OSMOREGULATION IN *ENTEROBACTERIACEAE*: ROLE OF PROLINE/BETAINE TRANSPORT SYSTEMS

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ABSTRACT

In a wide variety of organisms, L-proline and glycine betaine are amongst the compounds accumulated intracellularly as compatible solutes to counteract the effects of turgor reduction during growth in water-stressed environments. Two osmoregulatory transport systems, ProP and ProU, have been identified and characterized in the enterobacteria, each of which participates in the active concentration of both L-proline and glycine betaine from the culture medium in response to osmotic stress. The expression of genes encoding the components of the ProU porter is induced 400-fold upon growth in high-osmolarity medium; elucidation of the molecular mechanisms underlying such regulation would possibly enable understanding of the large class of processes that involve transduction of mechanical signals to chemical ones within biological systems. A complete understanding of the molecular basis by which ProP and ProU function in osmoregulation would also provide insight into the mechanisms of similar adaptation at the cellular level in the economically important genera of microbes and higher plants.

INTRODUCTION

In contrast to the situation observed in higher animals, wherein the maintenance of iso-osmolarity of the 'internal milieu' is one of the central features of homeostasis, all microbial cells and most plant cells are directly affected by changes in osmolarity of the environment; such cells have evolved strategies for adaptation that permit them to adjust to and grow in media within a range of osmolarity. Exposure of cells to an environment of high osmolarity leads to an immediate loss of intracellular water and a concomitant decrease in cell turgor [defined as intracellular osmotic pressure (π_i) − extracellular osmotic pressure (π_e)]. It is widely believed that the consequence of turgor loss is an inhibition of membrane-associated functions (such as respiration and transport) and therefore of growth[1], and that osmoregulation depends upon the accumulation of intracellular solutes in molar quantities so that π_i is incremented and cell turgor is restored[2–5].

Intracellular accumulation of specific solutes in response to osmotic stress might be expected to occur by increase either in biosynthesis or in active uptake. Substances that have been so identified include K^+ ions, L-proline, glutamate, γ-aminobutyrate, polyols and glycine betaine[2–4,6–8]. With the exception of K^+, these substances are also referred to as compatible solutes[2], because they are believed not to be inimical to macromolecular synthesis and function even when present in molar concentration within the cell. Even in the case of K^+, a recent report[9] indicates that under conditions of osmotic stress (when intracellular $[K^+]$ exceeds 0.9M), DNA-protein interactions remain unaffected, notwithstanding the fact that these interactions *in vitro* are extremely sensitive to ionic strength.

It has been noted that diverse organisms, amongst both microbes and plants, share the same limited number of compounds for the role of turgor-restoration in osmoregulation[3]; the mechanisms involved in turgor-sensing, or by which these substances accumulate intracellularly in response to osmotic stress are, however, little understood. It is interesting that the same set of compatible solutes accumulate also in cells of the renal inner medulla, which is

the only tissue in mammalian systems that is also constantly exposed to a hypertonic milieu[10].

In understanding the mechanisms involved in *any* physiological function, genetic studies have played a very important role. The classical genetic strategy to investigate any metabolic function would be to obtain mutants defective in their ability to exhibit that particular function, and then to examine the genes and functions affected by the mutations in these strains. In a study of osmoregulation, however, such a strategy suffers from a unique disadvantage arising out of the fact that osmolarity, like temperature, is a physical parameter of the environment that can influence the conformation of proteins in solution; consequently, mutants identified as osmosensitive are much more likely to harbour conditional-lethal missense mutations in any of a large number of essential genes (such as those for RNA polymerase, DNA gyrase, etc.) such that the gene product is functional in low-osmolarity medium and non-functional at elevated osmolarity, than they are to have mutations affecting adaptation to osmotic stress itself. Osmotic-remedial mutations of the former kind have indeed been described[11–13], and their preponderance in any search for osmosensitive mutants renders the study of osmoregulation itself by this approach far more difficult.

Alternative genetic strategies have therefore been tried, including one that has made use of the technique of gene fusions to identify so-called osmoresponsive genes, that is, genes whose expression is altered when the osmolarity of the growth medium is varied. The expectation has been that genes important in osmoregulation would be osmoresponsive with regard to their own expression, and would therefore be identified by such an approach. A large number of osmoresponsive genetic loci have in fact been mapped independently by several workers[14–21], and the challenge subsequently has been to determine the functions for each of them, a task which necessarily is an empirical one. This and other approaches, nevertheless, have in the last few years led to the identification in the enterobacteria of transport systems for K^+[14], choline[22], L-proline[15,16,20,23,24], and glycine betaine[8,17,18], and of a pathway for synthesis of glycine betaine from choline[22,25], all of which are activated under osmotic stress conditions.

