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Regulation of compatible solute accumulation in bacteria

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Summary

In their natural habitats, microorganisms are often exposed to osmolality changes in the environment. The osmotic stress must be sensed and converted into an activity change of specific enzymes and transport proteins and/or it must trigger their synthesis such that the osmotic imbalance can be rapidly restored. On the basis of the available literature, we conclude that representative Gram-negative and Gram-positive bacteria use different strategies to respond to osmotic stress. The main focus of this paper is on the initial response of bacteria to hyper- and hypo-osmotic conditions, and in particular the osmosensing devices that allow the cell to rapidly activate and/or to synthesize the transport systems necessary for uptake and excretion of compatible solutes. The experimental data allow us to discriminate the transport systems by the physicochemical parameter that is sensed, which can be a change in external osmotic pressure, turgor pressure, membrane strain, internal osmolality and/or concentration of specific signal molecule. We also evaluate the molecular basis for osmosensing by reviewing the unique structural features of known osmoregulated transport systems.

Introduction

The cytoplasmic membrane of bacteria is permeable to water but forms an effective barrier for most solutes present in the medium and metabolites present in the cytoplasm. A lowering of the external water activity (hyper-osmotic conditions) causes a rapid efflux of water and loss of turgor; ultimately, the cells may plasmolyse, i.e. the cytoplasmic membrane may retract from the cell wall. Similarly, upon hypo-osmotic shock water flows into the cell and increases the cytoplasmic volume and/or turgor pressure. To survive osmotic stresses, the cells need to adapt by accumulating specific solutes under hyperosmotic conditions and releasing them under hypo-osmotic conditions. Such solutes include K^+, amino acids (e.g. glutamate, proline), amino acid derivatives (peptides, N-acetylated amino acids), quaternary amines (e.g. glycine betaine, carnitine), sugars (e.g. sucrose, trehalose) and tetrahydropyrimidines (ectoines) (Csonka, 1989; Galinski and Trüper, 1994). These solutes are often referred to as *compatible solutes* because they can be accumulated to high levels by *de novo* synthesis or transport without interfering with vital cellular processes. In fact, many compatible solutes proved to be effective stabilizers of enzymes, providing protection not only against high salt but also against high temperature, freeze–thawing and drying (Yancey *et al.*, 1982). Solutes that are non-charged or zwitterionic are generally more favourable to protein stability than ionic solutes, and, consistently, upon accumulation of neutral compatible solutes one often observes an iso-osmotic displacement of ionic solutes such as K^+ and glutamate from the cell. In most eubacterial species glycine betaine is the preferred compatible solute and generally provides the highest level of osmotolerance, which may reflect – among others – its favourable interaction with macromolecules.

Osmoprotectants are those solutes that alleviate the inhibitory effect of hyperosmotic stress on the microorganisms when they are added to the medium, but often this term is also used for any solute that can overcome osmotic inhibition. As the term osmoprotectant is vague and not well defined, it is preferable to use only compatible solute for any compound that offers protection to high osmolality by accumulating to high cytoplasmic concentrations either by uptake from the medium or by *de novo* synthesis. Finally, *osmolality* describes the osmotic pressure of a solution in osmoles of osmolytes per kg of solvent. Notice that the osmole is not a defined number because the osmotic pressure is not solely determined by the particle number but also by their size, shape and charge (see

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Sweeney and Beuchat, 1993, for measurement of these parameters).

The cells may synthesize (some of the) compatible solutes after an osmotic upshock and degrade them after an osmotic downshift, but the initial response is much more rapid if compatible solutes can be taken up from the medium and/or released into the medium via semiconstitutive transport systems. The transport systems involved in osmoregulation can be subdivided into specific uptake systems, stretch-activated channels, specific efflux systems and aquaporins, and the regulation of the activity of these systems forms the topic of this paper. In many cases the synthesis of the transport systems is also under osmotic control, but often the effects of hyper- and hypo-osmotic conditions on expression and activity are not adequately discriminated or are poorly described. We prefer to use the term (in)activation to indicate a change in activity of a transport system or open probability of a channel protein, and use induction/repression to describe the phenomena that lead to altered expression. In this paper, we evaluate the main findings pertinent to the immediate or short-term response of transport systems for compatible solutes after a shift in the medium osmolarity. When appropriate, we compare the mechanism of activation of the system with the mechanism of induction of the corresponding gene(s). A more general overview of the genetics and physiology of prokaryotic osmoregulation is given in Csonka (1989), Csonka and Hanson (1991) and Lucht and Bremer (1994).

What are the regulatory signals for (in)activation?

Upon a change in the medium osmolality, a membrane transport system may be (in)activated by one or more of the following physicochemical parameters (Fig. 1): (i) *external osmolality or water activity*; (ii) *turgor pressure*, i.e. the difference between the extra- and intracellular potential of all osmotically active solutes, which affects the compression of the membrane against the cell wall; (iii) *membrane strain*, which occurs in response to a change in turgor pressure and affects the compression/expansion of the bilayer in the plane of the membrane; (iv) *internal hydrostatic pressure*; (v) *internal osmolality* or *water activity*; (vi) *cytoplasmic volume*; and (vii) *concentration of specific cytoplasmic signal molecule(s)*. Not all these parameters will act in the same time scale, which may be diagnostic in distinguishing some of the sensing mechanisms. The first three of the physicochemical parameters or signals are thought to act more rapidly than the last four as it will take some time to elicit significant changes in cytoplasmic concentration and volume (Csonka and Hanson, 1991; Stock *et al*., 1977). For instance, upon osmotic upshock, the increase in external osmolality will be instantaneous, the decrease in turgor pressure will be rapid but the intracellular volume may only decrease after turgor has fallen to zero. However, as pointed out by Csonka and Hanson (1991) what actually happens to the cell volume is highly dependent on the rigidity of the cell wall (peptidoglycan layer or sacculus). As the cell wall of

