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NOTES

Superimposition of TyrR Protein-Mediated Regulation on Osmoresponsive Transcription of *Escherichia coli proU* In Vivo

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Osmotic regulation of *proU* expression in the enterobacteria is achieved, at least in part, by a repression mechanism involving the histone-like nucleoid protein H-NS. By the creation of binding sites for the TyrR regulator protein in the vicinity of the σ^{70}-controlled promoter of *proU* in *Escherichia coli*, we were able to demonstrate a superposed TyrR-mediated activation by L-phenylalanine (Phe), as well as repression by L-tyrosine, of *proU* expression in vivo. Based on the facts that pronounced activation in the presence of Phe was observed even at a low osmolarity and that the affinity of binding of TyrR to its cognate sites on DNA is not affected by Phe, we argue that H-NS-mediated repression of *proU* at a low osmolarity may not involve a classical silencing mechanism. Our data also suggest the involvement of recruited RNA polymerase in the mechanism of antirepression in *E. coli*.

The *proU* operon in *Escherichia coli* and *Salmonella typhimurium* encodes a binding-protein-dependent transport system that mediates the active uptake of the compatible solutes glycine betaine and L-proline during growth in media of elevated osmolarities. Under such growth conditions, the expression of *proU* is induced 400-fold at the level of initiation of transcription, but the underlying regulatory mechanisms are not clearly understood (reviewed in references 6 and 14).

Analyses of the *cis* regulatory regions necessary for osmotic induction of *proU* have identified an extended sequence (more than 500 bp long) designated the negative regulatory element (NRE), whose proximal end is situated approximately 70 bp downstream of the σ^{70}-controlled promoter (P2) and which is required for the full repression of *proU* at a low osmolarity (7, 10, 22, 28, 29). Genetic and biochemical data suggest that the NRE mediates the repressor function of the histone-like nucleoid protein H-NS on *proU* (approximately 20- to 25-fold) (7, 10, 22, 29). Nevertheless, the NRE does not serve as a portable cassette for osmotic regulation when placed downstream of heterologous promoters (7, 28), indicating that sequences around and upstream of P2 are also required for its function. Furthermore, in mutants lacking H-NS or the NRE, or both, a residual 8- to 10-fold osmotic inducibility of *proU* is observed (7, 10, 22, 28, 29); this inducibility has been interpreted to represent a second distinct mechanism acting directly on the *cis* element(s) in the close vicinity of P2 (7, 14). Finally a σ^{s}-controlled promoter, P1 (situated 190 bp upstream of P2), has also been identified which, at least in *S. typhimurium*, is cryptic and whose relevance in *proU* regulation is as yet unclear (7, 33).

It has been suggested that H-NS-mediated repression of *proU* at a low osmolarity is achieved by promoter "silencing" and that relief of repression at a high osmolarity is the consequence of cytoplasmic potassium glutamate accumulation (6, 28). In the silencing model, the NRE serves as a position-independent silencer locus (10, 28, 49) akin to that described for the regulation of several eukaryotic genes (5, 27). The following features have been cited in support of this model. (i) H-NS is not a typical sequence-specific regulator protein (for reviews, see references 3 and 47), nor is the NRE a typical operator sequence. Indeed, there exist two regions of curved DNA in the vicinity of *proU* P2 (see Fig. 1B), one falling within the *proU* NRE and the other located about 150 bp upstream of the promoter (13, 29, 40, 41), to both of which H-NS exhibits preferential binding (22, 29, 40). (ii) The separation and phase angle of the NRE from *proU* P2 can be varied over a distance of 200 bp without affecting its ability to mediate repression (10, 18, 28). (iii) NRE-mediated repression is also observed for several different variants of the P2 promoter (19, 49). (iv) A role for H-NS binding has been implicated in the only locus (*bgl*) in *E. coli* where silencing has been unequivocally established (26, 38, 39); the protein has also been postulated to silence several other genes in the organism (12, 23). One question as yet unanswered is whether the repressive action, consequent to the binding of H-NS to *proU*, is direct (43) or indirect (18, 19, 29).