This review is concerned with the transport systems for L-proline and glycine betaine in the *Enterobacteriaceae*, with particular emphasis on their role and participation in osmoregulation; the other genetically characterized systems for transport of K^+ and choline, and for conversion of choline to betaine, have been reviewed recently[26,27].

OSMOPROTECTION BY L-PROLINE AND GLYCINE BETAINE

Members of the family *Enterobacteriaceae*, including *Escherichia*, *Salmonella*, *Serratia* and *Klebsiella*, are typical of a large number of non-halophilic microorganisms that are able to adapt to growth in environments whose osmolarity varies from 0 milliosmolal (mOsm) to about 1200 mOsm (the latter corresponding to minimal salts medium containing 0.5 M NaCl). Growth in high-osmolarity media (> 500 mOsm) is promoted, and the upper limit of osmotolerance is increased (up to 0.8 or 0.9 M NaCl) in the presence of submillimolar concentrations of compounds such as L-proline, glycine betaine or proline betaine (figure 1) in the medium, under both aerobic and anaerobic growth conditions[8,15,23–25,28–35]. These compounds are therefore referred to as osmoprotectants.

What is the property by which glycine betaine and L-proline exhibit osmoprotectant activity? One possibility is that these two compounds, when present in low concentrations in the growth medium, are capable of being actively concentrated within cells subjected to osmotic stress and that they then function as inert, intracellular compatible solutes tending to restore cell turgor and thereby promoting growth[4,8,23]. Two additional suggestions have been put forward in explaining osmoprotection by glycine betaine and L-proline. One is that their intracellular

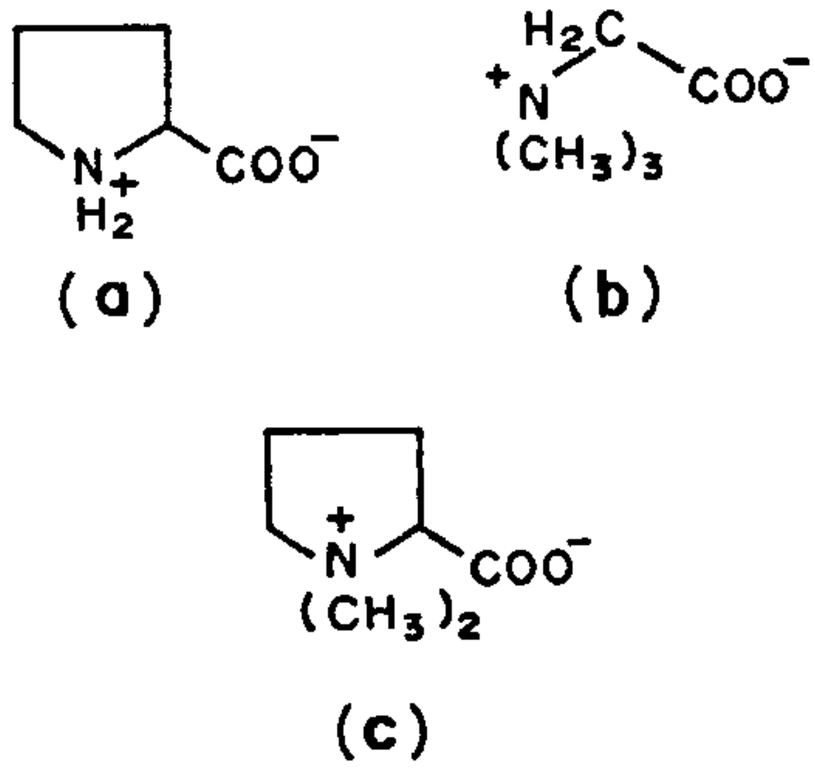

Figure 1. Compatible solutes: (**a**) L-proline, (**b**) glycine betaine, and (**c**) proline betaine (stachydrine).

accumulation might enable the cell to decrease the otherwise elevated concentration of K^+ within for maintenance of turgor, and that a lower [K^+] under these conditions has a growth-promoting effect[36,37]. The other is that both L-proline and glycine betaine in high concentration have the ability to stabilize the conformation and function of proteins in solutions of high ionic strength, perhaps by an effect of increasing the .local water activity around individual protein molecules under these conditions[3,38]; the physical properties of such solutions, however, is as yet incompletely understood, and this represents a fertile area for future experimental work.

All the considerations above are based upon the premise that the osmoprotectant compounds are actively transported into the cytoplasm of cells exposed to water stress. It is the recent identification of osmoresponsive transport systems for these substances that has excited much interest.

TRANSPORT SYSTEMS FOR L-PROLINE

Three distinct proline porters (PutP, ProP and ProU, encoded respectively by the *putP*, *proP* and *proU* loci) have been recognized by both genetic and physiological criteria in *Escherichia coli* and *Salmonella typhimurium*.

