Intracellular Organic Osmolytes: Function and Regulation*
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Cells of almost all organisms accumulate organic osmolytes when exposed to hyperosmolality, most often in the form of high salt or urea. In this review, we discuss 1) how the organic osmolytes protect; 2) the identity of osmolytes in Archaea, bacteria, yeast, plants, marine animals, and mammals; 3) the mechanisms by which they are accumulated; 4) sensors of osmolality; 5) the signaling pathways involved; and 6) mutual counteraction by urea and methylamines.

The function and regulation of intracellular organic osmolytes were already well described in a classical review 25 years ago (1). The basic concepts, as described then and still valid, are as follows. 1) All water-stressed organisms, except halobacteria, accumulate intracellular organic osmolytes in response to water stress. 2) The major systems of organic osmolytes include polyhydric alcohols, free amino acids and their derivatives, and combinations of urea and methylamines. 3) The evolutionary advantage of the organic osmolyte systems is compatibility with macromolecular structure and function at high and variable osmolyte concentrations without modifying cellular proteins to function in concentrated intracellular solutions. 4) Osmolyte compatibility results from non-perturbing or favorable effects of osmolytes on macromolecule-solvent interactions. In this review, we emphasize subsequent findings that expand upon these concepts in various species.

Mechanism of Protection by Organic Osmolytes

Two complementary mechanisms are recognized for the protective effects of organic osmolytes in cells exposed to hypertonicity (1).

“Perturbing” Versus “Compatible” Solute—Although many biochemical functions require specific inorganic ions, increasing the concentrations of these ions above those typically found in cells perturbs protein function. In contrast, organic osmolytes have much less effect, i.e., are compatible. Hypertonicity, as results from high NaCl, causes osmotic flux of water out of cells, elevating the concentration of all cellular constituents, including inorganic salts (2). Although cells can rapidly restore their volume (“regulatory volume increase”) by influx of inorganic salts followed by osmotic uptake of water, the intracellular inorganic ion concentration remains high. Organic osmolytes accumulate later, associated with a decreasing concentration of intracellular inorganic salts. Thus, perturbing inorganic ions are replaced by compatible osmolytes.

Stabilization of Native Protein Structure (3)—High concentrations of protective organic osmolytes stabilize protein structure. The mechanism of stabilization involves strong exclusion of the protective osmolytes from the surface of proteins. On the other hand, denaturing osmolytes accumulate at the surface of proteins. Protective osmolytes push the equilibrium of protein folding toward the native form, whereas denaturing osmolytes push it toward the unfolded form. The configuration of the protein backbone is the most important determinant of stabilization or denaturation (4).

Bacteria and Archaea

Bacteria and Archaea accumulate many different organic osmolytes in response to hypertonicity, including trehalose, proline, glutamate, ectoine, glycine betaine (betaine), and carnitine (5). The selection of osmolytes depends on the duration of the osmotic stress and the availability of substrates and osmolytes in the surroundings. Escherichia coli and Salmonella typhimurium have been particularly well studied in this regard. Following a sudden increase in osmolality, they rapidly take up large amounts of K+ via turgor-responsive transport systems. Glutamate synthesis increases virtually simultaneously, which provides a counterion. Other organic osmolytes are then accumulated more slowly by uptake from exogenous sources or synthesis, and the concentration of potassium glutamate falls. The organic osmolytes accumulated by E. coli and S. typhimurium depend on what is available in their surroundings. When exogenous organic osmolytes are lacking, they synthesize and accumulate trehalose. However, betaine or proline, if present, is preferentially accumulated by uptake, and synthesis of endogenously produced osmoprotectants diminishes.