In vitro tests of the silencing model are rendered difficult by the fact that no accepted method for the reconstitution of *proU* osmotic regulation in a cell-free system exists. One prediction of the silencing model, which would help distinguish it from other mechanisms of H-NS-mediated repression of *proU*, is that the silencing effect would extend sufficiently upstream of the *proU* promoter to interfere also with the recognition of closely linked binding sites for other DNA-binding proteins. This is the hypothesis which we have sought to test in this study, by first creating specific sites for binding of the regulator protein TyrR adjacent to the *proU* P2 promoter and then addressing the question of whether TyrR binds these sites in vivo at low and high osmolarities. Our results indicate that TyrR-mediated regulation can be superimposed on osmotic regulation of *proU* transcription and suggest in particular that the chromatin architecture in the *proU* P2 promoter region,

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even at a low osmolarity, is permissive to the binding of and activation by TyrR. To that extent, therefore, it appears to be unlikely that a silencing mechanism operates to achieve *proU* repression at a low osmolarity.

Overview of and rationale for choosing the TyrR regulation system. The TyrR protein in *E. coli* mediates the transcriptional regulation of several operons involved in the biosynthesis and transport of the aromatic amino acids (reviewed in references 30 and 31). The protein can act as either a repressor or an activator depending upon the promoter and the particular coeffector to which it is bound. We chose to work with the TyrR system primarily because of the fact that neither the affinity of the protein for its cognate binding sites on DNA nor the footprint obtained on such binding is altered in the presence of its coactivator L-phenylalanine (Phe) (2, 4, 31, 35). The need for imposing this constraint on our choice of system is explained below. In the case of other well-characterized activator proteins such as CRP (20), AraC (37), MalT (37), and the LysR family of proteins (36), the association of the proteins with their respective coactivators leads to an alteration of the DNA-binding characteristics of the proteins.

TyrR-mediated repression is achieved in the presence of the coeffector L-tyrosine (Tyr). Genes whose expression is repressed by TyrR often have two adjacent TyrR binding sites (TYR R boxes), one of which overlaps the promoter. (The TYR R box is 22 bp long, and its consensus sequence is described in the legend to Fig. 1.) The box overlapping the promoter has a relatively weak affinity for TyrR (weak TYR R box) and is bound only when it is close to and on the same face of the helix as the other box, which has a stronger binding affinity for the protein (strong TYR R box). For instance, in the case of the *tyrP* gene (encoding a Tyr-specific permease), which is repressed by TyrR-Tyr, the weak box overlaps the −35 region of the promoter, whereas the strong box is upstream of and separated from the weak box by 1 bp. It has been shown that, in the presence of Tyr, the protein self-associates to form a hexamer and that it binds cooperatively to both boxes to cause repression.

Transcriptional activation by TyrR in the presence of Phe requires only the presence of a strong box suitably positioned upstream of the promoter. A spacing of 18 bp between the TYR R box and the −35 hexamer is optimal for the purpose. The TyrR dimer remains constitutively bound to the strong box, and upon binding Phe it acquires the ability to activate transcription by the process of RNA polymerase recruitment (15, 32); under these specific conditions, TyrR has been shown to increase the affinity of binding of RNA polymerase to the adjacent promoter and to stimulate open-complex formation (16) by functioning as a class I transcription activator (21, 48).

In the case of native *tyrP*, the 1-bp separation between the two TYR R boxes (which is necessary for repression control) places the strong box 15 bp away from the −35 hexamer, which distance is suboptimal for Phe-mediated activation (1). The greatest activation effect at *tyrP* is observed for that template in which the strong box has been moved upstream by another 3 bp; in the latter situation, addition of Tyr also leads to an activation rather than a repression of *tyrP* expression (1).

Creation of the TYR R box(es) near *proU* P2. In this study, we chose to simulate at *proU* P2 the regulatory features described above for the *tyrP* gene (1, 2). In order to test whether TyrR could repress *proU*, it was necessary (i) to use site-directed mutagenesis to create a weak box overlapping the −35 region of the P2 promoter and (ii) to introduce a strong box sequence upstream of and 1 bp away from the weak box, as is the case in native *tyrP*. In order to test the ability of TyrR to activate *proU*, it was necessary to introduce a strong box sequence 3 bp farther upstream than in the previous construct and to leave the remainder of the *proU* regulatory region unaltered. For convenience, these two sets of alterations are referred to below as the repression tester and activation tester variant sequences, respectively.