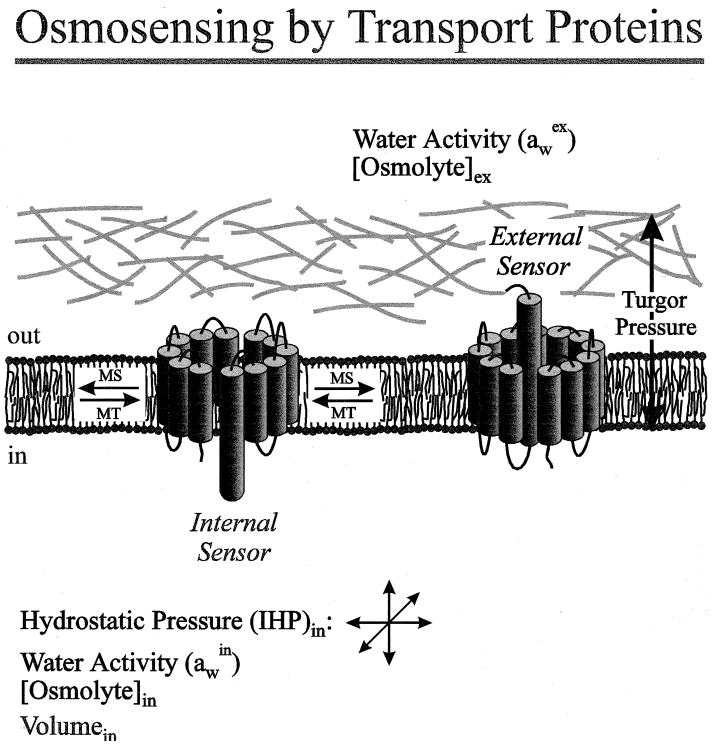

Fig. 1. Physicochemical parameters that may affect the activity of osmoregulated transport systems. Two transport systems, one with an external and one with an internal osmosensing domain, are depicted schematically. The cell envelope represents that of a Gram-positive bacterium, i.e. the cytoplasmic membrane and peptidoglycan layer are shown. MS and MT refer to membrane stretch and tension, respectively, which may occur when the turgor pressure changes. The isotropic nature of the hydrostatic pressure is indicated schematically, and contrasts the anisotropic nature of the turgor pressure (indicated by single arrow) (Csonka and Hanson, 1991). The water activity (a_w) refers to the mole fraction of water in solution.

Gram-negative bacteria is much thinner and the degree of cross-linking of the disaccharide units by peptides in the peptidoglycan is much lower than in Gram-positive bacteria, one would anticipate that its mechanical properties are quite different (Dijkstra and Keck, 1996). On the basis of studies with peptidoglycan purified from Gram-negative and Gram-positive bacteria, it is now well established that the peptidoglycan sacculus is an elastic, flexible, polyanionic restraining network (which behaves as a viscoelastic polymer) and not an inherently rigid structure as is often described in textbooks (Thwaites and Mendelson, 1989; Koch and Woeste, 1992; Doyle and Marquis, 1994). The cell wall is thus able to stretch as water flows into the cell and shrink when water flows out of the cell, which will result in changes in the cytoplasmic volume (and related parameters) along with the osmolality changes in the medium. Thus, opposite of what is often thought, volume changes may already occur when there is still turgor pressure. After the turgor pressure has dropped to zero, the bacteria may plasmolyse, i.e. the cytoplasmic membrane separates from the cell wall and the volume decreases further; at this point the cells behave as osmometers. In contrast to Gram-negative bacteria, Gram-positive do not plasmolyse (Mitchell and Moyle, 1956; Whatmore and Reed, 1990), which implies that the ultimate cytoplasmic volume under hyperosmotic conditions is set by the rigidity of the cell wall. The reason for the failure of Gram-positive bacteria to plasmolyse might be the strong adhesion between the cytoplasmic membrane and peptidoglycan. Alternatively, it has been proposed that these bacteria do not plasmolyse because of their very high internal osmotic pressure (turgor pressures of 15–25 atm), which means that much higher external osmolalities are needed before the turgor drops to zero than in Gram-negative bacteria (turgor pressures of 1–5 atm). Besides these anticipated differences in Gram-negative and Gram-positive bacteria when experiencing osmolality changes, it is obvious that most of the physicochemical parameters associated with an osmolality change of the medium are not mutually exclusive but rather interrelated, and these will change in parallel upon an osmotic shift.

Changes in each of the previously mentioned physicochemical signals may trigger the activation of osmoregulated transport proteins or alter the open probability of channel proteins, but they will not solely determine the fluxes of compatible solutes across the membrane. The ultimate activity of a system after the activation by a shift in medium osmolality will depend on the state of the cells with respect to (i) the internal osmotic pressure or related parameter at the time of the shift (as described above); (ii) the internal concentration of the compatible solute (and structural analogues), which may inhibit through 'feedback' or '*trans*' inhibition; and/or (iii) physiological parameters such as the energy status and the internal pH of the cell.

As a result of a change in the external osmolality or water activity, the structure of a membrane protein or a portion thereof (external sensing domain) could be deformed, which in turn may affect the activity. Similarly, as a result of a change in turgor pressure an integral membrane protein could be deformed through changes in protein–lipid interactions as a result of membrane stretch/tension or compression of the protein against the peptidoglycan layer. The other mechanisms may require a specific internally located domain that senses changes in hydrostatic pressure, intracellular osmolality or concentration of a specific solute. Below, we describe the likely mechanism(s) of osmotic regulation of well-studied uptake and efflux systems that, to our opinion, exemplify many other osmoregulated systems as well (Table 1).

The initial response to osmotic upshift: Gram-negative versus Gram-positive bacteria

The initial response of enteric bacteria to an osmotic upshock involves the uptake of potassium via Kdp and Trk, the main transport systems for K^+. With Trk we refer to the system that uses either TrkG or TrkH as the pathway for K^+ uptake in *Escherichia coli* (Bakker, 1992; Schlösser *et al*., 1995). To maintain electroneutrality, the accumulation of K^+ is accompanied by increases in the glutamate pool by *de novo* synthesis during growth at high osmolarity (Caylay *et al*., 1991; McLaggan *et al*., 1994), but other processes such as accumulation of other anions, putrescine excretion and proton efflux may contribute as well (Csonka, 1989). Although potassium transport has been studied most thoroughly in *E. coli* and *Salmonella typhymurium*, similar turgor-sensitive transport systems may be present in other organisms (Whatmore *et al*., 1990), and the initial response to high osmolarity is generally associated with potassium uptake. Displacement of K^+ (and counterions) for neutral compatible solutes may occur shortly after the accumulation of these ions, e.g. through the uptake of glycine betaine or proline via semiconstitutive transport systems such as ProP. Although K^+ and glycine betaine or proline uptake may proceed simultaneously, the accumulation of K^+ via Trk is more rapid and quantitatively most important in the initial phase of hyperosmotic stress. Other systems, e.g. for trehalose synthesis and high-affinity uptake of glycine betaine (*proU*), require induction and contribute to the restoration of turgor at later times of hyperosmotic stress (Booth, 1992; Lucht and Bremer, 1994).

Recent studies in *Lactobacillus plantarum* indicate that most of the potassium is bound (Glaasker *et al*., 1996a; 1998), which reduces its osmotic significance in this organism. Moreover, increases in the cellular concentrations of K^+ are only observed in KCl-stressed cells and not with equiosmolar concentrations of NaCl or sugar. It

Table 1. Properties of osmoregulated transport systems in bacteria.