Hyperosmolality increases transcription of genes encoding betaine and proline transporters. Transcription of proU, a binding protein-dependent transport system that is a member of the ABC superfamily of transporters in E. coli, is a much studied example (5, 6). The proU system transports proline, betaine, proline betaine, and other osmolytes. Hypertonicity increases proU transcription. Despite considerable effort, no classical regulatory protein that specifically controls proU transcription has been identified. Genetic searches yielded only mutants with alterations in DNA-binding proteins that have pleiotropic effects on gene expression and DNA superstructure. The recovery of such mutants and the finding that cells grown at high osmolality show an increase in DNA supercoiling of reporter plasmids led to models in which transcription initiation of proU is repressed at low osmolality by an oligomer of H-NS anchored to AT-rich curved DNA sites upstream and downstream of the transcription site, and this repression is relieved by hyperosmolality, perhaps as a result of modulation of the physical properties of H-NS and increased DNA supercoiling (6). In addition, both in vivo and in vitro studies suggested a direct effect of potassium glutamate on proU transcription. However, the sig-

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Significance of these findings was challenged because the effects might be indirect and might reflect a nonspecific stimulation of the transcription apparatus of the cell. Subsequently, a basis for specificity was proposed based on evidence that potassium glutamate might mediate a global transcription switch by acting differentially on RNA polymerase at sets of genomic promoters that differ in their responsiveness to this salt (7).

Hypertonicity also directly enhances bacterial osmolyte transport in vivo and in proteoliposomes in vitro (5, 8). Transporters from three different species have been investigated in detail (8), namely ProP from E. coli, BetP from Corynebacterium glutamicum, and OpuA from Lactococcus lactis. They are all activated at constant membrane area, so the stimulus is not change in membrane strain. Rather, it is the increased concentration of intracellular inorganic ions that results from osmotic efflux of water. The results also depend on the specific lipid composition of the proteoliposome. The equilibrium between inactive and active conformations of the transporters is influenced by properties of the internal ions, acting on their cytoplasmic C-terminal domains, but the pertinent aspect of the stimulus and the nature of the response by the receptor differ between transporters. OpuA senses ionic strength. Increasing internal ionic strength releases its C-terminal domains from the membrane surface, and this conformational change enhances transport. BetP is a proton-driven secondary transporter. It is a K⁺-specific chemosensor. ProP is a secondary transporter driven by the proton-motive force. Osmoregulation of transport occurs because of changes in its hydration. It activates when it is partially dehydrated, leaving water molecules that contribute to the pathway for H⁺ transport. It is important to note that all three transporters are directly activated by hypertonicity, meaning that they are osmosensors.

E. coli cells growing in the absence of exogenous osmoprotectants, an increase synthesis of trehalose and glycine betaine (5). Trehalose synthesis is determined by two enzymes encoded by the osmoregulated trehalose synthesis operon. Glycine betaine synthesis is determined by a membrane-bound, FAD-containing choline dehydrogenase that oxidizes the precursor, choline, to glycine betaine aldehyde. A second, highly substrate-specific enzyme converts glycine betaine aldehyde into glycine betaine.

Yeasts

Saccharomyces cerevisiae almost exclusively employ glycerol as an osmolyte (9). S. cerevisiae also accumulate trehalose and glycerophosphocholine (10), but to levels that are trivial compared with that of glycerol. Other yeast and fungi are known to produce and/or accumulate from the environment different polyols such as erythritol, ribitol, arabinitol, xyitol, sorbitol, mannitol, and galactitol. Glycerol is accumulated in response to hypertonicity by increasing its production and reducing its efflux (9). The increased production is signaled by the high osmolarity glycerol (HOG)² MAPK pathway, which activates transcription factors that increase expression of two key enzymes in glycerol biosynthesis from the glycolytic intermediate dihydroxyacetone phosphate. The protein levels and specificity of the enzymes increase rapidly following hypertonicity. On the other hand, glycerol transmembrane efflux occurs via a specific transport protein, the channels of which close rapidly upon a hyperosmotic shock, retaining the glycerol that is produced.

Two redundant transmembrane proteins, Snl1 and Sho1, independently activate the HOG pathway in response to hypertonicity (11). The SLN1 and SHO1 branches of the pathway converge to activate the MAPKK Pbs2, which in turn activates the MAPK Hog1. Hog1 then accumulates in the nucleus, where it modulates the transcription factors responsible for accumulation of glycerol. At the head of the SLN1 branch, Snl1 is a transmembrane protein homolog of bacterial two-component signal transducers. Low osmotic conditions continuously activate the Snl1 histidine kinase domain. The resulting histidine-bound phosphate transfers via phosphorelay through intermediaries to an aspartate residue in the Rec domain protein Ssk1. Sln1 senses changes in cellular turgor pressure. When hypertonicity reduces turgor pressure, the Snl1 histidine kinase domain is inactivated, which terminates the phosphorelay reaction. The phosphoaspartate residue in Ssk1 is intrinsically unstable and prone to spontaneous hydrolysis. Dephosphorylated Ssk1 binds to and activates the MAPKK upstream of Hog1. At the head of the SHO1 branch (12) are two mucin-like transmembrane proteins that may directly detect extracellular water activity by changes in their volume. These proteins individually form a complex with Sho1 and, upon osmotic stress, interact in a complex fashion to induce Sho1 to activate through intermediates the MAPKK upstream of Hog1.