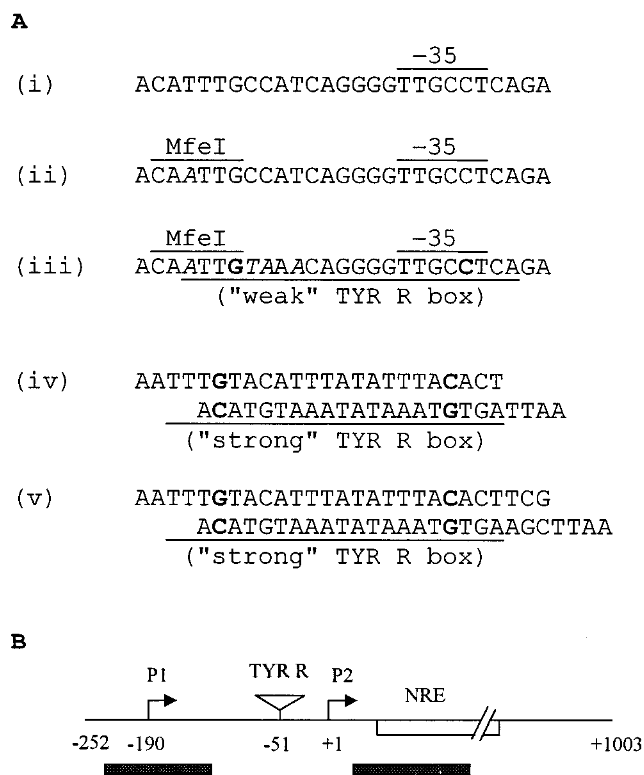

FIG. 1. Introduction of the TYR R box(es) near *proU* P2. (A) The nucleotide sequence upstream of the −35 region of the wild-type *proU* P2 promoter (i) and those following sequential site-directed mutagenesis to create first an *Mfe*I site (at position −51 relative to the start site of P2 transcription) (ii) and then a weak TYR R box (iii) are shown. The *Mfe*I and the −35 hexamer sequences are indicated, and the mutated base residues are in italics. Also shown are the pairs of annealed oligonucleotide sequences (iv and v) that were used to generate the double-stranded TYR R strong box sequences (identical to that in *tyrP*) flanked with 5′-AATT overhangs for construction of the repression tester and activation tester variants, following insertion into the *Mfe*I sites shown in sequences iii and ii, respectively. The 22-bp TYR R box sequences (whose consensus is the palindrome 5′-N_2**TGTAAA**N_6**TTTACA**N_2-3′, in which the residues shown in bold are invariant) are underlined, and the invariant residues are in bold. (B) Schematic depiction of the position of insertion of the TYR R strong box sequences in the two *proU* variants, relative to P1, P2, and the NRE. For nucleotide numbering, the start site of P2 transcription has been taken to be +1. Shaded bars indicate the regions of DNA curvature to which H-NS exhibits preferential binding.

The template used for the mutagenesis reactions was an M13 phage derivative bearing a *proU* fragment comprising P1, P2, and the NRE. This 1.26-kb *proU* fragment (see Fig. 1B) is identical to that earlier described for plasmid pHYD272 (7) and is known to carry all the *cis* elements involved in *proU* osmoresponsivity. Recombinant DNA manipulations were performed essentially as described previously (34). Site-directed substitution mutations were introduced by the method of Vandeyar et al. (44), using a commercially available kit from United States Biochemical Corp.

The sequence of wild-type *proU* P2 in the region of interest is shown in Fig. 1A, sequence i. To facilitate the introduction of the strong box sequence, a unique *Mfe*I site, CAATTG, was created by introducing a T-to-A substitution (underlined) 14

TABLE 1. β-Galactosidase expression in *tyrR366* strains from *lac* fusions to *proU* and its variants[a]

Plasmid	β-Galactosidase activity for:			
	hns$^+$		*hns-205*	
	−NaCl	+NaCl	−NaCl	+NaCl
pMU6441	0.5	1,450	34	1,582
pMU6442	1	351	68	720
pMU6443	<0.5	135	33	624

