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Over-expression of phage HK022 Nun protein is toxic for Escherichia coli

Augusto Uc-Mass[1], **Arkady Khodursky**[2], **Lewis Brown**[3], and **Max E. Gottesman**[1,*]

1*Institute of Cancer Research, Columbia University Medical Center, New York, NY 10032.*

2*Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, St. Paul, MN 55108.*

3*Comparative Proteomics Center, Department of Biological Sciences, Columbia University, New York, NY 10027.*

Abstract

The Nun protein of coliphage HK022 excludes superinfecting λ phage. Nun recognizes and binds to the N utilization (*nut*) sites on phage λ nascent RNA and induces transcription termination. Over-expression of Nun from a high-copy plasmid is toxic for *E.coli*, despite the fact that *nut* sites are not encoded in the *E.coli* genome. Cells expressing Nun cannot exit stationary phase. Toxicity is related to transcription termination, since host and *nun* mutations that block termination also suppress cell killing. Nun inhibits expression of wild-type *lacZ*, but not *lacZ* expressed from the Crp/cAMP–independent *lacUV5* promoter. Microarray and proteomics analyses show Nun down-regulates *crp* and *tnaA*. Crp over-expression and high indole concentrations partially reverse Nun-mediated toxicity and restore *lacZ* expression.

Keywords

phage HK022 Nun; transcription termination; *E. coli* stationary phase toxicity; *fis*; indole; Crp

Introduction

The relationships between bacteria and bacteriophages are dynamic. Interactions among phage and host factors are essential for phage development. All phage require and utilize the cellular machinery of the host to duplicate their genomes, synthesize viral proteins, and produce progeny. Bacteriophages compete for a given host, and several molecular mechanisms have evolved that allow a lysogenic phage to exclude superinfecting phages.[1] One of the best characterized mechanisms of exclusion occurs between the HK022 prophage and superinfecting λ phage. Exclusion of superinfecting λ phage by HK022 is mediated by the HK022-encoded Nun protein, which acts as a transcription terminator of λ early genes.[2] The mechanism entails the binding of the N-terminal arginine rich motif (ARM) of Nun to the *boxB* RNA sequences within in the *nut* regions of nascent transcripts produced from the λ*pL* and λ*pR* promoters.[3] The Nun C terminal domain (CTD) contacts RNA polymerase (RNAP),[4] and these interactions between Nun, the RNA *boxB* sites and RNAP promote the formation

*Contributing author

of a stalled transcription elongation complex. In addition, Nun CTD residue W108 interacts with λ DNA template ahead of the transcription elongation complex. Mutation of this amino acid to alanine abrogates Nun termination function. Nun CTD residues K106 and K107 facilitate interaction with template by electrostatic interactions with the phosphate groups of the DNA.[5, 6]

Nun-mediated transcription termination is stimulated by the *E. coli* factors NusA, NusB, NusE (ribosomal protein S10), and NusG. Mutations in Nus factors that abrogate Nun-mediated termination have been isolated.[7, 8] NusA is a 65kDA protein that binds core RNAP after transcription initiation and slows the elongation rate by stimulating pausing of the transcription elongation complex.[9] A NusA point mutant, NusA*E136K*, specifically blocks Nun-mediated termination in the λ*pR* operon, whereas termination of the λ*pL* transcript is unaffected. NusA*E136K* has been shown to bind more tightly to Nun than wild-type NusA.[7, 10]

In this work we show that Nun also affects *E. coli*. Nun overproduction blocks transition from stationary to logarithmic growth. We characterize the mechanisms underlying Nun-mediated toxicity, and identify several elements that play a critical role. We present evidence that the transcription termination function of Nun is responsible for toxicity, even though *E. coli* does not carry a *boxB* sequence. Mutations in NusA and Nun that compromise Nun function in transcription termination also inhibit Nun toxicity. We also show that high indole concentrations and Crp overproduction reduce toxicity. The mechanism of Nun toxicity appears to be mediated by global changes in transcription, and may not involve specific targets.

Results

Nun overexpression inhibits *E. coli* growth

Phage HK022 Nun protein excludes superinfecting phage λ by promoting premature termination of the λ*pL* and λ*pR* early transcripts. Nun is expressed from a dedicated weak promoter in HK022 *E. coli* lysogens, and is present at a concentration of 120–360 molecules per cell.[11] HK022 prophage do not appear to affect cell growth or any other aspect of cell physiology tested. However, Nun expressed from a multicopy plasmid under the control of the strong IPTG-induced *trc* promoter (AU222; see Table 1) severely inhibited *E. coli* growth (Fig. 1A). Strain AU221, carrying the empty vector, or uninduced AU222 had no effect (Fig. 1A; data not shown). Nun toxicity depends on two factors: Nun concentration and cell culture phase at the time of Nun overexpression. To determine the effect of Nun concentration, AU222 was grown overnight at 37°C, diluted 1:100, and treated with different IPTG concentrations (10 μM, 25 μM, 50 μM, 100μM and 1 mM). Figure 1A shows that cell growth, as determined by OD_{600}, negatively correlated with increasing inducer concentration. Figure 1B shows that Nun-sensitivity was growth-phase dependent. AU222 was diluted from an overnight culture and treated at different times with 1mM IPTG. We found that Nun blocked cells from exiting stationary phase, and was considerably less toxic to cells in early or late log phase.

