Bacteriophage HK022 Nun protein arrests transcription by blocking lateral mobility of RNA polymerase during transcription elongation

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Coliphage HK022 excludes phage λ by subverting the λ antitermination system and arresting transcription on the λ chromosome. The 12 kDa HK022 Nun protein binds to λ nascent transcript through its N-terminal Arginine Rich Motif (ARM), blocking access by λ N and arresting transcription via a C-terminal interaction with RNA polymerase. In a purified in vitro system, we recently demonstrated that Nun arrests transcription by restricting lateral movement of transcription elongation complex (TEC) along the DNA register, thereby freezing the translocation state. We will discuss some of the key experiments that led to this conclusion, as well as present additional results that further support it.

Results and Discussion

Temperate phage HK022 and λ are closely related. They have similar gene organizations and express proteins with similar functions. In one respect, however, they differ dramatically. λ promotes transcription of its genes by accelerating transcription through both factor dependent and factor independent termination sites.1,2 The λ N protein binds to sequences on λ nascent transcript (N-UTilization sites, or NUT Sites) and thereby modifies elongating RNA polymerase (RNAP) into a termination-resistant form.3,4 HK022 expresses a λ N homolog, Nun. The only known role of Nun is to exclude superinfecting λ and phage λ imm434, which have the same NUT sites.3 Nun does so both by competing with λ N for binding to the BoxB stem loop of the NUT RNA sites within the nascent λ transcript3,4 and by arresting transcription at pause sites on the λ chromosome.4 Nun-arrested RNAP is removed by the transcription-coupled repair protein MFD.5 Other than the effect of Nun on λ, no other biological function has been described for the protein. Lytic growth of HK022 is unaffected by nun mutations, and nun mutants lysogenize with normal frequency. The specificity of Nun exclusion is unique; other phage exclusion systems are active against a broad range of superinfecting phage.6 It has been suggested that the function of BoxB is to tether both λ N and Nun to increase the local concentration of protein on the λ transcript. Indeed, BoxB is dispensable for function of both proteins in vitro.1,7 Furthermore, Nun overproduction is toxic to E. coli, although λ NUT sites are not encoded in the bacterial chromosome.8 Toxicity is related to transcription termination, since host RNAP and Nun mutations that block Nun termination also suppress cell killing.

Nun interacts with RNAP, resulting in transcription arrest at pause sites, both in vivo and in vitro.1,4 In vivo, Nun arrest requires the four E. coli auxiliary transcription elongation factors, NusA, NusB, NusE, and NusG.9 Though these factors are not essential for Nun arrest in vitro, they enhance specific activity, reducing the concentration of Nun required to block elongation.4 The four Nus proteins enhance arrest when present in equimolar concentrations.

Keywords: Nun protein, transcription arrest, bacteriophage HK022, phage exclusion, bacteriophage λ

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In our recent studies, we explored further the Nun mechanism of action.1 We constructed scaffolds consisting of DNA template and non-template strands, and RNA complementary to the template strand to generate defined transcription elongation complexes (TEC). These TEC differed in the length and the sequence of the RNA primer. Importantly, these scaffolds included no λ DNA or RNA sequences. Nun arrested all TEC tested that carried an RNA:DNA hybrid 9 bp or larger. The TEC were incubated with rXTPs and were all transcriptionally active. For each TEC, Nun-mediated arrest occurred at a specific site, corresponding to an intrinsic pause site.

NusA inhibits Nun in the absence of the other Nun factors.4 NusA binds to the NUT sequence, to RNAP, and to the Nun C-terminus.9-11 It was not clear which of these interactions accounted for NusA inhibition of Nun. We confirmed that NusA inhibits Nun arrest when transcription is initiated from the λ pL promoter and the template includes the NUT site (Fig. 1A). In contrast, when transcription is performed on a template lacking λ NUT, NusA has no effect on Nun arrest. In Figure 1B, DNA scaffolds were assembled and transcription was initiated with A, C, U, and 3′deoxy GTP, which halts transcription 1 nt after the arrest site. NusA fails to inhibit Nun arrest in this system. We conclude that NusA inhibits Nun by preventing Nun binding to NUT, rather than through interaction with Nun or RNAP. This is consistent with our finding that NUT is dispensable in vivo and in vitro when Nun is provided in excess.

Previously we showed that TEC paused under nucleotide deprivation was a substrate for subsequent Nun arrest, although the site of arrest was different depending on the location of the pause.12 In subsequent work, we did functional mutational analysis on the Nun C-terminus, and found that a penultimate aromatic residue
that rapidly oscillates between post- and pre-translocated states.

Nun stabilization of pre- or post-translocated TEC explains why Nun-arrest TEC does not backtrack.1 Backtracking occurs when RNAP translocates toward the promoter along the DNA register. The RNA 3′ is displaced from the active center into the secondary channel. Because the 3′ end is no longer available for chemistry, this complex is resistant to both phosphodiester bond formation and pyrophosphorolysis. To rescue a backtracked complex, a new 3′ end must be generated.

Figure 2. Nun promotes misincorporation at position +11. Transcription elongation complexes were assembled from a 65-mer DNA template (65u10) hybridized to a 5′ P32 9-mer RNA with a 3′ end corresponding to the +9G position (TEC9G). TEC10U12 was then formed by the addition of 50 μM rUTP for 1′, then excess rUTP was washed off before the complex was eluted and incubated with 5 μM Nun or a comparable volume of Nun storage buffer (10′) transcription was initiated with 50 μM rCTP+rUTP, a combination that would promote misincorporation at the +11 position (again, the sequence is U3A4C5). Reactions were then stopped after 10′ with equal volume 2× Loading Buffer. +12C and +10U are indicated. Note that incorporation to +12 is dependent upon misincorporation at +11.

Figure 3. Nun inhibits Gre-induced endonuclease but Gre does not inhibit Nun arrest. Transcription elongation complexes were assembled from a 65-mer DNA template (65U10) hybridized to a 5′ P32 12-mer RNA with a 3′ end corresponding to the +12C position (TEC12C). TEC was then preincubated for 10′ at 25 °C with 5 μM Nun, GreA, GreB, Nun+GreA or Nun+GreB and reactions were either stopped at 10′ by addition of 2× loading buffer (lanes 1–5) or chased with 20 μM rATP/rCTP/rUTP and 60 μM 3′deoxy GTP for an additional 1′ before reactions were stopped (lanes 6–10). Readthrough is indicated by an arrow. RNA products were resolved on a 23% AA/7 m urea gel and imaged with Typhoon phospho-imager.
by endonucleolytic cleavage, which is performed by bacterial GreA and GreB factors. We tested the effect of Nun on GreA and GreB stimulated endonuclease activity. Since the substrate for this endonuclease is the RNA in backtracked TEC, and Nun blocks backtracking, Nun strongly inhibited GreA and GreB cleavage (Fig. 3, lanes 1–5). Conversely, GreA and GreB did not restart Nun-arrested TEC (Fig. 3, lanes 6–10). We conclude that Nun and Gre do not directly compete, but that Nun prevents formation of backtracked TEC.

Taken together, the results shown in Figures 2 and 3 confirm the earlier conclusion that Nun blocks TEC translocation and prevents backtracking. This action of a transcription factor has not been previously reported. There are clearly additional aspects of Nun that remain to be elucidated, and that are likely to reveal surprising aspects of this unusual protein.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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