System	Organism	Activation signal	Parameters that affect the activity			
			External osmolality	Internal osmolality	Internal [substrate]	Other factor
Osmosensor						
KdpD	*E. coli*	Low turgor (membrane strain)	–	–	–	Amphipaths
Uptake						
Trk	*E. coli/S. typhimurium*	Low turgor	No	[–]?	[–]	No
ProP	*E. coli/S. typhimurium*	Increased ext. osm. press.	[+]	No	No	K^+ (pH_{in})
ProU	*E. coli/S. typhimurium*	Internal factor	No	[+]	No	–
BetP	*C. glutamicum*	Low turgor? (membrane strain)	–	–	–	Amphipaths
QacT	*Lb. plantarum*	Low turgor	No	–	[–]	–
Betaine	*L. monocytogenes*	Low turgor?	–	–	[–]	–
Carnitine	*L. monocytogenes*	Low turgor?	–	–	[–]	pH_{in}
Efflux						
Comp. solutes	*E. coli/S. typhimurium* *Lb. plantarum* *C. glutamicum*	High turgor (membrane strain)	–	–	–	Amphipaths[a]
MscL	*E. coli*	High turgor (membrane strain)	–	–	–	Amphipaths

[–], decreases the activity; [+], increases the activity; ?, uncertain; –, unknown; pH_{in}, internal pH.
a. Not studied in all cases.

is therefore thought that this Gram-positive bacterium binds a large fraction of the K^+ extracellularly. Unlike the enteric bacteria and *B. subtilis*, *Lb. plantarum* and other lactic acid bacteria have limited or no possibilities to synthesize compatible solutes. Most of these organisms are multiple amino acid auxotrophs and reside in environments that contain these amino acids in some form as well as glycine betaine (plant origin) or carnitine (animal origin). The only way out to recover from hyperosmotic stress is via the uptake of compatible solutes such as glycine betaine, carnitine and/or proline, and the initial osmotic response in *Lb. plantarum* is formed by activation of the high-capacity–high-affinity semiconstitutive glycine betaine/carnitine/proline transport system QacT.

The findings made for *Lb.plantarum* may represent the basis for osmoregulation in Gram-positive bacteria in general, or at least in simple anaerobes such as the lactic acid bacteria. In fact, already in 1975 it was observed that in response to osmotic stress Gram-negative bacteria accumulate large amounts of glutamate (together with K^+), whereas the Gram-positive bacteria investigated accumulate proline (Measures, 1975). Under non-stressed conditions, the Gram-positive bacteria already have a high amino acid pool, of which a large proportion is glutamate. Similarly, the cellular concentrations of K^+ in non-stressed Gram-positive bacteria are usually much higher (often around 1 M) than in Gram-negative bacteria (Measures, 1975; Poolman *et al.*, 1987a; Kakinuma and Igarashi, 1988; Caylay *et al.*, 1991; McLaggen *et al.*, 1994; Glaasker *et al.*, 1996a; 1998), which is also reflected in the higher turgor pressure of the organisms (Csonka, 1989). It should be stressed, however, that the fractions of 'free' and 'bound' potassium have only been determined in a few cases (Caylay *et al.*, 1991; McLaggen *et al.*, 1994; Glaasker *et al.*, 1998). Given the differences in osmolality and the high concentrations of electrolytes, it seems that under hyperosmotic conditions, in particular Gram-positive bacteria are better off by accumulating proline or glycine betaine (or quaternary amines in general) than the electrolyte pair K–glutamate. Whereas some (Gram-positive) bacteria can synthesize proline and glycine betaine (from choline), others rely completely on uptake of these or other compatible solutes from the medium. We emphasize that the term accumulation as used in this section reflects increases in pool sizes through uptake and/or synthesis. In several cases (e.g. Measures, 1975), the cells were grown in rich media containing amino acids and peptides and accumulation through uptake or synthesis cannot be discriminated.

Osmotic regulation of uptake of potassium ions

The osmotic regulation of the Kdp system occurs at the level of catalytic activity as well as protein expression. There is evidence that Kdp mediates potassium exchange without net movement under osmostatic conditions, whereas the system switches to net uptake when turgor is low (Epstein, 1992). However, compared with the regulation of Kdp activity, much more is known about the transcriptional regulation of the *kdp* genes, which represents

one of the best-understood systems in terms of osmosensing (Booth, 1992; Nakashima *et al*., 1993; Sugiura *et al*., 1994; Jung *et al*., 1997). The induction of the *kdp* operon is mediated by a sensor kinase (KdpD)/response regulator (KdpE) system, but the sensing mechanism may be paradigmatic of that of some transport and channel proteins that are described below. A decrease in turgor pressure or related parameter results in autophosphorylation of KdpD. Subsequent transfer of the phosphoryl group to the transcription factor KdpE results in increased transcription of the *kdp* genes. As amphipaths that intercalate into the lipid bilayer elicit a similar effect as an osmotic upshock, it has been proposed that the osmotic signal that is sensed by KdpD corresponds to membrane stretch. In contrast to Kdp, the osmotic regulation of Trk is mainly at the level of transport activity but the signal for activation may be very similar to that of KdpD. The activity of Trk increases upon an osmotic upshift but the initial rate of influx does not depend on the size of the external osmolarity increase (Meury *et al*., 1985). Instead, it seems that the activity of Trk depends on the intracellular osmolality at the time the external osmolality is increased. As intracellular osmolality and potassium concentration are not well separated in the experimental set up, it is also possible that the actual rate is determined by the intracellular potassium concentration through feedback regulation. Nevertheless, the instantaneous activation of K^+ uptake upon an osmotic upshift and the dependence of the accumulation level on the size of the osmotic shift strongly suggests that the system is turgor controlled; the internal osmolality and/or K^+ concentration modulates the activity (Meury *et al*., 1985).

Osmotic regulation of uptake of organic compatible solutes

Glycine betaine and proline uptake in enteric bacteria follow the uptake of potassium(−glutamate), provided these compatible solutes are present in the medium, and thereby eventually replace the electrolytes. The uptake of these compatible solutes is effected by the proton motive force-driven ProP and ATP-driven binding protein-dependent ProU system. Upon osmotic upshock, the ProP protein is activated within seconds, the time resolution of the experiment, but the stimulation of activity requires the presence of K^+ in the medium (Koo *et al*., 1991). As 'activated' uptake occurs irrespective of whether the turgor has been restored via the uptake of K^+, it seems unlikely that ProP senses turgor *per se*. The requirement for K^+ is indicative for an important role of the internal pH in regulating ProP (Poolman *et al*., 1987a,b), but, as pointed out before, the increase in intracellular pH upon K^+ uptake is usually transient, whereas the 'activated' uptake lasts as long as the medium osmolarity is high (Koo *et al*., 1991). On the other hand, it should be stressed that an increased internal pH can be maintained when counterions that balance the charge during the uptake of K^+ are absent (McLaggan *et al*., 1994). In this regard, the putative activation of ProP by potassium ions requires further exploration. The osmotic regulation of ProP has also been studied in membrane vesicles, and although the authors conclude that the system is activated by low turgor pressure (Milner *et al*., 1988) the fact that membrane vesicles cannot withstand any (or at most very small) pressures argues against regulation by turgor pressure. The imposed osmotic shifts transiently disrupt the barrier function of the membrane and during this period external and internal solutes will equilibrate, thereby minimizing the turgor pressure (E. Glaasker and B. Poolman, unpublished). In our opinion, the data obtained with the membrane vesicles are most consistent with regulation by external osmotic pressure or related parameter, which also fits best the observations made by Koo *et al*. (1991).

