize halted transcription complexes and thus remove them from the site of a DNA lesion to provide access for the repair machinery.[27,28]

The high processivity of the transcription complex likely relies on the optimal configuration of the RNAP main (active site) cavity accommodating the nucleic acid chains; this state might not be achieved immediately upon the formation of the apo-holoenzyme (given the apparent flexibility of its multi-subunit and multi-domain architecture) and may require a "minimal" fragment of the nascent RNA. We speculate that abortive cycling might serve to exclude inactive RNAP conformers that form perpetually abortive, or "moribund," complexes[29] on one hand, and for the "polishing" of the interior of promising, potentially active RNAP molecules on the other. The optimal, highly processive RNAP state could presumably be achieved through iteratively induced gradual alterations in the protein structure, rather than through an abrupt, one-step transition. In this scenario, the moving nucleic acids would play an active, chaperone-like role in changing the enzyme conformation through dynamic interactions with the protein segments lying in the way of the growing RNA chain. The allosteric signals arising from these interactions at different stages of transcription initiation would serve to monitor the progress and provide feedback in the optimization of the RNAP structure. In fact, transcription initiation carried out by a single-subunit phage T7 RNAP (which is also characterized by abortive synthesis) provides a brilliant illustration of such a "chaperoning" function of the nucleic acids; transition to a stable elongation complex results in unprecedented rearrangement of the RNAP active cavity (involving a major refolding of the protein structure) from an initial state, in which the RNAP may accommodate only a 3 bp RNA/DNA hybrid[30] to a final processive conformation that buries 8-9 bp of the hybrid.[31,32] Interestingly, the nucleotide-induced refolding of the T7 enzyme has been also proposed to be a gradual, multi-step process, with possibly several structural intermediates whose conformations should be likely optimized during initiation to achieve high processivity of the elongation complex. Thus the abortive products in T7 RNAP transcription might also originate from an allosteric signaling, rather than from a steric competition

between the RNA and protein playing essentially the same role as we hypothesized for multi-subunit RNAPs.

CONCLUDING REMARKS

Structural studies of the multi-subunit RNAPs from bacterial and eukaryotic cells[33] have illuminated the presence of large, solvent-accessible surfaces with numerous functionally crucial cavities and channels, the blocking of which would likely inhibit transcription. In spite of the relatively high overall sequence and structural homology between the bacterial and eukaryotic enzymes, many of these structural elements differ substantially in shape and amino acid composition, thereby allowing for the development of a wide spectrum of inhibitors that would specifically target bacterial but not the host RNAP. However, no new RNAP inhibitors have been introduced into clinical practice during the last decade. In the past few years, the growing demand for novel antibiotics has led to several studies of transcriptional inhibitors that revealed the distinct binding sites on RNAP and a great variety of the inhibitory mechanisms.[14,15,17,34-40] Although none of these compounds meets all three criteria of the "ideal" antibiotic marked in the abstract of this article, those compounds for which the high-resolution structures of their complexes with RNAP have been solved (Rifs, sorangicin, streptolydigin, and tagetitoxin) are of special interest since a structurebased analysis might suggest their specific modifications to allow the creation of new inhibitors with the improved anti-bacterial properties in a relatively short time.

Another important conclusion that could be drawn from recent studies of the RNAP inhibitors is that although most of them possess unique mechanisms of action, they share one important functional feature: they are unable to stop RNAP (a powerful molecular motor capable of transcribing against a large (~25 pN) applied force),[41] directly through steric hindrance of the moving parts of the "motor" (the nucleic acids), but rather utilize an indirect approach, limiting the access of the "fuel" (the Mg^{2+} ions and substrates) to the "engine" (the RNAP active site). Such indirect modulation of the RNAP catalytic center may in fact represent a common theme in transcription regulation.