PutP: PutP represents a major proline permease in these organisms (apparent $Km = 2\ \mu M$), and is involved in the Na^+-coupled transport of L-proline for utilization as C or N source in growth[39–43]. The expression of *putP* is induced by exogenous L-proline[39,40], independent of osmolarity of the growth medium[44]. PutP does not play a role in osmoregulatory L-proline transport, and mutants in *putP* have been shown to be as proficient in their ability to be osmoprotected by this compound as are $PutP^+$ strains[23,24,30,44]. This may partly be attributed to the fact that induction of PutP expression by L-proline is associated also with induction of the catabolic proline dehydrogenase (encoded by *putA*) which would tend to decrease the intracellular L-proline levels. However, even in *putA* mutant strains, PutP does not contribute to cytoplasmic accumulation of L-proline during osmotic stress, probably because of an inhibition of porter activity under these conditions[44]. Such inhibition, which is demonstrable after growth in sucrose-containing media and probably reflects osmotic modulation of proter activity, is to be differentiated from inhibition that would be expected after growth in high [Na^+]-containing medium because of the Na^+ symport mechanism of functioning of the PutP transporter[43].

ProP: The second proline permease, ProP, has an apparent K*m* for L-proline uptake of 300 μM; it is inactivated by mutations at a locus, *proP*, that is presumed to represent the structural gene(s) for the porter[45–47]. *proP* expression is induced by amino acid limitation[46,47] and independently by growth in high-osmolarity medium[16,17,24,44], and L-proline uptake activity of ProP is also stimulated upon assay in medium of elevated osmolarity[16,24,44]. It has been shown with the aid of *proP-lac* operon fusions that osmoresponsive induction of ProP expression occurs at the transcriptional level, over a 2- to 3-fold range[16,17,20].

ProP shares with ProU (as discussed below) a participatory role in the exhibition of the osmoprotectant effect of L-proline; only the ProP system, however, is involved in mediating the osmoprotectant effect exhibited by an analogue of proline, 5-hydroxy L-pipecolic

acid[20]. Presumably, the latter compound serves as substrate for ProP but not the ProU transporter.

Kaback and Deuel[48] had earlier shown that L-proline uptake by washed membrane vesicle preparations of *E. coli* is also stimulated in a medium of elevated osmolarity. This observation could not have represented ProU-mediated transport because, as discussed below, ProU is a periplasmic binding protein-dependent porter; if their data are indicative of uptake through ProP, it would appear that the latter is entirely an inner-membrane porter, and also that its proline-transport activity is stimulated in high-osmolarity conditions.

ProU: The third porter, ProU, appears to be a very minor proline permease in comparison with the PutP and ProP transport systems; in fact, some workers have been able to show little or no [^{14}C]L-proline uptake in *putP proP* double mutants[18,24,44]. This inability might, however, reflect limitations in the assay procedure of filter-retention and wash employed, as another group had demonstrated ProU-mediated uptake of L-proline by the technique of flow dialysis[16]. The growth phenotype of *proU* mutants also clearly indicates that ProU is involved in transport of and osmoprotection by L-proline[15,18,19,23,24,49].

Operon fusion studies have shown that ProU is expressed at very low levels during growth in low-osmolarity medium, and that it is induced (at the transcriptional level) immediately after osmotic 'upshock' to reach levels that are 100- to 400-fold higher during growth at the new steady-state[15,16,18,19,21]. Thus, osmoresponsivity of *proU* expression is very much more marked than that of *proP*, but it is as yet not clear whether the activity of ProU with respect to L-proline uptake is also stimulated (akin to ProP) when assayed in high-osmolarity medium.

Although intracellular accumulation of exogenous L-proline has osmoprotective effect, the biosynthesis of this compound itself is not appreciably altered in cells growing at elevated osmolarity[30,50]. One mutation in the proline biosynthetic locus has been characterized that leads to constitutive elevated L-proline synthesis and confers osmotolerance when it is introduced into different strains of enterobacteria[30,31,51]. In this instance too, it is believed that the constitutive synthesis of L-proline merely substitutes for an exogenous supply of the amino acid, and that it is the induction and activation of the osmoregulatory proline porters at high osmolarity which serves to maintain elevated intracellular levels[30,51].

ProP AND ProU ALSO REPRESENT BETAINE TRANSPORT SYSTEMS

Cairney *et al*[17,18] were the first to demonstrate that both ProP and ProU serve also as transport systems for glycine betaine; the kinetic parameters obtained by them for glycine betaine uptake in cells grown and assayed in high-osmolairty medium were: K_m = 44 μM and V_{max} = 37 nmol min^{-1} mg^{-1} for ProP, and K_m = 1.3 μM and V_{max} = 12.5 nmol min^{-1} mg^{-1} for ProU. Although unexpected, their initial findings have since been confirmed and extended by several other workers, and ProP and ProU have indeed been shown to mediate the osmoprotectant effect of glycine betaine as well as that of L-proline discussed above[19,44,49,52]. The existence of transport systems common for glycine betaine and L-proline has been documented earlier in animals[53,54].