The hyperosmotic activation of ProU takes several minutes (Faatz *et al*., 1988), which makes it unlikely that increased external osmotic pressure or reduced turgor pressure triggers the activation. A closer look at the data suggests that activation of ProU is also dependent on the presence of substrate (glycine betaine) as the transport activity increases up to 3 min after an osmotic upshock in the absence but not in the presence of glycine betaine (Faatz *et al*., 1988). Despite the peculiarities in the time dependence of activation of the ProU system, the regulation of transport activity is clearly different from that of Trk and ProP and resembles that of transcription of *proU*, i.e. both are delayed upon an upshift. Initially, it was thought that K^+ and glutamate served a role as 'second messengers' in enhancing the *proU* transcription (Ramirez *et al*., 1989; Booth, 1992), but other studies have indicated that a large glutamate pool is not necessary for induction (Jovanovich *et al*., 1989; Csonka *et al*., 1994). It has been proposed (Csonka *et al*., 1994) that the stimulation of transcription of *proU in vitro* by K^+–glutamate is a manifestation of its favourable effect on macromolecular function (e.g. RNA polymerase–promoter interaction) and not unique to osmotic regulation of the *proU* promoter. In this view, the steady-state level of expression of *proU* increases with increasing intracellular osmolality, which takes time when the medium osmolarity is suddenly raised. The time dependence of activation of ProU is also consistent with an increased intracellular osmolality as signal for increased ProU activity, which in our opinion represents a more sensible (general) type of regulation of transport than one that requires some specific intracellular signal. Despite the attractiveness of a mechanism involving the sensing of intracellular osmolality by ProU, it needs to be said that the experimental evidence for this is limited and that even for the transcriptional regulation of *proU*

some questions about the specificity of the osmosensing device still persist (see also Rajkumari *et al*., 1996).

In *Lb. plantarum*, glycine betaine, carnitine, other quaternary ammonium compounds and proline are taken up via one and the same system (QacT), which is activated upon osmotic upshock (Glaasker *et al*., 1996a,b; E. Glaasker *et al*., unpublished). As the activated state lasts until the turgor is restored (Glaasker *et al*., 1998), e.g. via the uptake of sugars, this seems to be a clear case of turgor-regulated activity. Consistent with a turgor-controlled mechanism, the 'activated' state of the system lasts longer as the increase in medium osmolarity is higher. The increase in uptake rate from 'basal' to 'activated' mainly reflects an increase in maximal activity (V_{max} increases 5- to 10-fold), but it involves more than a single effect. It appears that the increase in V_{max} upon osmotic upshock is due to a diminished *trans*-inhibition (by proline and/or glycine betaine) as well as an effect that is independent of intracellular substrate (E. Glaasker *et al*., in preparation). The inhibition by intracellular substrate forms an additional level of control against excessive accumulation of glycine betaine, carnitine and proline. The linkage of the *trans*-inhibitory effect to the osmotic strength of the environment is also observed in other bacteria (next paragraph), and it may form a general strategy to tune the intracellular osmolality and maintain the cell turgor within certain limits.

In *Listeria monocytogenes* Scott A the structural analogues glycine betaine and carnitine are taken up via separate systems that respond to an osmotic upshock only when the cells have been pregrown in the presence of glycine betaine or carnitine (Verheul *et al*., 1997). These systems, although highly specific for their substrates, are inhibited by both glycine betaine and carnitine at the cytoplasmic face (*trans* site) of the membrane. Without intracellular glycine betaine and/or carnitine, the activity is maximal and not affected by medium osmolality. The inhibition by intracellular glycine betaine and carnitine is relieved upon osmotic upshock, which allows the cells to accumulate these compatible solutes further and restore turgor more rapidly. In kinetic terms, the activation of the glycine betaine and carnitine uptake systems of *L. monocytogenes* upon osmotic upshock is thought to reflect an increase in K_i^{App} (apparent inhibition constant) for the compatible solutes at the inner surface of the membrane. Apparently, a decrease in turgor alters the internal binding site for glycine betaine and carnitine, a phenomenon that is observed for both the ATP-driven carnitine and the ion motive force-driven betaine uptake system (Verheul *et al*., 1997).

Efflux of compatible solutes

Specific efflux systems

It has been shown for a number of microorganisms that compatible solutes are rapidly released from the cells upon a hypo-osmotic shock. For instance, in *E. coli* a rapid release of K^+, glutamate and trehalose is observed upon an osmotic downshock, whereas solutes such as alanine, lysine, arginine and sucrose are fully retained by the cells (Schleyer *et al*., 1993). When *Lb. plantarum* is subjected to an osmotic downshock, a rapid efflux of glycine betaine, proline and some glutamate occurs, whereas the pools of other amino acids remain unaffected (Glaasker *et al*., 1996a). Osmoregulated efflux activity with specificity for compatible solutes has also been described in *Corynebacterium glutamicum* (Lambert *et al*., 1995; Ruffert *et al*., 1997). Although the molecular nature of these efflux activities is unknown, the systems exhibit properties that mimic mechanosensitive channels (Sukharev *et al*., 1997; see below). Some features that discriminate the systems from 'ordinary' secondary carrier proteins (Poolman and Konings, 1993) are the following: (i) efflux is extremely fast and effected by an osmotic downshock as well as amphipaths that insert into the membrane; (ii) efflux is independent of metabolic energy; (iii) efflux is unaffected by substrate at the *trans* site of the membrane; and (iv) in many cases efflux is inhibited by gadolinium ions (Gd^{3+}), an unspecific channel blocker (Berrier *et al*., 1992; Lambert *et al*., 1995; Glaasker *et al*., 1996b; Ruffert *et al*., 1997). In the case of the glutamate excretion system from *C. glutamicum*, there is direct evidence that the effects of the osmotic gradients and the amphipath tetracaine are mutually compensative, i.e. the higher the medium osmolality the more tetracaine is needed to elicit efflux (Lambert *et al*., 1995). These and other experiments suggest that osmotic changes and amphipaths exert a similar type of mechanical stress on the membrane (Sheetz and Singer, 1974; Sheetz *et al*., 1976; Martinac *et al*., 1990).

The efflux of glycine betaine and proline by *Lb. plantarum* upon osmotic downshock is characterised by two kinetic components, i.e. the one described above with a $t_{1/2} < 1$ s and a slow one with a $t_{1/2}$ of 4–5 min. Similar observations have been made for the efflux of glycine betaine and carnitine in *L. monocytogenes*. The component with the slow kinetics is affected by the metabolic state of the cell and may represent a specific efflux system or, alternatively, exit of compatible solutes via the same system that effects the uptake. The glycine betaine uptake system of *L. monocytogenes* is a secondary transport protein that under the appropriate conditions (e.g. hypo-osmotic shock) may have a role in the release of compatible solutes. Secondary transport systems usually catalyse a reversible transport, of which the direction of net flux depends on the magnitudes of the ion motive force and the solute concentration gradients (Poolman and Konings, 1993). Thus, a lowering of the ion motive force as a result of an osmotic downshock would lead to

net efflux. The QacT system of *Lb. plantarum* and the carnitine uptake system of *L. monocytogenes* are ATP driven, and these systems are generally thought to operate unidirectionally and not to mediate efflux. However, it is worth mentioning that in *Rhizobium leguminosarum* an ABC-type binding protein-dependent amino acid uptake system (Aap) has been described that also affects efflux (Walshaw and Poole, 1996). At present it cannot be excluded that Aap-mediated efflux is indirect, i.e. through regulation of another channel or transport system, but the experimental data favour a direct role as the system mediated uptake and efflux in the heterologous host *E. coli*.