Plants

Plants subject to salt stress accumulate organic osmolytes, including proline, valine, isoleucine, ectoine, aspartic acid, betaine, glucose, fructose, sucrose, fructans, mannitol, pinitol, and myo-inositol (inositol) in the cytoplasm of their cells (13). Organic osmolytes generally are accumulated in plants by increasing the rate at which they are synthesized, but the osmo-regulatory mechanisms involved have been reported in only a few cases. Examples include that mannitol is synthesized via the action of a mannose-6-phosphate reductase in celery. Transformation of the non-mannitol producer Arabidopsis with this gene increases its tolerance for salt. Another example is that in tomato plants salinity enhances sucrose concentration and the activity of sucrose-phosphate synthase. Furthermore, in plants as in bacteria, glycine betaine is synthesized by two-step oxidation of choline via the intermediate betaine aldehyde (14). Its increased accumulation in leaves of stressed plants is regulated, at least in part, via changes in the expression of genes for the biosynthetic enzymes. However, the signals that provoke these changes in gene expression have not been identified.

As already mentioned, the Snl1 osmosensor histidine kinase in yeast monitors changes in turgor pressure (11). Reductions in turgor caused by hyperosmotic stress, nystatin, or removal of the cell wall activate the MAPK Hog1 specifically through the SLN1 branch, but not through the SHO1 branch of the high.

²The abbreviations used are: HOG, high osmolarity glycerol; MAPK, mitogen-activated protein kinase; MAPKK, MAPK kinase; GPC, glycerophosphocholine; AR,aldose reductase; SMIT, sodium myo-inositol transporter; NTE, neuropathy target esterase; OREBP, osmotic response element-binding protein; TAD, transactivation domain; PI3K-IA, phosphoinositide 3-kinase Class IA; ROS, reactive oxygen species.
osmolarity glycerol pathway (15). The activity of the plant histidine kinase Cre1 (cytokinin response 1) is regulated by changes in turgor pressure in a manner identical to that of Sln1 in yeast, and Cre1 complements the deficient Hog1 response in Sln1 mutant yeast cells (15). Based on these findings, it has been proposed that Cre1 has dual functions in plants, acting both as a cytokinin receptor and an osmosensor (15).

**Marine Animals**

In mollusks and crustaceans that exist at shallow depths, the most abundant osmolytes are betaine, taurine, trimethylamine oxide, glycine, alanine, proline, homarine, and arginine (16). In deep sea invertebrates, there are high levels of scylo-inositol, β-alanine, betaine, hypotaurine, and N-methyltaurine (17). Interestingly, trimethylamine N-oxide increases with depth of habitat in muscles of some teleosts,skates, and crustaceans, which apparently counteracts the perturbing effects of hydrostatic pressure (17).

The organic osmolyte system in elasmobranchs is particularly interesting in that these animals accumulate urea along with methylamines (trimethylamine N-oxide, betaine, sarcosine) in their tissues and extracellular fluid to balance the high osmolality of sea water (1). Urea is seemingly a particularly unlikely osmoregulatory partner because it denatures proteins and alters their enzymatic activity. However, the methylamines that accumulate with urea counteract its denaturing effects, explaining the utility of the combination. The theoretical basis for their counteractions has been intensively studied. As already discussed, the most important determinant of the difference between osmolytes that denature proteins and those that stabilize them is their opposite effects on the configuration of the protein backbone (4). These opposite actions are directly dependent on concentration, independent of each other, and additive (18), so at a particular ratio of stabilizing osmolyte to urea (generally 2:1), the denaturing effects counteract each other.