Just as for uptake of L-proline described above, the activity of the ProP transport system for uptake of glycine betaine is also stimulated several fold when transport assays are done in high-osmolarity medium, in comparison to the values obtained in low-osmolarity medium[17]. A comparison of the kinetic parameters for uptake by ProP of L-proline and of glycine betaine would suggest that this porter has higher affinity for transport of glycine betaine than of L-proline; such comparisons, however, must be done with caution, considering that these values have been calculated from measurements made on different strains in two different laboratories[17,46], and that only limited data are available.

Cairney *et al*[17] suggested that the specificity of the ProP transporter is altered by change in

osmolarity of the assay medium: that in low-osmolarity conditions, it transports exclusively L-proline whereas at elevated osmolarity, its affinity for glycine betaine is enhanced while that for L-proline is reduced considerably. On the other hand, Milner *et al*[44] studied the inhibition of ProP-mediated L-proline uptake by varying concentrations of glycine betaine, and concluded that there is no change in substrate specificity of this porter either after growth or upon assay in high- or low-osmolarity conditions. Their results also suggest that both L-proline and glycine betaine share roughly equal affinities as substrates for the ProP porter. A more extensive kinetic analysis will perhaps be required in resolving this question.

In the case of the ProU porter, it is clear from the kinetic data that it is a high-affinity transport system for glycine betaine[18,19]; competition experiments suggest that proline betaine (stachydrine, figure 1) is almost equally effective as substrate whereas L-proline itself is very weak in this regard[8,44]. As would be expected from the results on induction of *proU-lac* expression, ProU-mediated uptake of glycine betaine is demonstrable only in cells grown in medium of elevated osmolarity[18,19]; even after induction, the transport activity is seen only upon assay in high-osmolarity buffer, which has been interpreted as indicative of the fact that ProU is active as a porter only at elevated osmolarity. The latter result may, however, be artefactual in that Milner *et al*[44] have shown a general inhibition of transport of a variety of amino acids when cells grown in high-osmolarity medium are assayed after osmotic 'downshock', and also in that the periplasmic binding-protein of the ProU porter (see below) might have been inactivated or lost after such treatment. This question can be more appropriately addressed in the mutants now available that exhibit constitutive expression of *proU* even upon growth in low-osmolarity media[49].

That osmoprotection by L-proline and glycine betaine is mediated through the same porters is supported also by the observation that there is no additivity in their effects when strains are grown in high-osmolar medium in the presence of both of them as exogenous substrates[32]. Furthermore, the osmotolerance of strains carrying the mutant proline-biosynthetic gene that results in constitutive synthesis of L-proline is also not increased with the presence of glycine betaine in the growth medium[32].

Are there osmoregulatory transport systems for glycine betaine other than ProP and ProU? This possibility is still open, because *proP proU* double mutants continue to exhibit the osmoprotectant activity of this compound (but not of L-proline), albeit at a reduced level[32]. Additional genetic studies would be required to identify such other transport systems that might exist for this function.

It is known that glycine betaine is more effective than is L-proline as an osmoprotectant in the enterobacteria[8,17]. In the context of the discussion above, this has been explained in part on the much higher affinity of ProU for the former as substrate, and in part on the induction of the catabolic proline dehydrogenase when L-proline is present as osmoprotectant[44]; both these factors will tend to reduce the intracellular concentration of L-proline as compatible solute in comparison to that achievable by glycine betaine. On the other hand, Higgins, Booth and co-workers[36,37] suggested that the greater osmoprotective effect of glycine betaine is a consequence of its being superior to L-proline in stabilizing protein structure in solutions of low water activity; there is limited physical evidence to support this suggestion[55], but data from some other experiments are equivocal in this regard[56] and this question must still be regarded as an open one.

MOLECULAR STUDIES ON ProU

A major advantage of bacterial genetic studies is the ease and facility with which one can undertake molecular characterization of genes and their products that have originally been identified by classical techniques of mutant isolation and mapping. Two questions of interest that may be asked of the osmoregulatory transport systems above refer to the manner in which they function as porters and

to the mechanisms by which osmoresponsivity of their expression and activity is effected. Until now, both questions have been partially addressed in respect only of the ProU porter.

proU organization and gene-protein relationships: Bremer and co-workers[19,57] and Higgins *et al*[58] have shown respectively in *E. coli* and *S. typhimurium* that the *proU* locus encodes a glycine betaine-binding periplasmic protein of M_r approximately 32000. Neither group has reported on the ability or otherwise of this protein to bind L-proline. The identification of a periplasmic binding-protein as a component of the ProU porter has important implications for its possible structure, and for the mechanism of active transport through this porter.