Mutations that suppress Nun-mediated termination also reduce Nun toxicity

E. coli NusA protein is required for Nun-mediated termination in the λ*pR* operon. A *nusA* point mutant, *nusAE136K*, blocks Nun-mediated termination at λ*nutR* but not at λ*nutL*.[7, 10] We asked if Nun toxicity was also abrogated by *nusAE136K*. Overnight cultures were diluted 1:100 and grown for 6 hr in the presence or absence of 50 μM IPTG. As shown in Table 2, Nun reduced the growth rate in wild-type cells by 58% (rows 1 and 2). In contrast, Nun reduced growth by 24% in the *nusAE136K* background (rows 3 and 4).

We further tested the idea that Nun-induced transcription termination was lethal in *E. coli* by asking if the termination-deficient Nun*W108A* mutant was toxic.[5] Over-expression of

Nun*W108A* had no significant effect on cell growth (Table 2, rows 1 and 5). Taken together, these results suggest that transcription termination is at least partially responsible for Nun toxicity.

Nun inhibits cAMP/Crp – dependent promoters

Our results suggest that transcription termination accounts for Nun toxicity. In an attempt to discern general characteristics of the Nun effect on the physiology, we measured relative transcript and protein levels in cells overproducing Nun protein (see Supplementary file for microarray procedures and data analysis). Nun excess affected transcript levels of more than 450 genes: 184 down-regulated and 271 up-regulated (differentially expressed genes are listed in Supplementary Table S1). Further analysis revealed that the down-regulated genes were organized in longer operons than the upregulated ones (Fig.2), supporting an effect of Nun over-expression on transcription elongation. The set of down-regulated proteins in Nun overexpressing cells (See Supplementary Table S2 see Suppl. File for details of proteomics experiments) was enriched for CRP targets, 12 out of 28 proteins whose abundance decreased at least 2-fold (adjusted $p<10^{-2}$). The set of proteins whose abundance was increased more than 2-fold was not enriched for representatives of any functional class.

To investigate the role of CRP in mediating the Nun effect, we examined the activity of several CRP- dependent and CRP-independent *E. coli* operons after Nun induction. Table 3 shows the effect of Nun over-expression on *lacZ* transcription. β-galactosidase levels were determined 4 hr following induction of Nun and chromosomal *lacZ* with 100 μM IPTG. LacZ activity was reduced 88% compared to the empty vector control (Table 3: rows 1 and 2). Since the *lac* operon does not carry a λ *nut* site, we hypothesized that inhibition was mediated by a general transcription regulator(s). cAMP/Crp controls more than 100 *E. coli* operons, including *lac*.[12] To ask if Nun acted through cAMP/Crp, we determined the effect of Nun on a *lacZ* operon driven by the *lacUV5* promoter, which is independent of cAMP/Crp.[13] Our results show *lacUV5* partially relieved Nun inhibition (rows 3 and 4; 47% inhibition compared to the empty vector control).

We then asked whether Nun inhibition of catabolite-repressible gene expression was related to Nun termination activity. Accordingly, we measured *lac* operon induction in host and phage mutants that abrogate or reduce Nun-mediated termination. Table 3, rows 5 – 7, shows that *lacZ* expression is not reduced in *nunW108A nusA*$^+$ or *nusAE136K nun*$^+$ strains. Thus, Nun down-regulation of the *lac* operon correlates with Nun termination activity.

We extended our analysis to other catabolite-repressible operons. We tested the effect of Nun on *pmalE*, which is directly controlled by cAMP/Crp or on *pmalP*, which is under the control of MalT, whose expression is cAMP/Crp – dependent.[14] Overnight cultures carrying *pmalE* or *pmalP* fused to *lacZ* were diluted 1:100, grown for 1 hr, and treated with 100μM IPTG and 0.2% maltose for 3 hr. As shown in Table 4, Nun reduced *malE* and *malP* promoter activity 85% and 99%, respectively compared to Nun$^-$ controls. We conclude that Nun significantly represses cAMP/Crp – dependent promoters.

Crp over-expression reverses Nun inhibition of *lacZ* and partially overcomes toxicity

To determine whether Nun reduced cAMP levels or inhibited Crp, we induced *lacZ* in a strain carrying a multicopy, arabinose-inducible, plasmid that expressed Crp (pBWCRP; see Table 1). For unknown reasons, we were unable to introduce our Nun$^+$ plasmid into this strain, and therefore substituted a plasmid that expresses a *nun* derivative, *nunP9A*, isolated by alanine-scanning mutagenesis. The *P9A* mutation has no other evident phenotype; it did not reduce Nun toxicity or Nun inhibition of *lacZ* expression (Table 5). Table 5, column 4, rows 3 and 4, shows that over-expression of Crp completely reversed Nun inhibition of *lacZ* induction. These

results indicate that Nun reduces the concentration of Crp or its activity. Microarray analyses confirmed that Nun reduced *crp* expression 2.3-fold. Efforts to determine if Nun also reduced cAMP levels were inconclusive.