Mechanosensitive channels

The one mechanosensitive channel in the cytoplasmic membrane of a bacterium that has been studied in great detail is MscL of *E. coli* (Sukharev *et al*., 1997). Tension in the lipid bilayer is conveyed to MscL, which increases the open probability of the channel by several orders of magnitude (Sukharev *et al*., 1994). The pressure sensitivity of MscL is voltage dependent, i.e. the channel is more sensitive to membrane tension at more negative membrane potentials (Blount *et al*., 1997). The channel is blocked by Gd^{3+}. Support for the idea that the mechanical gating force comes from the lipids surrounding the protein is threefold (Martinac *et al*., 1990; Sukharev *et al*., 1994; Blount *et al*., 1997): (i) the purified MscL is fully functional after reconstitution into artificial lipid membranes, i.e. a bilayer is necessary and sufficient for mechanosensation; (ii) mutations within TMS1 of MscL alter the gating of the channel protein; and (iii) amphipaths that partition differently into the outer and inner leaflet of the membrane also activate the channel. It has been proposed that the lipid leaflet that has the highest tension gates the channel (Markin and Martinac, 1991). In addition to MscL, *E. coli* has other channel activities with conductances smaller than those of MscL (MscL has a conductance of ≈ 2.5 nS). Given the properties of the mechanosensitive channel proteins it seems likely that one or more of these activities correspond(s) to the observed efflux of compatible solutes upon hypo-osmotic shock as described under *Specific efflux systems*. In fact, mutations within MscL (K31→D and K31→E) evoke a large loss of K^+, which can largely be reversed by an increase in osmolality (Blount *et al*., 1997). The loss of K^+ parallels a strongly reduced growth rate that can partially be rescued by increasing the osmolality of the medium.

Aquaporins

One aspect of osmoregulation in bacteria that has been poorly documented concerns the molecular pathway of water movement across the cytoplasmic membrane. Although water may move passively across the membrane, it is evident from work in mammalian cells that aquaporins are crucial for the osmotic flow of water (Chrispeels and Agre, 1994). Bacterial aquaporins have only been known since the cloning and molecular characterization of *aqpZ*, the gene encoding the *E. coli* aquaporin. AqpZ is homologous to eukaryotic aquaporins and belongs to the major intrinsic protein (MIP) family, which also includes the glycerol facilitator GlpF (Calamita *et al*., 1995), but its physiological role in osmoregulation is unknown. Important insight into the structure and functioning of aquaporins has recently been obtained with the cryoelectron microscopy structure at 0.6 nm resolution of aquaporin-1 (Walz *et al*., 1997).

Regulation of pool sizes

In contrast to the K^+ uptake systems, there appears to be no feedback regulation ('*trans* inhibition') of the ProP and ProU systems in enteric bacteria, and the magnitude of the glycine betaine and proline pools is controlled by separate uptake and efflux systems. In the Gram-positive bacteria *Lb. plantarum* and *L. monocytogenes* the major osmoregulated transport systems for compatible solutes are feedback regulated, which prevents excessive uptake, but at the same time these organisms possess specific efflux/channel activity. It seems that irrespective of whether an organism can regulate the accumulation of compatible solutes through feedback inhibition of the corresponding uptake system, there is always a need for separate efflux pathways under conditions in which the turgor pressure becomes so great that the cells may break. To prevent futile cycling, the efflux systems must be highly regulated ('shut off') under normal osmotic and hyperosmotic conditions, whereas they must rapidly open to release turgor upon a sudden osmotic downshock. In fact, it has recently been shown that at osmostasis *Lb. plantarum* maintains basal flux of glycine betaine (but no net uptake or efflux), which amounts to about 10% of the maximal rate of uptake after an upshock (Fig. 2). Upon osmotic upshock the glycine betaine uptake system is rapidly activated to 100% and the basal efflux is completely inhibited (Glaasker *et al*., 1996b).

Molecular basis for osmosensing

So far, we have described what happens phenomenologically when a transport system experiences a change in medium osmolarity. How the environmental osmolality is sensed at the molecular level and converted into an altered activity is largely unexplored. The best-studied osmosensors are the KdpD and MscL proteins of *E. coli* (Sugiura *et al*., 1994; Blount *et al*., 1996). By mutational analysis it has been shown that the transmembrane segments (TMS) 3 and 4 (together with short, flanking

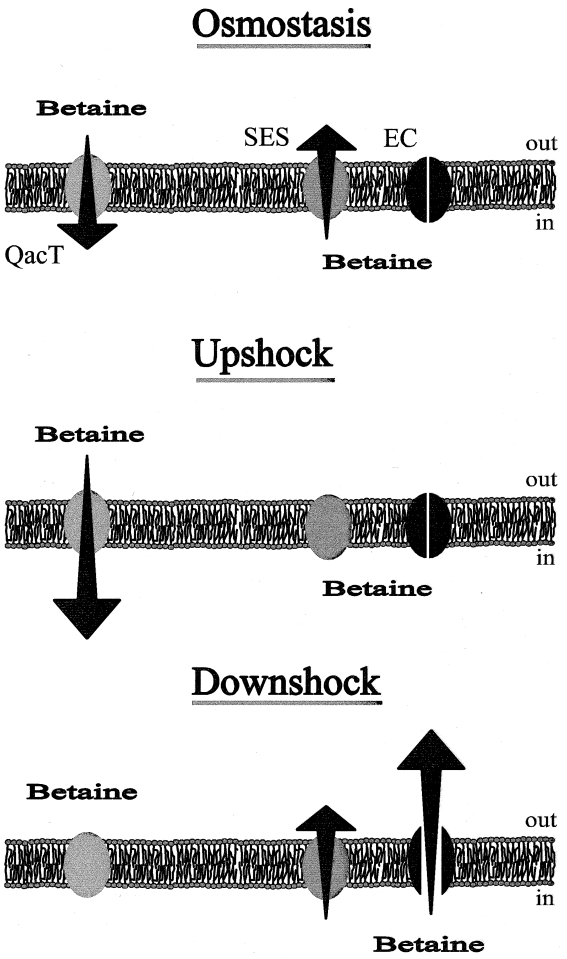

Fig. 2. Osmoregulation in *Lactobacillus plantarum*. Under osmostatic conditions there is a basal level of glycine betaine uptake and efflux. Upon osmotic upshock, the uptake system for glycine betaine (QacT) is activated, whereas the efflux is inhibited. Conversely, upon osmotic downshock, the QacT system is inhibited, whereas an specific efflux system (SES) is activated and a putative channel (EC) is opened. The sizes of the arrows reflect qualitatively the relative contribution to the total flux. Modified after Glaasker *et al.* 1996b.

regions) of KdpD are critical for the sensing of potassium (Sugiura *et al.*, 1994). Moreover, the sensing of K^+ could be separated mechanistically from medium osmolarity signals as mutants that failed to perceive the K^+ signal responded normally to hyperosmotic stress. The autophosphorylation of wild-type KdpD is negatively regulated by K^+, whereas medium osmolarity has a positive effect. As amphipathic compounds such as chlorpromazine and procaine effect the phosphorylation of KdpD in a similar manner as high-medium osmolarity, it is thought that the transmembrane segments of the protein sense osmolarity changes through stretch forces in the cytoplasmic membrane.