[a] Cultures of strains JP8042 (*tyrR366 hns*$^+$) and JP10939 (*tyrR366 hns-205*::Tn*10*) carrying the indicated plasmid derivatives were grown, for β-galactosidase assays, to mid-exponential phase in one-quarter-strength medium 56 (containing glucose [0.2%], thiamine [1 μg/ml], and trimethoprim [10 μg/ml]) without and with 0.3 M NaCl supplementation (−NaCl and +NaCl, respectively). Medium 56 was modified from the medium described by Monod et al. (25) and contains the following, per liter: K_2HPO_4 (10.6 g), $NaH_2PO_4 \cdot 12H_2O$ (6.1 g), $(NH_4)_2SO_4$ (2 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $Ca(NO_3)_2$ (10 mg), and $FeSO_4 \cdot 7H_2O$ (0.5 mg) (pH adjusted to 7.0). Enzyme specific activity values are reported in Miller units (24).

nucleotides upstream of the −35 hexamer (Fig. 1A, sequence ii). A weak TYR R box overlapping the −35 region was then created by site-directed mutagenesis of a CCAT sequence to TAAA (Fig. 1A, sequence iii). (The −35 hexameric sequence itself was left unaltered, although there is a natural match with the right arm of the palindromic TYR R box consensus at three of six positions and with the −35 region of *tyrP* at four of six positions.) The introduction of a strong TYR R box sequence flanked with 5′-AATT overhangs (shown in Fig. 1A, sequence iv) into the *Mfe*I site of sequence iii in Fig. 1A led to the creation of the repression tester variant, that is, with two TYR R boxes separated by 1 bp. On the other hand, the introduction of the strong TYR R box sequence flanked with 5′-AATT overhangs and containing an additional 3 bp (Fig. 1A, sequence v) into the *Mfe*I site of sequence ii in Fig. 1A resulted in the construction of the activation tester variant, that is, with the strong TYR R box positioned 18 bp upstream of the −35 hexamer.

After each step of mutagenesis, M13 phage clones carrying the correct mutation were identified by appropriate single-nucleotide sequence tracking. The complete sequence of the P2 promoter region for each of the two variants finally obtained was verified by automated DNA sequence analysis (data not shown). The structure and disposition of the regulatory elements in the *proU* variants constructed in this study are schematically depicted in Fig. 1B.

In order to undertake in vivo expression studies, the variant *proU* sequences were then subcloned upstream of the *lacZ* reporter gene in the very-low-copy-number trimethoprim resistance plasmid pMU2385 (46). The resultant plasmids were designated pMU6442 (with the activation tester variant sequence) and pMU6443 (with the repression tester variant sequence). As a control, plasmid pMU6441 was also constructed as a derivative of pMU2385 carrying the 1.26-kb wild-type *proU* regulatory region.

Effects of the TYR R box(es) on *proU* regulation in *hns*$^+$ and *hns* derivatives. The plasmids pMU6441, pMU6442, and pMU6443 were each transformed into a pair of isogenic *tyrR*$^+$ and *tyrR366* strains, JP7740 and JP8042, respectively, for *lacZ* expression studies. Both strains are prototrophic, Δ*lac*, and *recA* (46). In light of the role suggested for the H-NS protein in *proU* silencing, we also transformed the three plasmids into strains JP10938 (*tyrR*$^+$ *hns-205*::Tn*10*) and JP10939 (*tyrR366 hns-205*::Tn*10*), which are the *recA*$^+$ *hns* derivatives of JP7740 and JP8042, respectively. (The *hns* mutations were introduced by phage P1 transduction, with strain PD145 [8] serving as the donor.) The transformant derivatives were cultured in defined low- and high-osmolarity media supplemented when necessary with Tyr or Phe, and the specific activity of β-galactosidase in each culture was determined by the method of Miller (24). Each value reported is the mean of at least three independent measurements.

In order to test for the *cis* effects of the introduced sequence variations on *proU* osmotic regulation, we first determined the values for *lacZ* expression in the *tyrR* mutant derivatives (*hns*$^+$ and *hns*), that is, in which the possibility of a confounding effect caused by binding of TyrR was excluded. The results are presented in Table 1. In these *tyrR* host strains, supplementation of the culture medium with Tyr or Phe had no effect on β-galactosidase expression from any of the three plasmids (data not shown).