Crp over-production also partially overcame Nun toxicity, increasing the growth rate of Nun^+ cells more than 4-fold (Table 5, column 5, rows 3 and 4).

Nun up-regulates the *rrnE* operon

We showed above that Nun was most toxic to cells attempting to exit stationary phase, and had less effect on cells in logarithmic growth. We wondered if Nun might down-regulate ribosomal RNA synthesis, thus preventing entry into log phase. Additionally, *rrn* operons carry *boxA* sequences, which are also found in the λ*nut* sites. To measure the effect of Nun on *rrn* activation, we used fusions carrying various portions of the *rrnE* promoter region to *lacZ*. The results of these experiments are shown in Figure 3. Contrary to our expectations, Nun significantly increased *rrnE* expression (Figure 3A).

The reason for this increase was suggested by microarray analysis. We found that *fis* was up-regulated 2.6-fold by Nun (Supplementary Table S1). Fis positively regulates transcription of *rrn* operons.[15] To test the possibility that Nun enhancement of *rrnE* expression was mediated by Fis, we introduced the non-functional *fis::767* mutation into the strains shown in Figure 3A.[16] Figure 3B shows that *fis::767* completely reversed Nun up-regulation of *rrnE* transcription. Although the mutation blocked *rrnE* induction, it had no effect on Nun toxicity (data not shown). The role of Fis in Nun up-regulation of ribosomal RNA synthesis was also demonstrated using *rrnB-lacZ* fusions that lack the UP and Fis-binding regions. Nun had no effect on this mutant promoter (Figure 3C). Together, these data indicate that Nun induces ribosomal RNA transcription via an increase in Fis concentrations.

Nun down-regulates indole synthesis

Proteomic analyses of cells exposed to high Nun concentrations showed that the levels of tryptophanase, the enzyme responsible for indole production, were reduced 5.8-fold in response to Nun over-expression (see Supplementary Fig. S1). This is of particular interest, since indole synthesis increases as cells enter stationary phase and may promote this transition.[17] Figure 4 shows that Nun repressed indole synthesis 7-fold. Media from cultures carrying the empty vector contained 231μM indole, whereas the media indole concentration in cells expressing Nun was 31μM.

Our next experiment was to add indole to the medium and measure the effects of Nun on cell growth and *lacZ* induction. No effect was seen when we adjusted the indole concentration of the medium to 231μM (data not shown). However, indole at 1.15 mM partially reversed Nun toxicity (Table 6, column 5, compare rows 1 & 2, and 3 & 4). Note that at this concentration, indole inhibited cell growth by ~50%. In addition, indole restored β-galactosidase synthesis in Nun^+ cells to ~30% of Nun^- levels (Table 6, column 4, compare rows 1 & 2, and 3 & 4).

Discussion

The known role of phage HK022 Nun protein is to exclude superinfecting λ by terminating transcription prematurely on the λ chromosome. Exclusion is highly specific, affecting only phages that express λ *nut* RNA. We demonstrate in this report that over-expression of Nun blocked exit of *E. coli* from stationary phase. Surprisingly, since *E. coli* does not encode λ *nut*, the transcription termination activity of Nun was required for this toxicity. In HK022 lysogens, Nun is expressed from a dedicated promoter at very low levels, 120–360 molecules per cell. Nun concentrations increase as lysogens enter stationary phase.[11] However, HK022

lysogens grow normally and have no defect in exiting stationary phase (data not shown). No difference between HK022 and HK022 *nun* mutants in terms of lysogeny or prophage induction have been detected. We suggest that although the drastic effects of Nun over-production on cell growth and gene expression reported here may not have a counterpart in normal cell growth, it is nevertheless possible that subtle alterations of cellular metabolism are induced by physiological concentrations of Nun, and that these favor the propagation of HK022 under conditions that we have not reproduced in the laboratory.

Microarray and proteomic analyses reveal numerous changes induced by Nun in E. coli physiology (see Supplemental Table S1, S2, and Fig. S1). Microarray analysis indicates that genes encoding the tricarboxylic acid (TCA) cycle and chemotaxis are down-regulated, whereas some tRNA ligases, DNA damage repair genes and resident transposon-related genes are up-regulated. Certain genes related to oxidative damage, including *soxS* and *sodB*, are also up-regulated. It is plausible that cells exposed to Nun undergo an RpoE-associated stress response.[18, 19]

Nun over-expression also upregulates *fis* and down-regulates Crp and tryptophanase. These changes affect many cellular processes. Fis represses transcription of *rpoS*, which encodes the stationary-phase sigma factor. Nun induction of Fis, although it leads to increased *rrn* transcription, does not, however, account for Nun toxicity.