The mechanosensitivity of MscL is confined to the hydrophobic core (two TMS) and periplasmic loop in between the TMS (Blount *et al.*, 1996). The observation that amphipathic compounds activate MscL with an effectiveness that corresponds to their lipid solubility is taken as additional evidence that the mechanical gating force comes from the surrounding lipids (membrane stretch/tension) and that the signal is transferred to the TMS (Martinac *et al.*, 1990). The isolation of the K31→D and K31→E mutants with increased sensitivity to mechanical stress support this contention as Lys-31 is located near the middle of TMS1 (Blount *et al.*, 1997). Patch clamp studies have shown that the mutant channel proteins open more easily upon membrane tension as effected by osmotic downshifts. Finally, screening of MscL mutants with a 'slow' or 'no growth' phenotype has indicated that 14 of a total of 19 map in a region that constitutes two-thirds of TMS1. From a structural point of view the 'osmolality sensing' TMS of KdpD and those of MscL cannot be discriminated from those of any non-osmotically regulated membrane transport on the basis of primary sequence (motifs) or predicted secondary structure.

What are the putative osmosensing parts of other transport proteins that respond to changes in medium osmolarity? The known transport systems for the compatible solutes glycine betaine and proline can be subdivided in three families: (i) the binding protein-dependent ATP-driven systems that include ProU of *E. coli* and *S. typhimurium*, and OpuA and OpuC of *B. subtilis*; (ii) the ion motive force-driven transporters exemplified by ProP of *E. coli* and OusA of *Erwinia chrysanthemi*; and (iii) the ion motive force-driven transporters exemplified by OpuD of *B. subtilis*, BetT and CaiT of *E. coli*, BetP of *Corynebacterium glutamicum*, and the BetT-like protein of *Haemophilus influenzae*. Family 1 transporter ProU is activated by an osmotic upshock (Faatz *et al.*, 1988), but the protein(s)/site(s) of osmosensing are unknown. The ProW protein, that forms the putative translocation pathway of ProU, has an unusually long periplasmic tail that is predicted to form an amphiphilic α-helix (Haardt and Bremer, 1996). This protein domain has been implicated in osmosensing by monitoring alterations in membrane tension, but as we discussed above the physiological data are most compatible with the regulation of activity through changes in intracellular osmolality that may not be sensed by the periplasmic tail. Most of the extended hydrophilic amino-terminal region is lacking in OpuAB (homologue of ProW and part of the OpuA system) (Kempf and Bremer, 1995), but, unfortunately, the osmotic activation of OpuA has not been studied. The hyperosmotic stress-activated QacT transporter of *Lb. plantarum* and the carnitine transporter of *L. monocytogenes* are dependent on ATP and have all the properties of transport systems that belong to the ABC superfamily, but structural information of the components is not available. It is clear that more decisive experiments are needed to resolve the structure–osmoregulation relationships in these proteins.

Family 2 transporters ProP and OusA form part of a larger family that includes citrate and α-ketoglutarate transport systems but differ from these proteins by the presence of an extended central hydrophilic loop and a carboxy-terminal extension that is predicted to from an α-helical coiled coil; both structural elements are located internally (Culham *et al*., 1993; Gouesbet *et al*., 1996). It is tempting to speculate that the α-helical coiled coil is relevant for the osmoregulation of activity (Culham *et al*., 1993), but, unlike ProP, there is (preliminary) evidence that OusA is not affected by the osmolarity of the medium (Gouesbet *et al*., 1996). As possible activation of OusA has only been studied by comparing assay media with and without 0.5 M NaCl, the conclusion should be regarded with caution.

Family 3 transporters are generally quite specific for one or a few quaternary ammonium compounds, whereas family 1 and 2 transporters facilitate the uptake of glycine betaine, carnitine, proline, ectoine, pipecolic acid and other related compounds. Of family 3 transporters, OpuD, BetT and BetP are activated by hyperosmotic conditions, whereas CaiT is not (Lamark *et al*., 1991; Eichler *et al*., 1994; Kappes *et al*., 1996; Peter *et al*., 1996). Of these transporters, BetT, BetP and BetT-like of *H. influenzae* have a carboxy-terminal extension reminiscent of that in ProP and OusA, whereas such a 'domain' is lacking in OpuD and CaiT. Again, a correlation between osmoregulated activity and putative osmosensor 'domain' is lacking. It should be stressed, however, that osmoregulated activity can be overlooked easily as we have shown for glycine betaine uptake in *L. monocytogenes* (Verheul *et al*., 1997), in which the osmotic response is dependent on the internal concentration of glycine betaine and carnitine. Clear evidence that the carboxy-terminal extension has a role in osmosensing comes from studies of the BetP protein of *C. glutamicum* (Peter *et al*., 1998). The carboxy-terminal extension is 55 amino acids long and has a large excess of positively charged residues. Deletions in this putative domain result in complete loss of regulation. However, deletions in the hydrophilic amino-terminal tail also affect the osmoregulation of BetP by shifting the optimum of activation from 1.3 to 2.6 osmol kg^{-1}; the amino-terminal domain is 62 and has a large excess of negatively charged residues. These data could be interpreted as regulation through a single osmosensing device that is built up of the two extremities of the protein. On the other hand, the amino-terminal region is not conserved in the osmoregulated OpuD and BetT proteins of family 3 transporters, which points to a central role of the carboxyl-terminal region as the osmosensing device.

Concluding remarks

With the exception of Trk and ProP in enteric bacteria, BetP in *C. glutamicum*, and the transport systems for compatible solutes in *Lb. plantarum* (and *L. monocytogenes*), the hyperosmotic 'activation' of other uptake systems is in general only poorly described, and often based on one or a few experiments in which either a salt or sugar is used to raise the medium osmolality. Nevertheless, it is clear that some osmosensing mechanism forms an inherent property of the osmoregulated transport systems and channel proteins. A more rigorous kinetic analysis of the activation mechanism in relation to structural analysis of the system components should indicate whether or not 'added' loops and/or tails, present in a number of osmoregulated transport proteins, or specific TMS have a role in osmosensing. It should be stressed, however, that the osmosensing parts of the proteins may not be easily identified without the isolation of the appropriate mutants as suggested by the work on KdpD and MscL. Despite a lack of structural information, there are strong indications that the physicochemical signal that is sensed upon an osmolality change is not the same in all these systems, and that either external osmolality, turgor pressure (or a derived parameter such as membrane tension) and/or internal osmolality may be the trigger for activation. This implies that the molecular mechanism of osmosensing will vary among different transport systems, an area of research that is largely unexplored and deserves more attention than it has received thus far.

References

Bakker, E.P. (1992) Cell K^+ and K^+ transport systems in prokaryotes. In *Alkali Cation Transport Systems in Prokaryotes*. Bakker, E.P. (ed.). Boca Raton, FL: CRC Press, pp. 205–224.