References

1. Record Jr M, Reznikoff W, Craig M, McQuade K, Schlax P. *Escherichia coli* RNA polymerase ($E\sigma^{70}$), promoters, and the kinetics of the steps of transcription initiation. In: Umbarger H, ed. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. 2nd ed. Washington, DC: ASM Press, 1996:792-821.
2. Steitz TA, Steitz JA. A general two-metal-ion mechanism for catalytic RNA. Proc Natl Acad Sci USA 1993; 90:6498-502.
3. Hsu LM. Promoter clearance and escape in prokaryotes. Biochim Biophys Acta 2002; 1577:191-207.
4. Nudler E, Gusarov I, Avetissova E, Kozlov M, Goldfarb A. Spatial organization of transcription elongation complex in *Escherichia coli*. Science 1998; 281:424-8.
5. Komissarova N, Kashlev M. Functional topography of nascent RNA in elongation intermediates of RNA polymerase. Proc Natl Acad Sci USA 1998; 95:14699-704.
6. Mooney RA, Darst SA, Landick R. Sigma and RNA polymerase: An on-again, off-again relationship? Mol Cell 2005; 20:335-45.
7. Richardson J, Greenblatt J. Control of RNA chain elongation and termination. In: Neidhardt F, Curtiss IIIrd R, Ingraham J, Lin E, Low K, Magasanik B, Reznikoff W, Riley M, Schaechter M, Umbarger H, eds. *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. Washington, DC: ASM Press, 1996:822-48.
8. McClure WR, Cech CL. On the mechanism of rifampicin inhibition of RNA synthesis. J Biol Chem 1978; 253:8949-56.
9. Floss HG, Yu TW. Rifamycin-mode of action, resistance, and biosynthesis. Chem Rev 2005; 105:621-32.
10. Riva S, Silvestri LG. Rifamycins: A general view. Annu Rev Microbiol 1972; 26:199-224.
11. Rabussay D, Zillig W. A rifampicin resistent RNA-polymerase from *E. coli* altered in the beta-subunit. FEBS Lett 1969; 5:104-06.
12. Saito H, Tomioka H, Sato K, Emori M, Yamane T, Yamashita K, Hosoe K, Hidaka T. In vitro antimycobacterial activities of newly synthesized benzoxazinorifamycins. Antimicrob Agents Chemother 1991; 35:542-7.
13. Hudson A, Imamura T, Gutteridge W, Kanyok T, Nunn P. WHO Report, 2003.
14. Artsimovitch I, Vassylyeva MN, Svetlov D, Svetlov V, Perederina A, Igarashi N, Matsugaki N, Wakatsuki S, Tahirov TH, Vassylyev DG. Allosteric modulation of the RNA polymerase catalytic reaction is an essential component of transcription control by rifamycins. Cell 2005; 122:351-63.
15. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell 2001; 104:901-12.
16. Altmann CR, Solow-Codero DE, Chamberlin M. RNA cleavage and chain elongation by *Escherichia coli* DNA-dependent RNA polymerase in a binary enzyme:RNA complex. Proc Natl Acad Sci USA 1994; 91:3784-88.
17. Campbell EA, Pavlova O, Zenkin N, Leon F, Irschik H, Jansen R, Severinov K, Darst SA. Structural, functional, and genetic analysis of sorangicin inhibition of bacterial RNA polymerase. EMBO J 2005; 24:674-82.
18. Kireeva ML, Komissarova N, Waugh DS, Kashlev M. The 8-nucleotide-long RNA:DNA hybrid is a primary stability determinant of the *RNA* polymerase II elongation complex. J Biol Chem 2000; 275:6530-6.
19. Nudler E, Mustaev A, Lukhtanov E, Goldfarb A. The RNA:DNA hybrid maintains the register of transcription by preventing backtracking of *RNA* polymerase. Cell 1997; 89:33-41.
20. Fisher RF, Yanofsky C. Mutations of the beta subunit of *RNA* polymerase alter both transcription pausing and transcription termination in the trp operon leader region in vitro. J Biol Chem 1983; 258:8146-50.
21. Jin DJ, Walter WA, Gross CA. Characterization of the termination phenotypes of *rifampicin*-resistant mutants. J Mol Biol 1988; 202:245-53.
22. McDowell JC, Roberts JW, Jin DJ, Gross C. Determination of intrinsic transcription termination efficiency by *RNA* polymerase elongation rate. Science 1994; 266:822-25.