Down-regulation of Crp and tryptophanase together do account for Nun inhibition of stationary phase exit. Over-expression of Crp, or addition of indole to the medium, suppressed Nun toxicity. Note, however, that *crp* or *tnaA* mutants can exit stationary phase. We assume that down-regulation of Crp or TnaA is only toxic in the context of other changes in cellular physiology induced by Nun. Crp also fully restores induction of *lacZ* in Nun-inhibited cells and indole has a partial effect. Interestingly, phage λ encodes a gene, *hin*, whose product blocks *lacZ* induction. In contrast to Nun, however, Hin inhibition of *lacZ* expression is due to cAMP depletion subsequent to phage induction.[20] With respect to phage development, down-regulation of Crp or cAMP diverts temperate phage from the lysogenic into the lytic pathway.

Indole is synthesized as *E. coli* enters stationary phase and is also a quorum sensing signal that promotes this transition.[17] Indole stimulates expression of Crl, a positive regulator of RpoS.[21] Tryptophan is converted to indole by tryptophanase (TnaA). Since transcription of *tnaA* is controlled by RpoS, a positive regulatory loop amplifies indole secretion as cells transit to stationary phase. Interestingly expression of *E. coli* cryptic prophage genes are strongly down-regulated by RpoS.[22] It is tempting to speculate that accumulation of Nun in HK022 lysogens entering stationary phase reduces indole concentrations down-regulating RpoS activity and thus inducing prophage gene expression.

The pattern of gene deregulation induced by Nun, however, does not indicate a single target. At physiological concentrations, Nun binding to RNA is highly specific; only the *boxB* RNA sequence of the λ *nut* region is recognized. Although *boxB* is not found in the chromosome, *boxA*, another component of λ *nut*, is present at several sites. The *tnaA* operon, which is down-regulated by Nun, includes a *boxA* sequence, as do the *rrn* operons, which are up-regulated.

It is possible that at elevated levels, Nun binds RNA non-specifically. This could directly affect translation, and may explain why, despite overall consistency between transcriptomic and proteomic data (see Supplementary Discussion), some genes down-regulated by Nun are seen in proteomic but not in microarray analysis (e.g. TnaA).

Materials and Methods

Bacterial strains, plasmids, and phages

E. coli strains, bacteriophages, and plasmids used in this work are listed in the Table 1. Plasmid pAUM is a derivative of pTrc99 in which chloramphenicol resistance (*cat*) replaces ampicillin resistance (*bla*). Two *EagI* sites were introduced in pTrc99 with the Quickchange site-directed mutagenesis technique (Stratagene). The new *EagI* sites flank *bla*. The pair of primers used for the 5' region of *bla* were 99EagIF=CGC CCT TAT TCC CTT TTT TGC GGC CGT TTG CCT TCC TGT TTT TGC, and 99EagIR=GAG CAA AAA CAG GAA GGC AAA CGG CCG CAA AAA AGG GAA TAA GGG. The pair of primers used for the 3' region were 99EagI3F= CGT TCC ACT GAG CGT CAG CGG CCG TAG AAA AGA TCA AAG G, and 99EagI3R= CCT TTG ATC TTT TCT ACG GCC GCT GAC GCT CAG TGG AAC G. The *cat* gene from pBAD18 (see Table 1) was mutated changing codon 73 (GAA) to disrupt an *EcoRI* site. The primers used were Cm-EF= CTG ATG AAT GCT CAT CCG GAG TTC CGT ATG GCA ATG AAA GAC, and Cm-ER= GTC TTT CAT TGC CAT ACG GAA CTC CGG ATG AGC ATT CAT CAG. Then this pBAD18 derived plasmid was used as template to amplify by Polymerase Chain Reaction (PCR) a fragment of 930pb containing *cat*; the primers used were CmEaf= CCA GTC TAT TAA TTG CGG CCG GGA AGC, and CmEar= CAA CGC CAT GAG CGG CCG CAT TTC TTA. The PCR product containing *cat* and the pTrc99-derived plasmid were digested with *EagI* (New England Biolabs) and purified with QIAquick PCR purification kit and QIAquick Gel extraction Kit (QIAGEN) respectively. The PCR fragment and pTrc99-derived DNA fragment obtained were ligated by incubation with T4 DNA ligase (New England Biolabs) at 16°C overnight. The ligated mixture, containing plasmid pAUM, was used to transform XL1-Blue cells.

The plasmid pTrc-Nun was digested with *EcoRI* and *HindIII* (New England Biolabs) to release a fragment containing *nun*. pAUM was digested with *EcoRI* and *HindIII*, and the resulting pAUM-digested fragment was purified with the QIAquick Gel extraction Kit (QIAGEN). The *nun* and pAUM fragments were ligated by incubation with T4 DNA ligase (New England Biolabs). The ligation mixture containing plasmid pAUM-Nun was used to transform XL1-Blue cells.

Plasmid pAUM-NunP9A was constructed by the introduction of a mutation in codon nine in *nun* gene using the Quickchange site-directed mutagenesis technique (Stratagene). The primers used were P9Af= GAC TAT TTA TGT TAA TGC TGA CAG CGG ACA AAA CAG, and P9Ar= CTG TTT TGT CCG CTG TCA GCA TTA ACA TAA ATA GTC.