**Mammals (2, 19)**

The osmolality of mammalian blood is normally kept remarkably constant at ~290 mosmol/kg by a combination of thirst and varying urinary concentrations. Therefore, most mammalian cells are not normally exposed to the extreme osmolalities experienced by the cells discussed above. Nevertheless, certain mammalian cells contain considerable concentrations of organic osmolytes, and most of the rest are able to accumulate them if suitably stressed. Renal medullary cells contain the highest levels of organic osmolytes consequent to their being exposed to extremely high concentrations of NaCl and urea because of their roles in concentrating the urine. The principal organic osmolytes in renal medullary cells are sorbitol, betaine, inositol, taurine, and glycerophosphocholine (GPC). Cells in other tissues may also experience hyperosmolality, albeit to a lesser degree than in the renal medulla. Accordingly, they also accumulate organic osmolytes. The principal ones in brain are amino acids, choline, creatine, inositol, and taurine (20). Liver cells accumulate betaine, inositol, and taurine (21).

The mechanisms of accumulation and their regulation in response to high NaCl and urea have been most thoroughly studied in renal medullary cells. In these cells, sorbitol is synthesized from glucose, catalyzed by aldose reductase (AR) (2). Hypertonicity increases transcription of the AR gene, resulting in elevation of its mRNA and protein.

Betaine increases with hypertonicity because more is taken up from extracellular fluid (2). It is synthesized from choline both in liver and kidney. The rate of its synthesis, unlike that of sorbitol, however, is not affected by hypertonicity. Rather, hypertonicity increases the number of betaine transporters, called BGT1 (betaine/GABA transporter 1). BGT1 couples transport of betaine to that of chloride and sodium. Hypertonicity elevates transcription of the BGT1 gene, followed by an increase in its mRNA and transport activity. Thus, tonicity regulates betaine transporter activity by affecting BGT1 transcription. In addition BGT1 is also regulated by plasma membrane insertion. The relatively small amount of BGT1 that exists under normotonic conditions is mainly in the cytoplasm. Following hypertonicity, BGT1 localizes to basolateral plasma membranes.

Inositol also increases with hypertonicity because more is taken up from extracellular fluid (2). Although it is synthesized by renal cells, the inositol accumulated following hypertonicity is transported into the cells rather than synthesized. Hypertonicity increases inositol transport by increasing the number of inositol transporters, called sodium myo-inositol transporters (SMITs). SMIT couples transport of inositol to that of sodium. Hypertonicity elevates transcription of the SMIT gene, followed by increases in its mRNA and transport activity.

Taurine also increases with hypertonicity because more is taken up from extracellular fluid (2). The osmoregulated taurine transporter, called TauT, is sodium- and chloride-dependent. Hypertonicity increases its transcription and the abundance of its mRNA and protein.

GPC is unique among the renal medullary organic osmolytes in that it accumulates in response to high urea as well as to hypertonicity (2). It is synthesized from phosphatidylcholine and broken down into choline and α-glycerophosphate. High NaCl increases GPC synthesis and also reduces its degradation. Its synthesis from phosphatidylcholine is catalyzed by neuropathy target esterase (NTE), a phospholipase B. High NaCl increases NTE activity by elevating its transcription and thereby its mRNA and protein. High NaCl also reduces degradation of GPC by decreasing the activity of GPC-choline phosphodiesterase, the enzyme that degrades it. In contrast, high urea elevates GPC solely by inhibiting GPC-choline phosphodiesterase activity. Being a methylamine, the GPC that accumulates in renal medullary cells counteracts the perturbing effects of the high concentration of urea that exists there, just as methylamines counteract urea in elasmobranchs (see above). The methylamine betaine also accumulates in renal medullary cells and counteracts urea. However, although high urea increases GPC in these cells, it decreases betaine. The difference is striking, but its evolutionary basis remains conjectural.