Under the conditions of growth and assay used in this study, we observed a >1,000-fold osmotic induction of wild-type *proU* expression in the *tyrR hns*$^+$ strain (Table 1). Neither *proU* variant (in pMU6442 or pMU6443) was affected in low-osmolarity-medium repression in the *hns*$^+$ strain; on the other hand, the expression levels in the NaCl-supplemented medium were lower than that for wild-type *proU* itself (Table 1). Nevertheless, at least a 250-fold osmotic inducibility was still observed for both mutant derivatives in the *hns*$^+$ strain. A possible explanation for this partial loss of osmoresponsivity is that the strong TYR R box insertions in pMU6442 and pMU6443 (27 and 24 bp, respectively) fortuitously introduce half-integral turns of the DNA helix in the region, which in an earlier study was shown also to be correlated with reduced expression of *proU* at a high osmolarity (41).

In the *tyrR hns* background, the expression profiles for the three plasmids were more or less similar to one another (Table 1). Consistent with the data from earlier studies (7, 10, 18, 22, 29), absence of the H-NS protein led to a moderate increase in *proU-lacZ* expression in low-osmolarity medium. All three plasmids exhibited residual osmotic inducibility in the *hns* strain, although once again the absolute values for *lacZ* expression from pMU6442 and pMU6443 in the NaCl-supplemented medium were less than that for the wild-type *proU* control.

We then measured the levels of *lacZ* expression from the three plasmids in the *tyrR*$^+$ strains (*hns*$^+$ and *hns*) to determine the regulatory role of TyrR on the mutant *proU* promoters. The results are presented in Table 2. In concord with earlier practice (2), the magnitude of TyrR-mediated regulation by the two coeffectors was calculated as the ratio of β-galactosidase activity in the *tyrR* mutant to that in the *tyrR*$^+$ strain in the presence of the particular coeffector (repression) or its reciprocal (activation).

As expected, *lacZ* expression from the wild-type *proU* regulatory region was not affected by TyrR at low or high osmolarities in either the *hns*$^+$ or *hns* background (compare the values for pMU6441 in Tables 1 and 2). Furthermore, even for the plasmids pMU6442 and pMU6443 in both the *hns*$^+$ and

TABLE 2. β-Galactosidase expression in *tyrR*$^+$ strains from *lac* fusions to *proU* and its variants[a]

Plasmid(s)	Coeffector	β-Galactosidase activity for:			
		hns$^+$		*hns-205*	
		−NaCl	+NaCl	−NaCl	+NaCl
pMU6441 (wild type)	None	1.3	1,471	29	1,683
pMU6442 (activation tester)	None	1	309	51	708
	Tyr	22	1,320	82	1,274
	Phe	198	2,352	626	2,411
pMU6443 (repression tester)	None	<0.5	133	17	448
	Tyr	<0.5	61	11	215
	Phe	<0.5	166	ND[b]	ND
pMU6443 and pMU1065 (repression tester and multicopy *tyrR*$^+$)	None	<0.5	100	ND	ND
	Tyr	<0.5	20	ND	ND

[a] Methods for growth and enzyme assays were as described in the footnote to Table 1. The plasmids were present in strain JP7740 (*tyrR*$^+$ *hns*$^+$) or JP10938 (*tyrR*$^+$ *hns-205*::Tn*10*). The coeffectors were present at a final concentration of 1 mM. Growth media for the strain derivatives carrying plasmid pMU1065 (46) were supplemented with kanamycin at 20 μg per ml. Enzyme specific activity values are reported in Miller units (24).

[b] ND, not determined.

hns derivatives, TyrR did not exert any significant regulatory effect during growth in the low- or high-osmolarity minimal media that were not supplemented with Phe or Tyr.