Plasmid pHY0 (see Table 1) was digested with *EcoRI* and *HindIII* to release a fragment containing *crp* which was ligated to pBAD30 digested with the same nucleases, yielding pBWCRP.

Determination of growth rate

Fresh overnight cultures were diluted 1:100 in LB+ 50 μg/ml chloramphenicol. To induce samples, IPTG was added at time 0 at 50μM final concentration, Growth rate was determined by measuring OD_{600} at time 0, and 6 hr later. The difference in OD_{600} was divided by 6 to yield growth rate hr^{-1}. Note that what is being determined is, in fact, the lag time for stationary phase exit.

Transcription from chromosomal promoters

lacZ fusions were used to measure transcription from chromosomal promoters. Overnight cultures were diluted 1:100 in LB supplemented with the appropriate antibiotic chloramphenicol or chloramphenicol + ampicillin, each at 50 μg/ml. Cultures were grown 1

hr at 37°C, and then the inducers were added simultaneously. The inducers used were IPTG (100μM), maltose (0.2%), and arabinose (0.02%). In the case of the determination for the ribosomal fusions the addition of the inducer IPTG (100μM) was done 4 hr after dilution, and the cells were grown at 30°C. β-galactosidase activity is reported as Miller Units.[23]

Indole assays

Indole was determined in *E. coli* culture supernatants as described.[24] Briefly, 400μl aliquots of growing cultures were removed every 2 hr and centrifuged to remove cells. 200μl of supernatant were added to 200μl of 0.5 M perchloric acid. After centrifugation to remove precipitated proteins, 400μl of Ehrlich's reagent was added. The samples were incubated for 30 min at 37°C, after which absorbance at 571 nm was determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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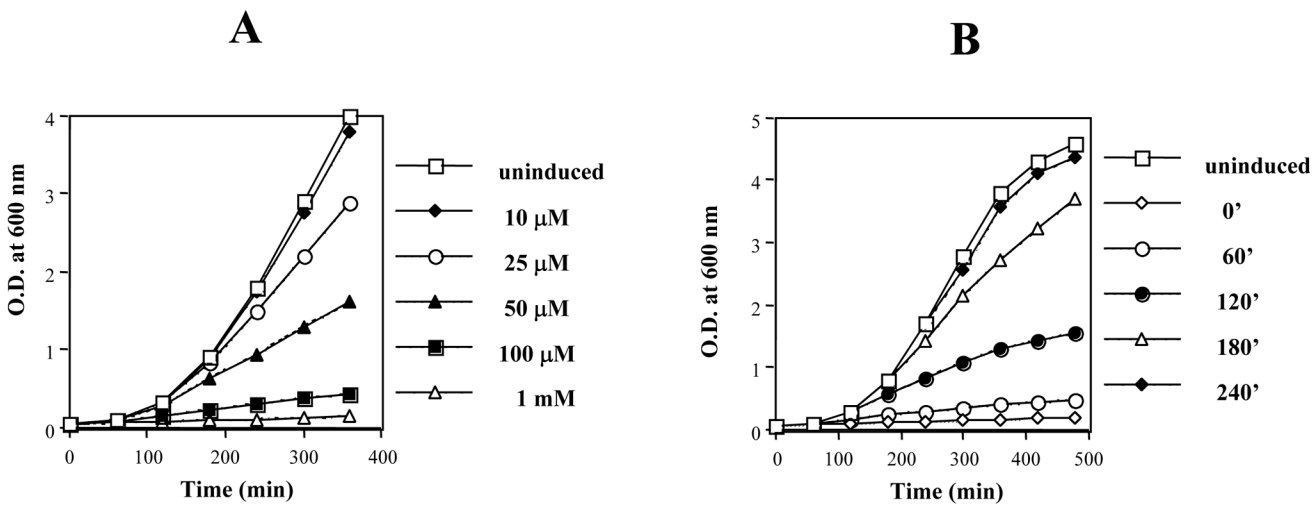

Figure 1. ***E. coli*** **outgrowth from stationary phase is inhibited by Nun overproduction**

Strain AU222 was grown overnight at 37 °C in LB + chloramphenicol (50μg/ml) and diluted 1:100. A) IPTG was added at time 0 at the indicated concentrations to induce Nun; B) Nun was induced with 1 mM IPTG at the indicated times following dilution. All experiments were at 37 °C.