TonEBP/OREBP (also named NFAT5) is the transcription factor that mediates hypertonicity-induced transcription of AR, BGT1, SMIT, tauT, and NTE (2). Each TonEBP/OREBP target gene contains in its regulatory regions at least one DNA consensus motif called an osmotic response element or TonE
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(tonicity-responsive enhancer). TonEBP/OREBP forms stable dimers (or higher order oligomers) in cells and in solution, independent of tonicity. Dimerization is necessary for its binding to DNA as a homodimer that completely encircles the DNA target. Dimerization is also required during hypertonicity for its "proper" phosphorylation and for transactivation of downstream genes. Hypertonicity elevates the transcriptional activity of TonEBP/OREBP by increasing its mRNA and protein abundance, by phosphorylating it, by translocating it to the nucleus, and by increasing its transactivating activity as follows.

The mRNA abundance of TonEBP/OREBP increases following hypertonicity because of a transient increase in its mRNA stability, mediated by elements within the 5′-untranslated region (2). Translation increases in proportion to the increase in mRNA, whereas the rate of TonEBP/OREBP protein degradation is unaffected. How tonicity regulates TonEBP/OREBP mRNA stability is unknown.

Phosphorylation of TonEBP/OREBP increases within 30 min of hypertonicity (2). Phosphorylation apparently contributes to hypertonicity-induced activation of TonEBP/OREBP, but the specific amino acids that are phosphorylated are not yet identified, which limits our understanding. Nevertheless, we know that the binding of TonEBP/OREBP to its cognate DNA element, as measured by electrophoretic mobility shift assay, is eliminated by a serine/threonine phosphatase. Thus, serine/threonine phosphorylation is involved in TonEBP/OREBP binding to its DNA element. Furthermore, hypertonicity increases phosphorylation of TonEBP/OREBP within amino acids 548–1531, which contain a transactivation domain (TAD) that is activated by hypertonicity. Serine/threonine kinase inhibitors reduce the hypertonicity-dependent transactivating activity of TonEBP/OREBP.

Nuclear accumulation of TonEBP/OREBP starts within 30 min after an increase in tonicity (2). Recombinant TonEBP/OREBP comprising only the N-terminal amino acids (1–547) localizes to the nucleus when NaCl is increased, indicating that the C terminus is not required. The N terminus contains functional nuclear localization and export signals. TonEBP/OREBP shuttles constantly between the cytoplasm and nucleus, and its distribution at any particular osmolality is determined by the balance of import and export. Mutation of nuclear export signals in recombinant TonEBP/OREBP causes it to accumulate in the nucleus regardless of tonicity. Hypertonicity-induced nuclear translocation of TonEBP/OREBP is signaled at least in part by the DNA damage response protein ATM (gammaH2AX, mutant) kinase. High NaCl increases DNA strand breaks in cell culture and in vivo and activates ATM. ATM contributes to high NaCl-induced nuclear translocation of TonEBP/OREBP through interaction at sites within amino acids 1–547.

The transactivating activity of TonEBP/OREBP is increased by hypertonicity (2). TonEBP/OREBP contains a NaCl-dependent TAD within amino acids 1039–1249. There are additional TADs that are not sensitive to tonicity and also modulation domains that potentiate the activity of the TADs but are not independently active in stimulating transcription. Many hypertonicity-induced signaling molecules are known to regulate transactivation of its target genes by TonEBP/OREBP. Each is necessary for full TonEBP/OREBP activity, but not one of them alone is sufficient. They include 1) CAM-dependent kinase, which is activated by high NaCl in a CAM-independent manner, and 2) p38, a MAPK homolog of yeast Hog1. Hypertonicity activates MAPK kinases, which in turn activate p38 in a multienzyme complex attached to the scaffolding protein CCM2 (also named OSM). This activation of p38 is associated with hypertonicity-induced cytoskeletal rearrangement. The multienzyme complex includes actin, Rac1, MEKK3, and M KK3.

Also included are 3) Fyn, a member of the Src family of non-receptor cytoplasmic protein-tyrosine kinases that is activated by the cell shrinkage that results from hypertonicity, and 4) ATM. Multiple agents activate ATM without activating TonEBP/OREBP, including urea, ultraviolet radiation, ionizing radiation, and low NaCl. Thus, ATM is necessary for full TonEBP/OREBP activity, but activation of ATM alone is not sufficient to induce it. 5) Phosphoinositide 3-kinase Class IA (PI3K-IA) is a lipid kinase that phosphorylates the 3′-hydroxyl moiety of phosphatidylinositol and phosphoinositides, generating phosphatidylinositol 3,4,5-triphosphate. High NaCl activates the PI3K-IA catalytic subunit, p110α, resulting in increased production of phosphatidylinositol 3,4,5-triphosphate. This effect of PI3K-IA is mediated by ATM. 6) Reactive oxygen species (ROS) also convey signals from cytokines, hormones, and ions to downstream effectors, including transcription factors. High NaCl increases intracellular ROS in renal cells in culture and in the renal medulla. ROS might regulate TonEBP/OREBP via p38 because the antioxidant N-acetyl-L-cysteine prevents high NaCl-induced phosphorylation of p38.