Berrier, C., Coulombe, A., Szabo, I., Zoratti, M., and Ghazi, A. (1992) Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. *Eur J Biochem* **206:** 559–565.

Blount, P., Sukharev, S.I., Schroeder, M.J., Nagle, S.K., and Kung, C. (1996) Single residue substitutions that change the gating properties of a mechanosensitive channel in *Escherichia coli*. *Proc Natl Acad Sci USA* **93:** 11652–11657.

Blount, P., Schroeder, M.J., and Kung, C. (1997) Mutations in a bacterial mechanosensitive channel change the cellular response to osmotic stress. *J Biol Chem* **272:** 32150–32157.

Booth, I.R. (1992) Regulation of gene expression during osmoregulation: the role of potassium glutamate as a secondary signal of osmotic stress. In *Alkali Cation Transport Systems in Prokaryotes*, Bakker, E.P. (ed.). Boca Raton, FL: CRC Press, pp. 205–224.

Calamita, G., Bishai, W.R., Preston, G.M., Guggino, W.B., and Agre, P. (1995) Molecular cloning and characterisation of AqpZ, a water channel from *Escherichia coli*. *J Biol Chem* **270:** 29063–29066.

Caylay, S., Lewis, B.A., Guttman, H.J., and Record, M.T. (1991) Characterisation of the cytoplasm of *Escherichia coli* K-12 as a function of external osmolarity. *J Mol Biol* **222:** 281–300.

Chrispeels, M.J., and Agre, P. (1994) Aquaporins: water channel proteins of animal and plant cells. *Trends Biochem Sci* **19:** 421–425.

Csonka, L.N. (1989) Physiological and genetic responses of bacteria to osmotic stress. *Microbiol Rev* **53:** 121–147.

Csonka, L.N., and Hanson, A.D. (1991) Prokaryotic osmoregulation: genetics and physiology. *Annu Rev Microbiol* **45:** 569–606.

Csonka, L.N., Ikeda, T.P., Fletcher, S.A., and Kustu, S. (1994) The accumulation of glutamate is necessary for optimal growth of *Salmonella typhimurium* in media of high osmolarity but not induction of the *proU* operon. *J Bacteriol* **176:** 6324–6333.

Culham, D.E., Lasby, B., Marangoni, A.G., Milner, J.L., Steer, B.A., van Nues, R.W., and Wood, J.M. (1993) Isolation and sequencing of the *Escherichia coli* gene *proP* reveals unusual structural features of the osmoregulatory proline/betaine transporter, ProP. *J Mol Biol* **229:** 268–276.

Dijkstra, A.J., and Keck, W. (1996) Peptidoglycan as barrier to transenvelope transport. *J Bacteriol* **178:** 5555–5562.

Doyle, R.J., and Marquis, R.E. (1994) Elastic, flexible peptidoglycan and bacterial cell wall properties. *Trends Microbiol* **2:** 57–60.

Eichler, K., Bourgis, F., Buchet, A., Kleber, H.-P., Mandrand-Berthelot, M.-A. (1994) Molecular characterisation of the *cai* operon necessary for carnitine metabolism in *Escherichia coli*. *Mol Microbiol* **13:** 775–786.

Epstein, W. (1992) Kdp, a bacterial P-type ATPase whose expression and activity are regulated by turgor pressure. *Acta Physiol Scand* **607** (Suppl.): 193–199.

Faatz, E., Middendorf, A., and Bremer, E. (1988) Cloned structural genes for the osmotically regulated binding-protein-dependent glycine betaine transport system (ProU) of *Escherichia coli* K-12. *Mol Microbiol* **2:** 265–279.

Galinski, E.A., and Trüper, H.G. (1994) Microbial behaviour in salt-stressed ecosystems. *FEMS Microbiol Rev* **15:** 95–108.

Glaasker, E., Konings, W.N., and Poolman, B. (1996a) Osmotic regulation of intracellular solute pools in *Lactobacillus plantarum*. *J Bacteriol* **178:** 575–582.

Glaasker, E., Konings, W.N., and Poolman, B. (1996b) Glycine-betaine fluxes in *Lactobacillus plantarum* during osmostasis and hyper- and hypoosmotic shock. *J Biol Chem* **271:** 10060–10065.

Glaasker, E., Tjan, F.S.B., Ter Steeg, P.F., Konings, W.N., and Poolman, B. (1998) The physiological response of *Lactobacillus plantarum* towards salt and non-electrolyte stress. *J Bacteriol* (in press).

Gouesbet, G., Trautwetter, A., Bonassie, S., Wu, L.F., and Blanco, C. (1996) Characterisation of the *Erwinia chrysanthemi* osmoprotectant transporter gene *ousA*. *J Bacteriol* **178:** 447–455.

Haardt, M., and Bremer, E. (1996) Use of *phoA* and *lacZ* fusions to study the membrane topology of ProW, a component of the osmoregulated ProU transport system of *Escherichia coli*. *J Bacteriol* **178:** 5370–5381.

Jovanovich, S.B., Record, M.T., and Burgess, R.R. (1989) In an *Escherichia coli* coupled transcription-translation system, expression of the osmoregulated gene *proU* is stimulated at elevated potassium concentrations and by an extract from cells grown at high osmolality. *J Biol Chem* **264:** 7821–7825.

Jung, K., Tjaden, B., and Altendorf, K. (1997) Purification, reconstitution, and characterisation of KdpD, the turgor sensor of *Escherichia coli*. *J Biol Chem* **272:** 10847–10852.

Kakinuma, Y., and Igarashi, K. (1988) Active potassium extrusion regulated by intracellular pH in *Streptococcus faecalis*. *J Biol Chem* **263:** 14166–14170.

Kappes, R.M., Kempf, B., and Bremer, E. (1996) Three transport systems for the osmoprotectant glycine betaine operate in *Bacillus subtilis:* characterisation of OpuD. *J Bacteriol* **178:** 5071–5079.

Kempf, B., and Bremer, E. (1995) OpuA, an osmotically regulated binding protein-dependent transport system for the osmoprotectant glycine betaine in *Bacillus subtilis*. *J Biol Chem* **270:** 16701–16713.

Koch, A.L., and Woeste, S. (1992) Elasticity of the sacculus of *Escherichia coli*. *J Bacteriol* **174:** 4811–4819.

Koo, S.-P., Higgins, C.F., and Booth, I.R. (1991) Regulation of compatible solute accumulation in *Salmonella typhimurium*. *J Gen Microbiol* **137:** 2617–2625.

Lamark, T., Kaasen, I., Eshoo, M.W., Falkenberg, P., McDougall, J., and Ström, A.R. (1991) DNA sequences and analysis of the *bet* genes encoding the osmoregulatory choline-glycine betaine pathway of *Escherichia coli*. *Mol Microbiol* **5:** 1049–1064.

Lambert, C, Erdmann, A., Eikmanns, M., and Krämer, R. (1995) Triggering glutamate excretion in *Corynebacterium glutamicum* by modulating the membrane state with local anesthetics and osmotic gradients. *Appl Env Microbiol* **61:** 4334–4342.