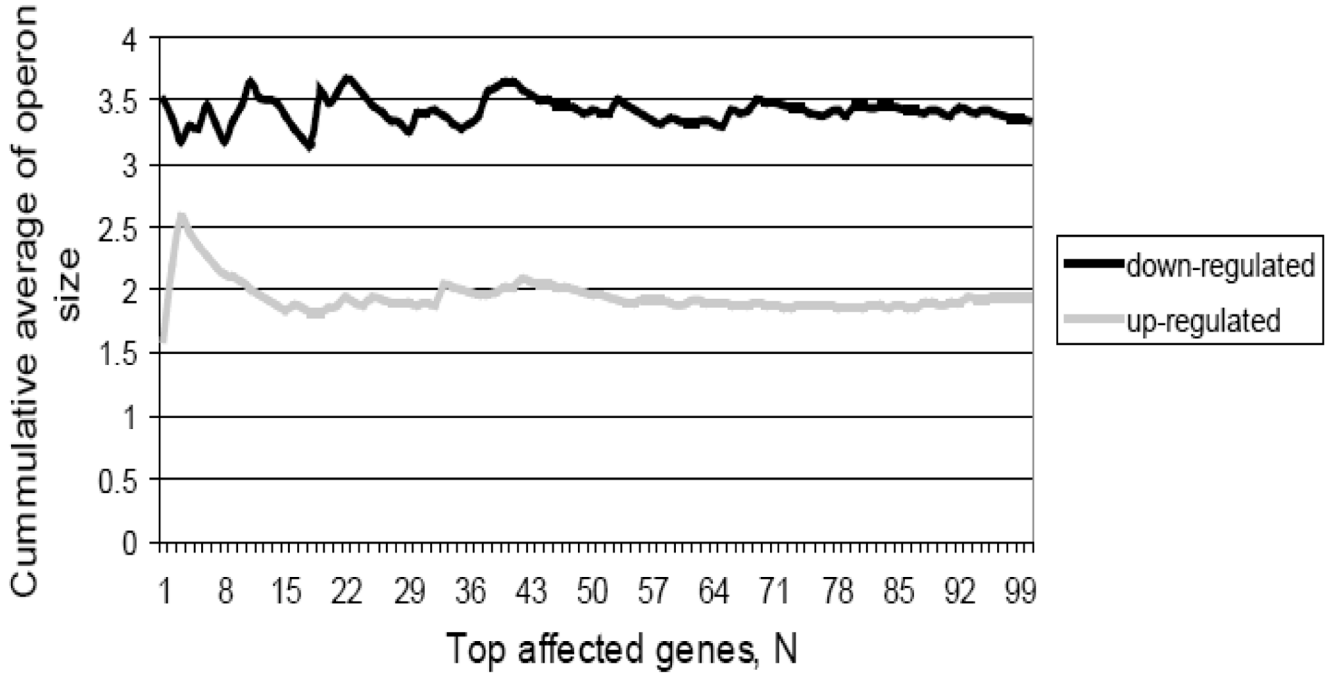

Figure 2. Length of operons affected by Nun

The average length of down- and up-regulated operons was calculated by applying a sliding window of size 5 to the lists of significantly affected genes sorted (from most to least affected) according to the strength of the transcriptional effect. The average size of an unaffected operon with similar signal intensity was 1.9 ± 0.1 genes.

