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Ploughing a lonely furrow: the curious case of the P1 promoter in the osmotically regulated *proU* operon of *Escherichia coli**

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Abstract

Evidence is reviewed for the existence of a σ^s-controlled promoter for the osmotically regulated *proU* operon in *Escherichia coli* and *Salmonella typhimurium*. Expression from the promoter is rendered cryptic in both the organisms, most likely by a mechanism of factor-dependent termination of transcription (attenuation) in the region upstream of the structural genes. The possible role of P1 in *proU* expression and regulation is discussed.

Keywords: Osmoregulation, *proU*, σ^s-controlled promoter

1. Osmoregulation

The term osmoregulation is used to refer to the set of physiological processes by which organisms adapt to survive and grow in environments of varying water activity. In unicellular organisms, such as bacteria and yeasts as also in plants, a decrease in water activity of the environment (that is, a hyperosmotic stress) results in an equivalent decrease in water activity of the cytoplasm; and the purpose of osmoregulation is then to achieve restoration of cell volume and turgor by cytoplasmic accumulation of 'compatible solutes' so as to restore osmotic balance between intra- and extracellular compartments without affecting vital cell processes such as protein synthesis or DNA replication.[1]

2. Introduction to *proU* regulation

One genetic locus that is involved in osmoregulation in *Escherichia coli* is *proU*. The *proU* operon encodes a binding protein-dependent transport system that is involved in active transport and cytoplasmic accumulation of compatible solutes, glycine betaine and L-proline during growth of *E. coli* in media of elevated osmolarity. The remarkable feature about *proU* is that its transcription is induced several hundred fold during growth in a high-osmolarity medium, making it by far the most osmoresponsive of all genes known.[2, 3] However, our understanding of the mechanism(s) of osmotic regulation of *proU* transcription is far from complete.

A schematic depiction of *cis* regulatory elements in *proU* regulation, based on data from studies in our laboratory, is given in Fig. 1. We have identified in *E. coli* a σ^{70}-controlled pro-

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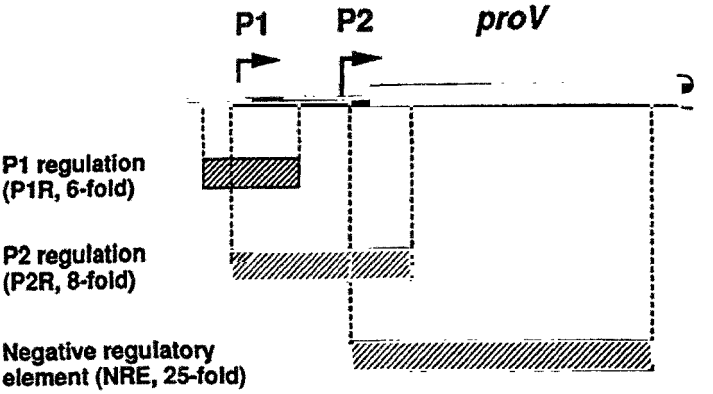

FIG. 1. Schematic depiction of *cis* regulatory elements in *proU* regulation: P1R, sequence conferring osmotic regulation on isolated P1 promoter; P2R, sequence conferring osmotic regulation on isolated P2 promoter; and negative regulatory element (NRE), sequence situated downstream of P2. Magnitude of osmotic regulation contributed by each element is indicated in parentheses. The positions of promoters P1 and P2 and the first structural gene *proV* are marked.

moter for *proU*, situated approximately 70 base pairs (bp) upstream of the first structural gene *proV*, which is required for *proU* expression.[4, 5] We have also identified a *cis*-acting negative regulatory element (NRE), extending over a region of approximately 600 bp and situated downstream of the promoter (overlapping *proV*), deletion of which results in a 25-fold derepression of *proU* expression at low osmolarity.[6] Constructs which carry the *proU* promoter without the NRE continue to exhibit an eight-fold osmotic regulation of transcription, indicating the existence of multiple mechanisms that contribute to *proU* osmoresponsivity.[6] Substantially similar results have been obtained by other groups subsequently working with the closely related bacterium *Salmonella typhimurium*.[7, 8]

2.1. *The proU P1 promoter*

In initial experiments employing primer-extension analysis on total cellular RNA, our group had also identified an additional presumptive promoter for *proU*, 190 bp farther upstream of and transcribed in the same direction as that described above.[4] For convenience in description, we have designated this additional promoter as P1 and the promoter that is more proximal (to the structural genes) as P2. By appropriate deletion and subcloning experiments, we have obtained a fragment of *proU* that carries only the P1 promoter region in the absence of P2. We have also demonstrated that this fragment is able to drive expression of a *lacZ* reporter gene *in vivo* and that such expression is moderately osmotically inducible (around 6 fold); primer-extension analysis was used to confirm that the promoter activity identified in this fragment was the same as that of P1.[5] We had earlier interpreted these results as indicative of the existence of a third independent and additive mechanism contributing to overall osmoresponsivity