Lucht, J.M., and Bremer, E. (1994) Adaptation of *Escherichia coli* to high osmolarity environments: osmoregulation of the high-affinity glycine betaine transport system ProU. *FEMS Microbiol Rev* **14:** 3–20.

McLaggan, D., Naprstek, J., Buurman, E.T., and Epstein, W. (1994) Interdependence of K^+ and glutamate accumulation during osmotic adaptation of *Escherichia coli*. *J Biol Chem* **269**, 1911–17.

Markin, V.S., and Martinac, B. (1991) Mechanosensitive ion channels as reporters of bilayer expansion: a theoretical model. *Biophys J.* **60:** 1120–1127.

Martinac, B., Adler, J., and Kung, C. (1990) Mechanosensitive ion channels of *E. coli* activated by amphipaths. *Nature* **348:** 261–263.

Measures, J.C. (1975) Role of amino acids in osmoregulation in non-halophilic bacteria. *Nature* **257:** 398–400.

Meury, J., Robin, A., and Kepes, A. (1985) Turgor-controlled K^+ fluxes and their pathways in *Escherichia coli*. *Eur. J Biochem* **151:** 613–619.

Milner, J.L., Grothe, S., and Wood, J.M. (1988) Proline porter II is activated by a hyperosmotic shift in whole cells and membrane vesicles of *Escherichia coli* K1. *Biol Chem* **263:** 14900–14905.

Mitchell, P., and Moyle, J. (1956) Osmotic structure and function in bacteria. In *Bacterial Anatomy, sixth Symposium Soc. Gen. Microbiol.* Cambridge: Cambridge University Press, pp. 150–180.

Nakashima, K., Sugiura, A., Kanamaru, K., and Mizuno, T. (1993) Signal transduction between the two regulatory components involved in the regulation of the *kdpABC*

operon in *Escherichia coli*: phosphorylation-dependent functioning of the positive regulator KdpE. *Mol Microbiol* **7:** 109–116.

Peter, H., Burkovski, A., and Krämer, R. (1996) Isolation, characterisation, and expression of the *Corynebacterium glutamicum betP* gene, encoding the transport system for the compatible solute glycine betaine. *J Bacteriol* **178:** 5229–5234.

Peter, H., Burkovski, A., and Krämer, R. (1998) Osmo-sensing by N- and C-terminal extensions of the glycine betaine uptake system BetP of *Corynebacterium glutamicum*. *J Biol Chem* **273:** 2567–2574.

Poolman, B., and Konings, W.N. (1993) Secondary solute transport in bacteria. *Biochim Biophys Acta* **1183:** 5–39.

Poolman, B., Hellingwerf, K.J., and Konings, W.N. (1987a) Regulation of the glutamate-glutamine transport system by intracellular pH in *Streptococcus lactis*. *J Bacteriol* **169:** 2272–2276.

Poolman, B., Driessen, A.J.M., and Konings, W.N. (1987b) Regulation of solute transport in Streptococci by external and internal pH values. *Microbiol Rev* **51:** 498–508.

Rajkumar, K., Kusano, S., Ishihama, A., Mizuno, T., Gowrishankar, J. (1996) Effect of H-NS and potassium glutamate on σ^{S}- and σ^{70}-directed transcription *in vitro* from osmotically regulated P1 and P2 promoters of *pro*U in *Escherichia coli*. *J Bacteriol* **178:** 4176–4181.

Ramirez, R.M., Prince, W.S., Bremer, E., and Villarjo, M. (1989) *In vitro* reconstitution of osmoregulated expression of *proU* of *Escherichia coli*. *Proc Natl Acad Sci USA* **86:** 1153–1157.

Ruffert, S., Lambert, C., Peter, H., Wendisch, V.F., and Kramer, R. (1997) Efflux of compatible solutes in *Corynebacterium glutamicum* mediated by osmoregulated channel activity. *Eur J Biochem* **247:** 572–580.

Schleyer, M., Schmid, R., and Bakker, E.P. (1993) Transient, specific and extremely rapid release of osmolytes from growing cells of *Escherichia coli* K-12 exposed to hypoosmotic shock. *Arch Microbiol* **160:** 424–431.

Schlösser, A., Meldorf, M., Stumpe, S., Bakker, E.P., and Epstein, W. (1995) TrkH and its homolog, TrkG, determine the specificity and kinetics of cation transport by the Trk system of *Escherichia coli*. *J Bacteriol* **177**, 1908–10.

Sheetz, M.P., and Singer, S.J. (1974) Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. *Proc Natl Acad Sci USA* **71:** 4457–4461.

Sheetz, M.P., Painter, R.G., and Singer, S.J. (1976) Biological membranes as bilayer couples. III. Compensatory shape changes induced in membranes. *J Cell. Biol* **70:** 193–203.

Stock, J.B., Rauch, B., and Roseman, S. (1977) Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. *J Biol Chem* **252:** 7850–7861.

Sugiura, A., Hirokawa, K., Nakashima, K., and Mizuno, T. (1994) Signal-sensing mechanisms of the putative osmosensor KdpD in *Escherichia coli*. *Mol Microbiol* **14:** 929–938.

Suhharev, S.I., Bount, P., Martinac, B., Blattner, F.R., and Kung, C. (1994) A large conductance mechanosensitive channel in *E. coli* encoded by *mscL* alone. *Nature* **368:** 265–268.

Sukharev, S.I., Blount, P., Martinac, B., and Kung, C. (1997) Mechanosensitive channels of *Escherichia coli*: the MscL gene, protein, and activities. *Annu Rev Physiol* **59:** 633–657.

Sweeney, T.E., and Beuchat, C.A. (1993) Limitations of methods of osmometry: measuring the osmolality of biological fluids. *Am J Physiol* **264**, R469-R480.

Thwaites, J.J., and Mendelson, N.H. (1989) Mechanical properties of peptidoglycan as determined from bacterial thread. *Int J Biol Macromol* **11:** 201–206.

Verheul, A., Glaasker, E., Poolman, B., and Abee, T. (1997) Betaine and L-carnitine transport in response to osmotic signals in *Listeria monocytogenes* Scott A. *J Bacteriol* **179:** 6979–6985.

Walshaw, D.L., and Poole, P.S. (1996) The general L-amino acid permease of *Rhizobium leguminosarum* is an ABC uptake system that also influences efflux of solutes. *Mol Microbiol* **21:** 1239–1252.

Walz, T., Hirai, T., Murata, K., Heymann, J.B., Mitsuoka, K., Fujiyoshi, Y., *et al.* (1997) The three-dimensional structure of aquaporin-1. *Nature* **387:** 624–627.

Whatmore, A.M., and Reed, R.H. (1990) Determination of turgor pressure in *Bacillus subtilis*: a possible role for K^+ in turgor regulation. *J Gen Microbiol* **136:** 2521–2526.

Whatmore, A.M., Chudek, J.A., and Reed, R.H. (1990) Effect of osmotic upshock on intracellular solute pools of *Bacillus subtilis*. *J Gen. Microbiol* **136:** 2527–2535.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., and Somero, G.N. (1982) Living with water stress: evolution of osmolyte systems. *Science* **217:** 1214–1222.