In the case of plasmid pMU6442 (bearing the activation tester variant of *proU*), we found that β-galactosidase expression was activated by TyrR in the presence of Phe, and less so in the presence of Tyr, in both the low- and the high-osmolarity media (Table 2). In the low-osmolarity medium, the magnitudes of activation mediated by TyrR-Phe for the *hns*$^+$ and *hns* strains were approximately 200- and 10-fold, respectively. The corresponding values for activation mediated by TyrR-Tyr were around 22- and 1.5-fold, respectively. The marked TyrR-mediated activation for pMU6442 could not be demonstrated for another related plasmid variant (designated pMU6445) in which the strong TYR R box was positioned 3 bp closer to the P2 promoter (data not shown).

A moderate level of TyrR-mediated repression in the presence of Tyr (around twofold) was demonstrated for plasmid pMU6443 (bearing the repression tester variant of *proU*) in the *hns*$^+$ strain at a high osmolarity and the *hns* mutant at both low and high osmolarities (Table 2). Repression in the *hns*$^+$ strain at a low osmolarity could not be demonstrated because of the very low levels of basal expression in these cultures. Repression was rendered more pronounced (6.8-fold) in the *hns*$^+$ strain additionally carrying a multicopy *tyrR*$^+$ plasmid pMU1065 (46) (Table 2). As expected, growth in the presence of Phe did not repress *lacZ* expression from pMU6443 in the *tyrR*$^+$ strain (Table 2).

Absence of correlation between intrinsic promoter strength and degree of Phe-mediated activation. The level of activation by TyrR-Phe of *proU* in plasmid pMU6442 is at least an order of magnitude higher than that reported earlier for *tyrP* or other genes for aromatic amino acid metabolism (even after optimization of spacing between the strong TYR R box and the −35 region). We considered the possibility that this difference (in degree of activation) merely reflects the fact that the promoter for *proU* is inherently weaker than the TyrR-activable promoters of the native TyrR regulon. This hypothesis is rendered more plausible by the data in Table 2, which reveal that even in *proU* the degree of activation is most pronounced when the level of basal expression is the lowest (that is, in the *hns*$^+$ strain grown in low-osmolarity medium).

We sought to test this hypothesis by creating a down-promoter mutation in *tyrP* and then examining the degree of activation by TyrR at the mutated promoter. For this purpose, the A residue (underlined) in the −35 hexamer (TTG<u>A</u>CG) of *tyrP* was converted to the noncanonical C, which is found in *proU* P2 (Fig. 1A, sequence i), by site-directed mutagenesis. The *tyrP* template into which this mutation was introduced is identical to one described in an earlier study (48) that has the strong TYR R box situated 18 bp upstream of the −35 region (that is, at a location optimal for studying activation).

The expression of the *lacZ* reporter gene on each of two isogenic plasmids, pMU6449 and pMU2055, carrying the mutant and wild-type *tyrP* promoter sequences, respectively, was then determined in transformants of JP7740 (*tyrR*$^+$) and

TABLE 3. β-Galactosidase expression from *tyrP-lac* fusions on plasmids pMU2055 and pMU6449[a]

Plasmid	*tyrP* −35 region (sequence)	β-Galactosidase activity for:			
		tyrR366	*tyrR*$^+$		
			MM	MM + Tyr	MM + Phe
pMU2055	Wild type (TTGACG)	130	138	850	1,560
pMU6449	Mutant (TTGCCG)	8	4	31	67

[a] The plasmids were present in strain JP8042 (*tyrR366*) or JP7740 (*tyrR*$^+$). Methods for growth and enzyme assays were as described in the footnote to Table 1, with the modification that the minimal salts medium (MM) used was prepared from half-strength medium 56. Tyr or Phe supplementation was at 1 mM. Enzyme specific activity values are reported in Miller units (24).

JP8042 (*tyrR*). Consistent with the results of earlier work (1), the wild-type *tyrP* promoter was activated 12- and 6.5-fold by Phe and Tyr, respectively, in the *tyrR*$^+$ host (Table 3). The mutant *tyrP* promoter exhibited a 16-fold reduction in basal expression in the *tyrR* strain, but the levels of activation supported by TyrR (8- and 4-fold with Phe and Tyr, respectively) were more or less similar to those for the wild-type promoter (Table 3). We therefore conclude that there is no correlation, at least in *tyrP*, between promoter strength and the magnitude of TyrR-mediated activation.