In gram-negative bacteria, transport systems that each include a periplasmic binding-protein constitute a distinct class of transporters with several shared features[59,60]. For example, each of them is a multicomponent porter that includes a binding-protein and three or four additional proteins that are either integrated in or associated with the inner membrane, the genes for all of which are organized as part of a single operon; primary structure-homology is evident between the membrane proteins of different transport systems within this class[61,62]. It appears that the energization of active transport through these porters is not by the proton-motive force, but by phosphate-bond energy. These features are likely also to apply to the ProU porter.

The *proU* locus of *E. coli* has been cloned on multicopy plasmids, and its function shown to reside on a segment of DNA approximately 4 kilobase-pairs long (figure 2)[52]. An earlier study had suggested that the locus is comprised of two cistrons[52]; more recent evidence indicates that it consists of at least three genes organized as part of a single operon (C.S. Dattananda, unpublished; E. Bremer, unpublished, cited in ref. 19). The product of the first gene has been identified as a 44-kilodalton protein; the size of this protein is truncated to 42-kilodaltons in plasmids carrying

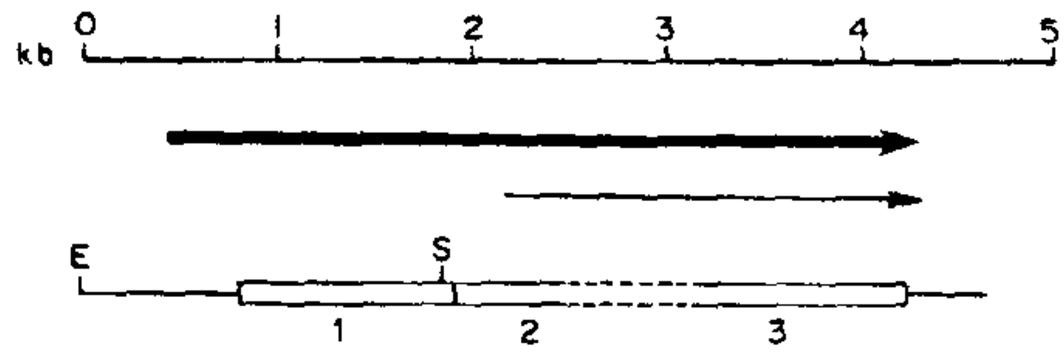

Figure 2. Molecular organization of the *proU* locus in *E. coli*. The DNA encoding ProU is represented on the lower line, with the protein-coding regions of genes 1, 2 and 3 designated by the open boxes. The interrupted-line segment indicates the extent of uncertainty (from genetic complementation studies) in the demarcation between genes 2 and 3; it is also possible that this portion encodes an additional structural gene. Two unique restriction enzyme cleavage-sites are marked: *Eco*RV(E) and *Sal*I(S). The thick arrow represents the direction and extent of the major transcript (under osmotic control) from this locus; the thin arrow represents transcription from a constitutively expressed, weak internal promoter. A kilobase-pair (kb) scale is included.

chromosomal DNA only to the left of the *Sal*I site in figure 2 (K. Rajkumari, unpublished), but despite such truncation, it is still able to effectively substitute for the native protein in mediating osmoprotection by glycine betaine and L-proline[52]. The second gene in the operon encodes a 37-kilodalton protein, and the periplasmic binding-protein described above is the product of the third gene (Dattananda, unpublished). Given the sizes of DNA comprising the *proU* locus and of the three proteins identified as its products, it seems unlikely that there is a fourth gene in the operon. Mutations in this locus that block expression only of the periplasmic protein result in abolition of osmoprotection by both glycine betaine and L-proline, suggesting that it indeed functions as binding protein for both substrates.

That the gene for the binding-protein is the last of three in the *proU* operon is interesting, because it represents the lone exception to the general observation in other members of this class that the gene encoding the binding-protein is the first within the transcription unit[60]. The first-gene arrangement in each of these cases has been explained as one arising out of the necessity that the binding-protein be expressed in greater molar proportion than the membrane components of the porter, and that

this is best achieved by a mechanism of premature termination of a given percentage of transcripts after the first gene in the operon has been transcribed[59,60]. In the case of the ProU porter, it is possible that the binding-protein is not expressed in excess of the other components, or that other mechanisms are operative in permitting the preferential expression of the third gene. An additional constitutive promoter has indeed been localized within the second gene that is active only for synthesis of the binding-protein (figure 2), but it is far too weak to be of physiologic significance in strains with haploid dosage of the *proU* locus (Dattananda, unpublished.)