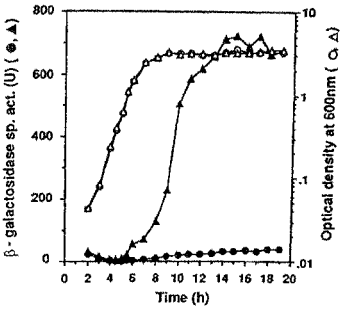

FIG. 2. RpoS-dependent induction of P1 in stationary phase. Derivatives of *rpoS*$^+$ (Δ, ▲) and *rpoS* (○, ●) strains carrying a P1-*lac* fusion construct were grown in batch cultures, and the optical density (open symbols) and specific activity of β-galactosidase (closed symbols) for each was plotted as a function of time of incubation. Note the marked induction of *lac* expression in the *rpoS*$^+$ derivative at the transition from late exponential to stationary growth phase. Reprinted from Manna and Gowrishankar.[9]

of *proU* (Fig. 1), but more recent data (see later part) may necessitate a rethinking on this question.

In a search designed to obtain *trans*-acting mutations that result in reduced expression of both a *proU-lac* fusion and the native *proU*$^+$ locus in the same cell, a mutation was identified by Manna and Gowrishankar[9] that was shown subsequently to virtually abolish expression from the P1 promoter-bearing fragment. The mutation was mapped to the *rpoS* gene, encoding the alternative stationary-phase sigma factor (σ^S) of RNA polymerase in *E. coli*. Based on this finding, we examined the modulation by growth phase of expression from the isolated P1 promoter and were able to show that the promoter exhibits strong stationary phase induction (about 100 fold) *in vivo* in an RpoS-dependent manner (Fig. 2).[9] *In vitro* transcription experiments were used to confirm that P1 is indeed transcribed by σ^S-bearing RNA polymerase holoenzyme, as opposed to P2 which is transcribed by its σ^{70}-bearing counterpart.[5]

3. A lonely furrow...

Despite the wealth of evidence obtained by us on P1 promoter in *E. coli*, its study has evinced little interest from other research groups and its role in expression, let alone osmotic regulation, of the *proU* locus remains an enigma.[3] First, *cis* constructs of *E. coli proU* bearing only P2 and NRE without P1 promoter behave no different from constructs that also include P1, for both expression and regulation of a downstream reporter gene.[10] Second, in contrast to the isolated P1-promoter fragment, constructs comprising P1, P2 and NRE do not exhibit any growth phase

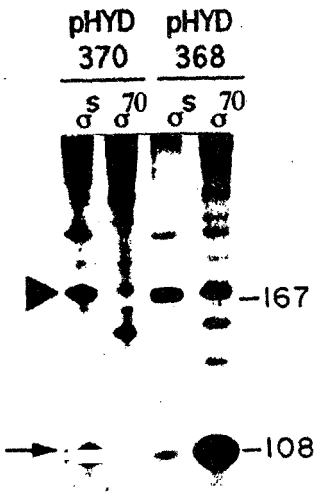

FIG. 3. *In vitro* transcription from plasmid templates carrying the P1 promoter of *E. coli* (pHYD368) and of *S. typhimurium* (pHYD370), with RNA polymerase core enzyme reconstituted with either σ^s or σ^{70}. Arrow and arrowhead denote, respectively, the RNA-I transcript from the plasmid vector and transcript from *proU* P1. The positions of migration of two RNA size markers (167 and 108 bases) are depicted on the right. Reprinted from Rajkumari *et al.*[13]

dependence of transcriptional expression, nor is the magnitude of such expression affected in an *rpoS* mutant.[9] Third, a mutation in the –10 or –35 region of P2 (in any construct carrying both promoters) is sufficient to abolish all expression.[11, 12] Finally, a DNA fragment from *S. typhimurium*, equivalent in size and position to that from *E. coli* described above as bearing the isolated P1 promoter, exhibited no promoter activity *in vivo* even in *rpoS*$^+$ strains.[7, 13]

The question that therefore arises is: is P1 an irrelevance, a mere artefact of the experimental manipulations that led to its original identification? Such a conclusion might be somewhat of an extreme position, a verdict whose severity is not in proportion to the magnitude of the crime! However, I must also admit to a degree of partiality for this orphan promoter.