Conclusions. In this study, we have successfully designed and created modified *proU* regulatory regions that have now acquired an additional facet of activation or repression control by the TyrR protein and that still retain substantial osmoresponsivity in the *tyrR* mutant background. These results establish, for the first time, that appropriately positioned TYR R boxes are sufficient to confer TyrR-mediated regulation on a heterologous promoter in vivo.

Although the *proU* regulatory region used in this study carries two promoters, several lines of evidence suggest that osmoresponsivity and TyrR control are both exerted at promoter P2. (i) As mentioned above and reviewed earlier (6, 14), no role for P1 in normal *proU* osmotic regulation has yet been established. Mutations that abolish P2 promoter activity abolish *proU* expression. Conversely, *rpoS* mutations that abolish P1 promoter activity do not affect normal *proU* regulation. Furthermore, there is no evidence that transcription from P1 traverses past P2 into the NRE region (33). (ii) The placement of the TYR R box(es) in the activation tester and repression tester variant plasmids pMU6442 and pMU6443, respectively, was designed specifically to exert regulation at the P2 promoter. (iii) Finally, the osmoresponsivity of *lacZ* expression from plasmids pMU6442 and pMU6443 was not affected in an *rpoS*::Tn*10* mutant (data not shown), thereby excluding a role for the P1 promoter in such regulation.

The striking finding in this study was the 200-fold stimulation of *proU* expression at a low osmolarity achieved with TyrR-Phe in the activation tester variant. The fact that the binding of the TyrR protein dimer to the strong TYR R box is constitutive, that is, independent of Phe (2, 4, 31, 35), with the latter merely serving to convert the bound protein into an active conformation for the recruitment of RNA polymerase, allows us to make two inferences: (i) the strong TYR R box upstream of P2 is accessible for TyrR protein binding even at a low osmolarity, and (ii) TyrR binding by itself (in the absence of Phe) has no effect on *proU* repression under these conditions. Our results therefore indicate that if silencing does occur at the *proU* P2 promoter, it does not extend to this upstream TYR R box region.

Our findings may also be important for an understanding of antirepression as a mechanism of activation of gene expression in *E. coli*. An antirepressor may be operationally defined as a factor which promotes transcription by interfering with a system of repression. Antirepression may be said to exist when the magnitude of transcriptional activation mediated by the factor is higher in the presence of a particular repressing condition than in its absence. Examples of transcriptional activation by RNA polymerase recruitment and antirepression may not be mutually exclusive.

Several instances in which DNA-binding regulator proteins act as antirepressors of H-NS in mediating transcriptional activation are known. These include cyclic AMP-cyclic AMP receptor protein for the divergently transcribed promoters in the *pap* locus (11) and perhaps too for *bgl* (26, 39), CfaD for the promoter of the *cfaABCE* operon (17), IHF for the early promoter of phage Mu (45), and FIS for the P1 promoter of each of the rRNA operons (42) and perhaps for the *hns* promoter itself (9). In each case, it has been assumed that binding of the specific regulator protein to DNA directly alters the nucleoprotein topology in a manner that renders H-NS incapable of repression.

Earlier results obtained with TyrR also suggest that the protein acts as an antirepressor of HU and IHF in mediating activation at the *mtr* and *tyrP* promoters (48). In the present study as well, we found that the magnitude of TyrR-mediated activation of *proU* in pMU6442 at a low osmolarity, in the presence of either Tyr or Phe, is much higher in the *hns*$^+$ strain (where H-NS serves to repress *proU* expression) than in the *hns* mutant (Table 2). Therefore, TyrR fulfils the operational definition of an antirepressor of H-NS in this situation. Yet, as argued above, TyrR binding by itself (in the absence of Phe) does not alter the repressive nucleoprotein topology at *proU* during growth in low-osmolarity medium. Therefore, our findings implicate, for the first time, recruited RNA polymerase as a component in the mechanism of antirepression.

Finally, the results in Table 3 also indicate that the substantially enhanced magnitude of stimulation at *proU* by TyrR-Phe may not simply be a consequence of *proU* bearing a weaker promoter than that of *tyrP*. One could speculate, therefore, that this difference is a reflection of the relative degrees of basal repression to which different promoters, including those of the native TyrR regulon (48), are subjected by the binding of the nucleoid proteins.

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