Osmosensitivity and enhanced osmotolerance with ProU plasmids: An interesting correlation exists between the overexpression of one or more genes of the *proU* locus and growth phenotype of the corresponding strains. Complementation of a chromosomal *proU* deletion mutation in respect of osmoprotection by L-proline and glycine betaine is seen only with plasmids that carry the entire locus intact[52]. The evidence also indicates that in the multicopy-proU$^+$ strains, turgor-restoration during growth in high-osmolarity medium supplemented with L-proline or glycine betaine is achieved at a level of gene induction which is much lower than that which occurs in a haploid ProU$^+$ strain under the same growth conditions; as a corollary, maximal induction in the multicopy strain is associated with significantly enhanced osmotolerance in osmoprotectant-supplemented media[52]. This suggests that an increase in the number of ProU porters in the cell envelope can contribute to an increased intracellular accumulation of compatible solutes in the face of osmotic stress.

On the other hand, in medium not supplemented with either L-proline or glycine betaine, the multicopy-ProU$^+$ strain exhibits marked osmosensitivity, and is unable to grow in minimal salts medium supplemented with 0.15 M NaCl—that is, at an osmolarity (550 mOsm) which is easily tolerated even by isogenic ProU$^-$ strains, and at which each of the *proU* loci is expressed at 50% of the maximally induced level (unpublished data). Thus, it would appear that overexpression of the ProU porters in the absence of the osmoprotectants is inimical to growth.

TURGOR-REGULATION OF ProU EXPRESSION

The case for turgor-regulation: Induction of *proU* expression in *E. coli* and *S. typhimurium* is obtained only with impermeable solutes in the growth medium, and equiosmolar concentrations of different substances result in equivalent levels of induction of this operon[15,16,18,19,57]. It is, therefore, clear that its expression is controlled, directly or indirectly, by the turgor pressure of the cell; the mechanism by which a physical parameter such as turgor is sensed and quantitated by the cell, and then transduced to a signal that affects gene expression, is obviously of considerable interest.

Sutherland *et al*[36] have shown that the regulation of *proU* expression differs in many respects from that of a K$^+$ -transport operon, *kdp*, which is also believed to be turgor-regulated[14,26], and that osmoresponsive induction of *proU* expression does not occur under conditions where cells are starved for K$^+$. On the basis of these results, they have argued that it is the expression of *kdp* and intracellular accumulation of K$^+$ that are primarily turgor-regulated, and that *proU* induction is indirect, being secondary to the increased [K$^+$] that occurs within the cells.

Such an interpretation, however, does not explain all the observations. For one, several lines of evidence suggest that the expression of *kdp* is, in fact, not regulated by turgor pressure but is controlled by intracellular [K$^+$], and these arguments have been presented elsewhere[63]; therefore, the fact that *proU* regulation is different from that of *kdp* may not indicate that the former is not turgor-regulated. In addition, a critical assumption of the model of Sutherland *et al*[36] for turgor-regulation of *kdp* is that turgor is completely restored in cells that have adapted to osmotic stress, so as to account for the observation that *kdp* induction is not a sustained response during growth in

high-osmolarity media[15,36]. This assumption can be questioned on the *a priori* consideration that a compensatory response which contributes to adaptation cannot be transient but must continue to be exhibited so long as the stress condition persists; furthermore, Koch and Pinette[64] have shown, in the first example of direct turgor pressure measurement in a gram-negative heterotrophic bacterium, that upon adaptation to osmotic stress, the extent of turgor restoration is only partial and never complete. Finally, the observation that increased ProU functioning (in the multicopy-*proU*$^+$ strains) in the presence of exogenous L-proline or glycine betaine serves to markedly decrease the expression of individual copies of the locus even during minimal osmotic stress[52], cannot be accommodated in the framework of a [K^+]-regulation model, because intracellular [K^+] is not altered significantly under these conditions (Rajkumari, unpublished).

Instead, it is possible to explain all the data on ProU regulation in a model that assumes it to be directly controlled by turgor pressure, along with the proviso that its induction requires intracellular [K^+] to be above a threshold level. In support of this notion, a correlation has also been shown between the effect of turgor-restoration consequent to betaine accumulation in high-osmolarity media on *proU* expression and that on another gene, *ompF*, earlier characterized as being under turgor pressure-control[57]; however, the mechanism by which the two genes are regulated by turgor pressure appears to be different, because the two genes regulating *ompF* expression, *ompR* and *envZ*, do not participate in the control of *proU*[18,19].

Mechanism of turgor-regulation: The cloning studies on *proU* have established that the *cis* sequences involved in *proU* induction are present in a 830 base-pair region of DNA upstream of the first structural gene of the operon, which represents the upper size limit for the promoter-operator region[19]. Is there a *trans*-acting regulatory protein that is also involved in the osmoresponsivity of *proU* expression? A model which assumes that changes in turgor pressure lead to alteration in conformation of a protein that binds to the *proU* operator would represent the simplest extension of the paradigm of operon control; however, although putative operator-constitutive mutants have been isolated, attempts to obtain mutants in the gene encoding the hypothetical regulatory protein have not been successful[49]. This might be because such mutations have unexpected pleiotropic effects or are even lethal.