23. Zhou YN, Jin DJ. The *rpoB* mutants destabilizing initiation complexes at stringently controlled promoters behave like "stringent" *RNA* polymerases in *Escherichia coli*. Proc Natl Acad Sci USA 1998; 95:2908-13.
24. Vo NV, Hsu LM, Kane CM, Chamberlin MJ. In vitro studies of transcript initiation by *Escherichia coli RNA* polymerase. 3. Influences of individual DNA elements within the promoter recognition region on abortive initiation and promoter escape. Biochemistry 2003; 42:3798-811.
25. Vo NV, Hsu LM, Kane CM, Chamberlin MJ. In vitro studies of transcript initiation by *Escherichia coli RNA* polymerase. 2. Formation and characterization of two distinct classes of initial transcribing complexes. Biochemistry 2003; 42:3787-97.
26. Hsu LM, Vo NV, Kane CM, Chamberlin MJ. In vitro studies of transcript initiation by *Escherichia coli RNA* polymerase. 1. RNA chain initiation, abortive initiation, and promoter escape at three bacteriophage promoters. Biochemistry 2003; 42:3777-86.
27. Roberts J, Park JS. *Mfd*, the bacterial transcription repair coupling factor: Translocation, repair and termination. Curr Opin Microbiol 2004; 7:120-5.
28. Trautinger BW, Jaktaji RP, Rusakova E, Lloyd RG. *RNA* polymerase modulators and DNA repair activities resolve conflicts between DNA replication and transcription. Mol Cell 2005; 19:247-58.
29. Sen R, Nagai H, Shimamoto N. Polymerase arrest at the lambdaP(R) promoter during transcription initiation. J Biol Chem 2000; 275:10899-904.
30. Cheetham GM, Steitz TA. Structure of a transcribing T7 *RNA* polymerase initiation complex. Science 1999; 286:2305-9.
31. Yin YW, Steitz TA. Structural basis for the transition from initiation to elongation transcription in T7 *RNA* polymerase. Science 2002; 298:1387-95.
32. Tahirov TH, Temiakov D, Anikin M, Patlan V, McAllister WT, Vassylyev DG, Yokoyama S. Structure of a T7 *RNA* polymerase elongation complex at 2.9 A resolution. Nature 2002; 420:43-50.
33. Cramer P. Multisubunit *RNA* polymerases. Curr Opin Struct Biol 2002; 12:89-97.
34. Temiakov D, Zenkin N, Vassylyeva MN, Perederina A, Tahirov TH, Kashkina E, Savkina M, Zorov S, Nikiforov V, Igarashi N, Matsugaki N, Wakatsuki S, Severinov K, Vassylyev DG. Structural basis of transcription inhibition by antibiotic streptolydigin. Mol Cell 2005; 19:655-66.
35. Tuske S, Sarafianos SG, Wang X, Hudson B, Sineva E, Mukhopadhyay J, Birktoft JJ, Leroy O, Ismail S, Clark Jr AD, Dharia C, Napoli A, Laptenko O, Lee J, Borukhov S, Ebright RH, Arnold E. Inhibition of bacterial *RNA* polymerase by streptolydigin: Stabilization of a straight-bridge-helix active-center conformation. Cell 2005; 122:541-52.
36. Sarubbi E, Monti F, Corti E, Miele A, Selva E. Mode of action of the microbial metabolite GE23077, a novel potent and selective inhibitor of bacterial *RNA* polymerase. Eur J Biochem 2004; 271:3146-54.
37. Artsimovitch I, Chu C, Lynch AS, Landick R. A new class of bacterial *RNA* polymerase inhibitor affects nucleotide addition. Science 2003; 302:650-4.
38. Adelman K, Yuzenkova J, La Porta A, Zenkin N, Lee J, Lis JT, Borukhov S, Wang MD, Severinov K. Molecular mechanism of transcription inhibition by peptide antibiotic Microcin J25. Mol Cell 2004; 14:753-62.
39. Mukhopadhyay J, Sineva E, Knight J, Levy RM, Ebright RH. Antibacterial peptide microcin J25 inhibits transcription by binding within and obstructing the *RNA* polymerase secondary channel. Mol Cell 2004; 14:739-51.
40. Vassylyev DG, Svetlov V, Vassylyeva MN, Perederina A, Igarashi N, Matsugaki N, Wakatsuki S, Artsimovitch I. Structural basis for transcription inhibition by tagetitoxin. Nat Struct Mol Biol 2005; 12:1086-93.
41. Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, Block SM. Force and velocity measured for single molecules of *RNA* polymerase. Science 1998; 282:902-7.