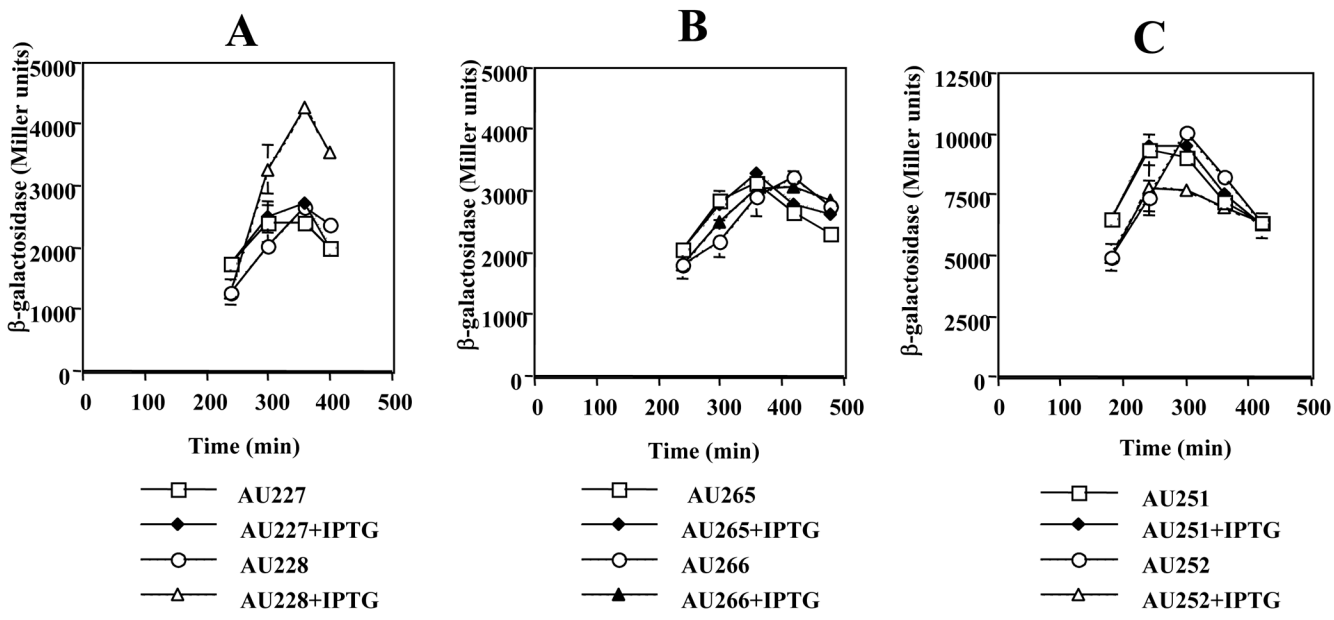

Figure 3. Nun induces ribosomal operon transcription in a Fis-dependent fashion

Strains grown overnight at 30 °C were diluted 1:100 in LB and grown at 30 °C. Four hr later 100 μM IPTG was added where indicated and β-galactosidase (MU) were determined hourly. Strains in panels A and B carry the *p1-p2* promoters of *rrnE* fused to *lacZ* (Table 1). A) *fis*$^+$; B) *fis*::767; C) *fis*$^+$, the *p2* promoter of *rrnB* is fused to *lacZ* - Fis binding sites are deleted. The above data are an average of two independent experiments.

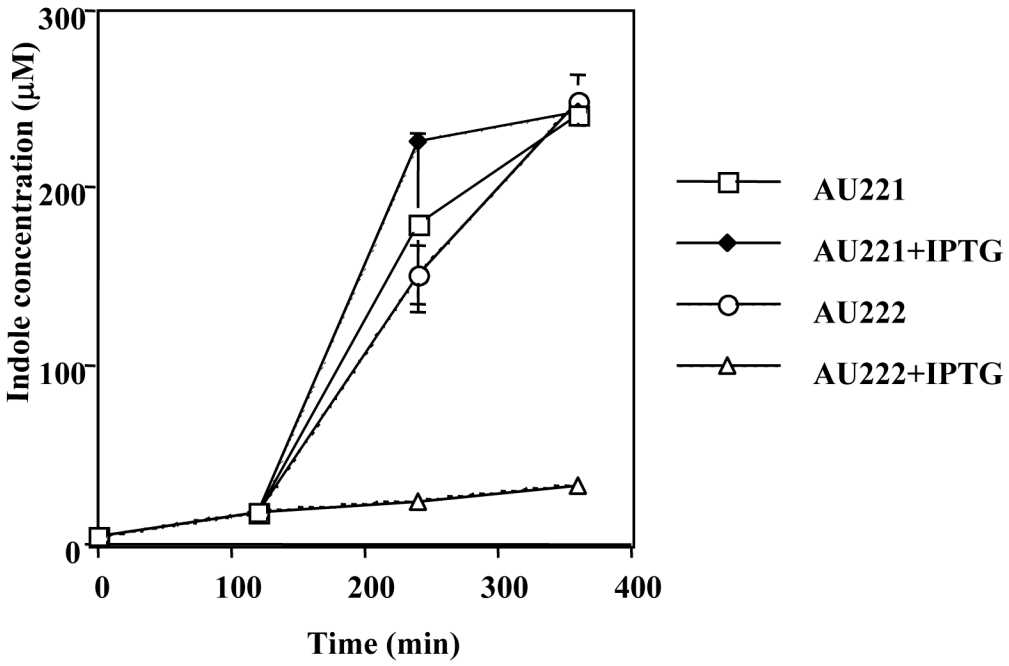

Figure 4. Nun inhibits indole production

Overnight cultures grown at 37 °C were diluted 1:100 and 100μM IPTG was added where indicated. Samples were collected at 2 hr intervals, and the concentration of indole in the supernatant was determined. The above data are an average of two independent experiments. AU221, vector; AU222, pAUM-nun

TABLE 1

Strains, plasmids and phages used in this study

Strains, plasmids, and phages Strains	Genotype	Reference or source
N99	W3102; Wild-type	NIH collection
RJ1800	MG1655 *fis::767*	16
CA8224	HfrH *placUV5- lacZ*	Beckwith J. collection
AU221	N99/pAUM	This work
AU222	N99/pAUMNun	“
AU223	N99/pAUMNunP9A	“
AU227	N99 *lacZXA21(λ 423)*/pAUM	“
AU228	N99 *lacZXA21(λ 423*)/pAUMNun	“
AU230	N99 *nusAE136K*/pAUM	“
AU231	N99 *nusAE136K*/pAUMNun	“
AU246	N99/pAUMNunW108A	“
AU251	N99 *lacZXA21(λ 4253)*/pAUM	“
AU252	N99 *lacZXA21(λ 4253)*/pAUM Nun	“
AU260	N99 *lacZXA21 lacUV5-lacZ*+/pAUM	“
AU261	N99 *lacZXA21 lacUV5-lacZ*+/pAUMNun	“
AU265	N99 *lacZXA21(λ 423), fis*:767/pAUM	“
AU266	N99 *lacZXA21(λ 423), fis*:767/pAUMNun	“
AU269	N99 *lacZXA21(λ HS3120)*/pAUM	“
AU270	N99 *lacZXA21(λ HS3120)*/pAUMNun	“
AU271	N99 *lacZXA21(λ HS3134)*/pAUM	“
AU272	N99 *lacZXA21(λ HS3134)*/pAUMNun	“
AU278	AU221/pBAD30	“
AU297	AU221/pBWCRP	“
AU284	AU223/pBAD30	“
AU299	AU223/pBWCRP	“
Plasmids		
pTrc99	*ColE1ori amp*^{R}	Amersham Biosciense
pTrcNun	pTrc99-*nun*$^+$	Our lab collecton
pBAD30	pACYC184 ori *amp*^{R}	25
pBAD18	*ColEIori cam*^{R}	25
pHY0	pEXT20-*crp*	26
pAUM	pTrc99, *amp*^{S}, *cam*^{R}	This work
pAUMNun	pAUM-*nun*$^+$	“
pAUMNunP9A	pAUM-*nunP9A*	“
pAUMNunW108A	pAUM-*nunW108A*	“
pBWCRP	pBAD30-*crp*	“
Phages		
λ 423	λ*cI857 rrnEP1P2*	27
λ4253	λ*cI857 rrnBP2*	28
λHS3120	λ *pmalE*	H. Shuman collection
λHS3134	λ *pmalP*	H. Shuman collection