An alternative possibility is that osmoresponsivity of *proU* expression is not protein-mediated but is a direct consequence of change in intracellular ionic strength on the supercoiled structure of DNA in the region of the *proU* promoter[37]. One mutation that affects *proU* expression and confers temperature-sensitivity has been mapped close to or within *topA*, that encodes topiosomerase I, suggesting that super-coiling might indeed be involved in regulation[65]. Whether it is sufficient in itself to explain osmoresponsivity is, however, not established.

CONCLUSIONS AND SUMMARY

Two transport systems, ProU and ProP, that participate in the osmoprotectant actions of L-proline and glycine betaine have recently been identified in *E. coli* and *S. typhimurium*. ProU has been better characterized, and represents a periplasmic binding protein-dependent transport system with high affinity for glycine betaine uptake and a low affinity for L-proline. The three genes encoding this porter are organized in a single operon whose transcription is markedly induced when the cell turgor is reduced, but the mechanism of signal transduction in the control of gene expression is as yet not elucidated. The osmoprotectant ability of L-proline and glycine betaine is enhanced in strains with multiple copies of the *proU* genes, presumably as a consequence of an increase in the ability of these strains to accumulate these solutes against a concentration gradient.

ProP appears to be a monocomponent porter with equivalent affinities for L-proline and glycine betaine. Its expression is only marginally increased in high-osmolarity

medium, but the porter activity is significantly stimulated under these conditions.

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1. Roth, W. G., Leckie, M. P. and Dietzler, D. N., *Biochem. Biophys. Res. Commun.*, 1985, **126,** 434.
2. Brown, A. D., *Bacteriol. Rev.*, 1976, **40,** 803.
3. Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D. and Somero, G. N., *Science*, 1982, **217,** 1214.
4. Le Rudulier, D., Strom, A. R., Dandekar, A. M., Smith, L. T. and Valentine, R. C., *Science*, 1984, **224,** 1064.
5. Roth, W. G., Porter, S. E., Leckie, M. P., Porter, B. E. and Dietzler, D. N., *Biochem. Biophys. Res. Commun.*, 1985, **126,** 442.
6. Epstein, W. and Schultz, S. G., *J. Gen. Physiol.*, 1965, **49,** 221.
7. Measures, J. C., *Nature* (*London*), 1975, **257,** 398.
8. Perroud, B. and Le Rudulier, D., *J. Bacteriol.*, 1985, **161,** 393.
9. Richey, B., Cayley, D. S., Mossing, M.C., Kolka, C., Anderson, C. F., Farrar, T. C. and Record, M. T. Jr., *J. Biol. Chem.*, 1987, **262,** 7157.
10. Bagnasco, S., Balaban, R., Fales, H. M., Yang, Y. M. and Burg, M., *J. Biol. Chem.*, 1986, **261,** 5872.
11. Hawthorne, D. C. and Friis, J., *Genetics*, 1964, **50,** 829.
12. Russell, R. R. B., *J. Bacteriol.*, 1972, **112,** 661.
13. Kohno, T. and Roth, J., *Biochemistry*, 1979, **18,** 1386.
14. Laimins, L. A., Rhoads, D. B. and Epstein, W., *Proc. Natl. Acad. Sci. USA*, 1981, **78,** 464.
15. Gowrishankar, J., *J. Bacteriol.*, 1985, **164,** 434.
16. Dunlap, V. J. and Csonka, L. N., *J. Bacteriol.*, 1985, **163,** 296.
17. Cairney, J., Booth, I. R. and Higgins, C. F., *J. Bacteriol.*, 1985, **164,** 1218.
18. Cairney, J., Booth, I. R. and Higgins, C. F., *J. Bacteriol.*, 1985, **164,** 1224.
19. May, G., Faatz, E., Villarejo, M. and Bremer, E., *Mol. Gen. Genet.*, 1986, **205,** 225.
20. Gowrishankar, J., *J. Bacteriol.*, 1986, **166,** 331.
21. Guttierez, C., Barondess, J., Manoil, C. and Beckwith, J., *J. Mol. Biol.*, 1987, **195,** 289.
22. Styrvold, O. B., Falkenberg, P., Landfald, B., Eshoo, M. W., Bjornsen, T. and Strom, A. R., *J. Bacteriol.*, 1986, **165,** 856.
23. Csonka, L. N., *J. Bacteriol.*, 1982, **151,** 1433.
24. Grothe, S., Krogsrud, R. L., McClellan, D. J., Milner, J. L. and Wood, J. M., *J. Bacteriol.*, 1986, **166,** 253.
25. Landfald, B. and Strom, A. R., *J. Bacteriol.*, 1986, **165,** 849.
26. Epstein, W., *FEMS Microbiol. Rev.*, 1986, **39,** 73.
27. Strom, A. R., Falkenberg, P. and Landfald, B., *FEMS Microbiol. Rev.*, 1986, **39,** 79.
28. Christian, J. H. B., *Aust. J. Biol. Sci.*, 1955, **8,** 75.
29. Christian, J. H. B., *Aust. J. Biol. Sci.*, 1955, **8,** 490.
30. Csonka, L. N., *Mol. Gen. Genet.*, 1981, **182,** 82.
31. Le Rudulier, D. and Valentine, R. C., *Trends Biochem. Sci.*, 1982, **7,** 431.
32. Le Rudulier, D. and Bouillard, L., *Appl. Environ. Microbiol.*, 1983, **46,** 152.
33. Le Rudulier, D., Bernard, T., Goas, G. and Hamelin, J., *Can. J. Microbiol.*, 1984, **30,** 299.
34. Sugiura, M. and Kisumi, M., *Appl. Environ. Microbiol.*, 1985, **49,** 782.
35. Sugiura, M. and Kisumi, M., *J. Gen. Microbiol.*, 1985, **131,** 2515.
36. Sutherland, L., Cairney, J., Elmore, M. J., Booth, I. R. and Higgins, C. F., *J. Bacteriol.*, 1986, **168,** 805.
37. Higgins, C. F., Cairney, J., Stirling, D. A., Sutherland, L. and Booth, I. R., *Trends Biochem. Sci.*, 1987, **12,** 339.
38. Schobert, B. and Tschesche, M., *Biochim. Biophys. Acta*, 1978, **541,** 270.
39. Ratzkin, M., Grabnar, M. and Roth, J., *J. Bacteriol.*, 1978, **133,** 737.
40. Ratzkin, M. and Roth, J., *J. Bacteriol.*, 1978, **133,** 744.
41. Wood, J. M. and Zadworny, D., *Can. J. Biochem.*, 1979, **57,** 1191.
42. Wood, J. M., *J. Bacteriol.*, 1981, **146,** 895.
43. Cairney, J., Higgins, C. F. and Booth, I. R., *J. Bacteriol.*, 1984, **160,** 22.
44. Milner, J. L., McClellan, D. J. and Wood, J. M., *J. Gen. Microbiol.*, 1987, **133,** 1851.
45. Menzel, R. and Roth, J., *J. Bacteriol.*, 1980, **141,** 1064.

