SYNOPSIS

NusA is a highly conserved bacterial transcription elongation/termination factor that is involved in various steps of the transcription process. It enhances intrinsic termination at weak terminators by facilitating the hairpin-formation (Schmidt and Chamberlin 1987; Gusarov and Nudler 2001) and induces pausing during transcription elongation especially at the RNA hairpin-dependent pauses (Farnham et al. 1982; Lau et al. 1983; Sigmund and Morgan 1988). It gets converted into an antiterminator in the presence of bacteriophage antiterminator proteins like N and Q (Santangelo and Artsimovitch 2011). Recently, it has also been shown that it is involved in DNA repair process (Cohen et al. 2009; Cohen and Walker 2010). NusA is a ~55 kDa protein, having multiple functional domains; N-terminal RNA polymerase (RNAP)-binding domain (NTD), three RNA binding domains (S1, KH1, and KH2) and two C-terminal acidic repeats (AR1 and AR2) (Worbs et al. 2001; Shin et al. 2003). A concave surface of the NTD interacts with the flap domain of the β-subunit of RNAP (Yang et al. 2009; Ha et al. 2010), whereas the AR2 binds to the C-terminal domain (UCTD) of the α-subunit of RNAP (Mah et al. 2000).

Lambdoid phages encode proteins, like N and Q, those make the transcription elongation complex (EC) resistant to terminators. This phenomenon is called transcription antitermination (Weisberg and Gottesman 1999; Santangelo and Artsimovitch 2011). The antiterminator, N, is a small basic protein that binds to a specific stem-loop structure [box B of nut (N utilization) site] of the mRNA, through its N-terminal arginine rich motif (ARM) (Lazinski et al. 1989; Chattopadhyay et al. 1995) and interacts with the RNAP through its C-terminus (Nodwell and Greenblatt 1993; Mogridge et al. 1998). N requires several host-factors, called Nus factors, for processive antitermination (Weisberg and Gottesman 1999). N and these Nus factors assemble on the nut site of the mRNA, where N and NusA make specific interactions (Santangelo et al. 2003). In case of Q, the Q-NusA interaction occurs on the surface of the elongation complex (EC) that facilitates the action of the former (Shankar et al. 2007).

Among the Nus factors, NusA-N interaction is the most important for the function of N and this interaction transforms NusA into an antiterminator from its natural role as a facilitator of termination. Earlier studies have shown that GST-tagged N protein from phage λ selectively interacts with the AR1 domain of NusA ((Mah et al. 1999). N-AR1 interaction was further established by crystal structure of NusA C-terminal repeats in complex with a lambda N peptide (residues 34-47) (Bonin et al. 2004b; Prasch et al. 2006). All these interactions were
demonstrated outside the EC. However, the functional relevance of this interaction is doubtful (Mah et al. 1999) and it is also debatable that how the interaction with AR1 exerts a long-range effect on the NusA-NTD-RNAP interaction. The objective of this study is to understand the modes of interactions of NusA with N as well as with RNAP during antitermination.

In chapter III, we attempted to revisit the N-NusA interaction in solution as well as on EC and found that the AR1/AR2 regions of NusA have no functional role to play in *in vivo* and *in vitro* transcription antitermination process and simple mixing of different fragments of NusA can non-specifically interact with N with varied efficiency. Using a genetic screen combined with site-directed mutagenesis approaches, we identified several NusA-NTD mutations, V8A, V8E, A7D, A11D, V12D and L31E located inside a hydrophobic patch of the N-terminal RNAP binding domain of NusA which impairs N-mediated antitermination. These NusA-NTD mutations are not defective for any other properties of NusA.

In chapter IV, RNA foot-printing analyses of the *nut* site of the N/NusA modified transcription elongation complex (EC) revealed that these NusA mutants affect the N binding at the *nut* site. In the N/NusA-modified EC, a Cys-53 (S53C) from the convex surface of the NusA-NTD forms a specific disulfide (S-S) bridge with a Cys-39 (S39C) of the NusA binding region of the N protein. Therefore, we concluded that when bound to the EC, the N interaction surface of NusA shifts from the AR1 domain to its NTD domain. This occurs due to a massive away movement of the adjacent AR2 domain of NusA upon binding to the EC. Thus, we proposed that the close proximity of this altered N-interaction site of NusA to its RNAP binding surface, enables N to influence the NusA-RNAP interaction during transcription antitermination that in turn facilitates the conversion of NusA into an antiterminator.

In chapter V, we attempted to decipher the conformational changes imparted by NusA to the RNAP during N mediated antitermination. We hypothesized that in the presence of N, i) NusA binds to a new site in the transcription EC and/or, ii) NusA changes the conformations of the flap-domain. *In vitro* competition assays were performed on the stalled EC, which revealed that the association of NusA to the EC, stabilized ~2 fold in the presence of N is dependent on *nut* site. The mutant, G1045D in the ȳ-flap domain was known to affect N-mediated antitermination (Cheeran et al. 2005). N-mediated antitermination defect of flap mutant was further demonstrated by *in vivo* and *in vitro* antitermination assays on both Rho-dependent and Rho-independent terminators. This mutant unlike RNA-exit channel mutants (P251S, P254L, G336S, and R270S; likely to be part of the N-CTD interaction sites), was not suppressed by a
suppressor, L108F, present in the RNAP-binding domain of N (Cheeran et al. 2005). Here, we made some other mutants in flap domain like P1044A, V1046E, V839E and R1058L. These point mutations in β flap domain specifically impair the N-antitermination process and they are defective in antitermination due to impaired β flap-NusA interaction. RNase H cleavage assays revealed that accessibility of the RNA exit channel is affected in the presence of N and this is due to N-NusA interaction, which is likely because of spatial re-orientation of the flap domain over the exit channel. Altered Fe-BABE cleavage patterns of RNAP from NusA also support the proposition of N-induced spatial orientation of the flap domain. Fe-BABE labelled NusA-generated cleavage of emerging RNA in stalled EC protected by N revealed that N modulates NusA NTD-exit channel interaction. Based on these aforementioned evidences we proposed that one of the way of achieving N-antitermination is to change the conformation of flap through altering the interaction of the latter domain with NusA. We also propose that this N-NusA interaction changes the orientation of flap over the exit channel in such a way that it renders a constricted channel.

Publications