Table 2

nusAE136K and *nunW108A* suppress Nun toxicity

Strain	Host mutation	Plasmid	Growth rate(hr^{-1})[a]
AU221	*nusA*$^{+}$	vector	.71 +/− .01
AU222	*nusA*$^{+}$	*nun* $^{+}$	.30 +/− .01
AU230	*nusAE136K*	vector	.70 +/− .01
AU231	*nusAE136K*	*nun* $^{+}$	.53 +/− .04
AU246	*nusA*$^{+}$	*nunW108A*	.68 +/− .02

Strains were grown overnight and diluted 1:100 into 50μM IPTG.

[a]The initial OD_{600} was subtracted from the OD_{600} at 6 hr and divided by 6.

Table 3

Nun inhibits *lac* operon induction.

Strain	Promoter	Host mutation	Nun	β-galactosidase	% inhibition
AU221	*plac*+	*nusA*+	−	522 +/− 44	-
AU222	*plac*+	*nusA*+	+	62 +/− 6	88
AU260	*placUV5*	*nusA*+	−	1248 +/− 7	-
AU261	*placUV5*	*nusA*+	+	660 +/− 45	47
AU230	*plac*+	*nusAE136K*	−	606 +/− 42	-
AU231	*plac*+	*nusAE136K*	+	649 +/− 45	< 1
AU246	*plac*+	*nusA*+	*nunW108A*	505 +/− 15	3

Overnight cultures were diluted 1:100, grown for 1 hr at 37 °C, and induced with 100 μM IPTG. Cultures were then grown at 37 °C for 4 hr and β-galactosidase units (Miller units) were determined. The above data are an average of three independent experiments.

Table 4

Nun inhibits *malE* and *malP* promoters

Strain	*lacZ* fusion	nun	β-galactosidase	% inhibition
AU269	*pmalE*	−	2933 +/− 106	-
AU269	*pmalE*	+	565 +/− 88	85
AU270	*pmalP*	−	662 +/− 72	-
AU270	*pmalP*	+	< 1	>99

Overnight cultures were diluted 1:100, grown for 1 hr at 37 °C, and then brought to 100μM IPTG and 0.2% maltose to induce *nun* and *mal* promoters, respectively. β-galactosidase activity (MU) was determined after an additional 3 hr at 37 °C.

The above data are an average of two independent experiments.

Table 5

Crp overproduction suppresses Nun toxicity and restores *lac* transcription.

Strain	Nun	Crp	β-galactosidase	Growth rate(hr^{-1})[a]
AU278	−	−	425 +/− 70	.68 +/− .01
AU297	−	+	505 +/− 88	.67 +/− .02
AU284	+	−	146 +/− 10	.11 +/− .01
AU299	+	+	502 +/− 90	.39 +/− .02

Overnight cultures were diluted 1:100 in LB +ampicillin (50μg/ml) + chloramphenicol (50μg/ml), grown for 1 hr at 37 °C, and treated with 100 μM IPTG and 0.02%arabinose to induce *nun* and *crp*, respectively. β-galactosidase activity (MU)) was determined after an additional 5 hr at 37 °C. The above data are an average of two independent experiments.

[a]Growth rate was determined as in Table 2.

Table 6

Indole suppresses Nun.

Strains	Nun	Indole	β-galactosidase	Growth rate (h^{-1})
AU221	−	−	463 +/− 30	.69 +/− 0.01
AU222	+	−	25 +/− 2	.11 +/− 0.01
AU221	−	+	164 +/− 11	.34 +/− 0.01
AU222	+	+	142 +/− 29	.19 +/− 0.02

Overnight cultures grown at 37°C were diluted in fresh LB supplemented with 1.15 mM indole and 100μM IPTG. β-galactosidase (MU)) was determined after 4 hr at 37°C. Growth rate was determined as described in Table 2. The above data are an average of two independent experiments.