46. Anderson, R. R., Menzel, R. and Wood, J. M., *J. Bacteriol.*, 1980, **141**, 1071.
47. Stalmach, M. E., Grothe, S. and Wood, J. M., *J. Bacteriol.*, 1983, **156**, 481.
48. Kaback, H. R. and Deuel, T. F., *Arch. Biochem. Biophys.*, 1969, **132**, 118.
49. Druger-Liotta, J., Prange, V. J., Overdier, D. G. and Csonka, L. N., *J. Bacteriol.*, 1987, **169**, 2449.
50. Munro, G. F., Herkules, K., Morgan, J. and Sauerbier, W., *J. Biol. Chem.*, 1972, **247**, 1272.
51. Le Rudulier, D., Yang, S. S. and Csonka, L. N., *Biochim. Biophys. Acta*, 1982, **719**, 273.
52. Gowrishankar, J., Jayashree, P. and Rajkumari, K., *J. Bacteriol.*, 1986, **168**, 1197.
53. Hagihira, H., Wilson, T. H. and Lin, E. C. C., *Am. J. Physiol.*, 1962, **203**, 637.
54. Franklin, C. S., Evered, D. F. and Nunn, P. B., *Biochem. J.*, 1970, **118**, 41P.
55. Pollard, A. and Wyn Jones, R. G., *Planta*, 1979, **144**, 291.
56. Arakawa, T. and Timasheff, S. N., *Biophys. J.*, 1985, **47**, 411.
57. Barron, A., May, G., Bremer, E. and Villarejo, M., *J. Bacteriol.*, 1986, **167**, 433.
58. Higgins, C. F., Sutherland, L., Cairney, J. and Booth, I. R., *J. Gen. Microbiol.*, 1987, **133**, 305.
59. Ames, G. F–L. and Higgins, C. F., *Trends Biochem. Sci.*, 1983, **8**, 97.
60. Ames, G. F–L., *Annu. Rev. Biochem.*, 1986, **55**, 397.
61. Higgins, C. F., Hiles, I. D., Whalley, K. and Jamieson, D. J., *EMBO J.*, 1985, **4**, 1033.
62. Dassa, E. and Hofnung, M., *EMBO J.*, 1985, **4**, 2287.
63. Gowrishankar, J., *J. Genet.*, 1987, **66**, 87.
64. Koch, A. L. and Pinette, S., *J. Bacteriol.*, 1987, **169**, 3654.
65. Diblasio, E. A. and Vinopal, R. T., *Abstr. Annu. Meeting Am. Soc. Microbiol.*, 1986, K-123.