4. P1 as a cryptic promoter

Recent work from our group has provided at least a partial explanation for the inability to detect the P1 promoter equivalent in *proU* of *S. typhimurium*.[13] Our results indicate that *S. ty-*

phimurium proU does indeed bear a P1 promoter which is specifically recognized by σ^S-bearing RNA polymerase during *in vitro* transcription, and as efficiently as the *E. coli* P1 promoter itself (Fig. 3). Furthermore, we have also demonstrated that a 22-bp deletion mutation downstream of the *S. typhimurium* P1 (between +63 and +84; Fig. 4), in the fragment that is otherwise inactive for promoter activity *in vivo*, is able to confer RpoS-dependent *lacZ* reporter gene expression *in vivo* from the P1 promoter. Finally, we have shown that RpoS-dependent P1 promoter activity even from the 'inactive' wild-type fragment is detectable *in vivo* by experiments of primer extension analysis on total cellular RNA, but only with a primer situated proximal to +60 and not with the one situated distal to +85 (primers P and D, respectively, in Fig. 4); on the other hand, P1 promoter activity from the mutant fragment was detectable with either primer.[13]

The above results indicate that *S. typhimurium* does also have a P1 promoter upstream of *proU* but is cryptic *in vivo*. Our observations are consistent with a model in which promoter crypticity is conferred by a novel mechanism of attenuation (that is, premature termination of transcription) that operates at a site around 70 bp downstream of P1. The fact that attenuation did not occur in a purified *in vitro* transcription system suggests that the mechanism of transcription termination may require the mediation of an additional factor present within the cells.[13]

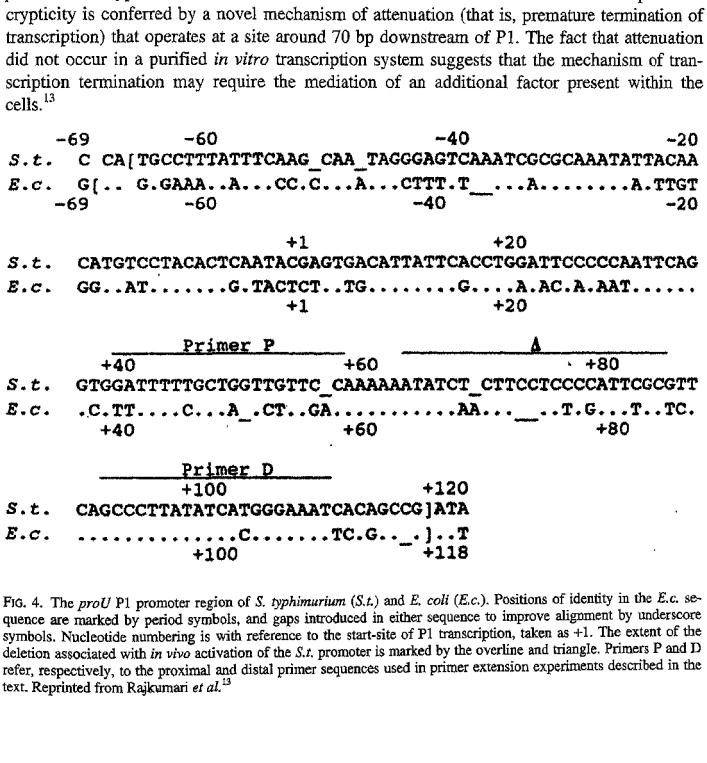

FIG. 4. The *proU* P1 promoter region of *S. typhimurium* (*S.t.*) and *E. coli* (*E.c.*). Positions of identity in the *E.c.* sequence are marked by period symbols, and gaps introduced in either sequence to improve alignment by underscore symbols. Nucleotide numbering is with reference to the start-site of P1 transcription, taken as +1. The extent of the deletion associated with *in vivo* activation of the *S.t.* promoter is marked by the overline and triangle. Primers P and D refer, respectively, to the proximal and distal primer sequences used in primer extension experiments described in the text. Reprinted from Rajkumari *et al.*[13]

I would like to suggest that the results obtained with P1 in *S. typhimurium* may hold a clue to our understanding of the role of P1 in *E. coli* as well. As described above, even the *E. coli* P1 promoter does not appear to contribute to *proU* expression *in vivo* from constructs that include additional downstream sequences such as P2, although active P1 transcription is demonstrable from such constructs *in vitro*.[13] Recent work from our laboratory has also shown that in comparison with the *E. coli* DNA fragment carrying the *in vivo* active P1 promoter, another fragment which carries just 30 bp of additional downstream sequence exhibits considerably reduced promoter activity *in vivo*.[14] Thus, it appears most likely that transcription from P1 in *E. coli* is also subject to attenuation *in vivo*, albeit at a site about 100 bp downstream from that in *S. typhimurium*.

5. Conclusions

There is conclusive evidence for the existence of a σ^S-controlled P1 promoter upstream of the *proU* structural genes in both *E. coli* and *S. typhimurium*. With reference to *proU* expression, however, this promoter appears to be cryptic and promoter crypticity is most likely a consequence of attenuation of transcription occurring in the region between the promoter and the structural genes. The major unanswered questions are the identity of the factor(s), if any, that mediates the attenuation of transcripts from P1, and the environmental conditions, if any, that modulate the efficiency of attenuation (in other words, are there conditions in which preferential read-through of transcripts from P1 might occur into the *proU* structural genes?). Alternatively, is transcription from P1 involved in modulating the rate of transcription from P2? When the answers come in, it is my belief that the curious case of the *proU* P1 promoter may be poised to reveal truths that are stranger than the fiction of its genre.

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