Interactions with the extracellular matrix also contribute to hypertonicity-induced activation of TonEBP/OREBP (2). Integrins are extracellular matrix receptors that link the internal actin cytoskeleton to the extracellular matrix. Hypertonicity increases β1-integrin mRNA and protein. αiβi-Integrin-null mice accumulate less organic osmolytes and less SMIT and AR mRNAs in their inner medullas in response to dehydration than do wild-type mice. Furthermore, hypertonicity-induced increase in TonEBP/OREBP protein is reduced in cultures of renal inner medullary collecting duct cells that lack αiβi-integrin.

Other proteins that physically associate with TonEBP/OREBP and affect its activity include RNA helicase A (2), Hsp90 (22), and PARP-1 (poly(ADP-ribose) polymerase-1) (22). Binding of RNA helicase A to TonEBP/OREBP inhibits its transcriptional activity. Hypertonicity decreases the binding, which releases TonEBP/OREBP from the inhibition. PARP-1 expression reduces TonEBP/OREBP transcriptional activity and the activity of its transactivating domain (22). This inhibition by PARP-1 is independent of its enzymatic and DNA-binding activities. Hsp90 enhances TonEBP/OREBP transcriptional and transactivation activity and sustains the increased abundance of TonEBP/OREBP protein in cells exposed to high NaCl (22).

Many different organic osmolytes occur, even in the same cell. In so far as the protection provided by an osmolyte does not depend on specific chemical interactions, any of them should in principle be able to replace any of the others. In support of this idea, when renal cells in culture are maintained at a constantly high NaCl concentration, an experimentally induced change in an organic osmolyte is reciprocated by an opposite change in
the amount of another, and they protect equally well (19). Given their interchangeable protective effects, it is not clear why one or another should be preferred. The explanation may be that even though the protective effects of osmolytes are nonspecific, an osmolyte or the mechanisms that regulate it may also have specific effects. For example, although synthesis of sorbitol in renal cells (catalyzed by AR) protects the cells from hypertonicity, AR can also be harmful (2). Its activity is high in diabetes, which has been implicated in the microvascular complications of this disorder, and as a result, there are major efforts to develop and use inhibitors of AR to minimize these complications. Furthermore, AR is activated during myocardial ischemic injury, and AR inhibitors protect rat hearts from ischemia-reperfusion injury. Adverse effects of increased traffic through the AR pathway have been attributed to elevation of the cytosolic NADH/NAD⁺ ratio.

What is the sensor of hypertonicity that activates protective osmoregulatory responses in mammalian cells? Hypertonicity causes a rapid decrease in cell volume, an increase in concentration of all intracellular components (including inorganic ions and macromolecules), and reorganization of the cytoskeleton (2). Each of these changes has been shown separately to signal an osmoregulatory response in one setting or another. Macromolecular crowding and ionic strength, in particular, have profound effects on biochemical processes, which could contribute to the increased ROS, DNA breaks, and altered activity of kinases and other enzymes that are involved in activating TonEBP/OREBP. No specific mammalian osmosensor has yet been identified. It is possible that none exists. If so, rather than a specific sensor, it may be the complex array of changes that occur that accounts for the osmoregulatory responses.

What is the stimulus for maintaining a high level of organic osmolytes after cells have adapted to hyperosmolality? Initial responses to hypertonicity include regulatory volume increase, which normalizes cell volume, followed by a slower accumulation of intracellular organic osmolytes, which additionally normalize intracellular ionic strength. These responses presumably have occurred in cells chronically adapted to hyperosmolality, yet the cells maintain a high level of organic osmolytes (2), leaving us to wonder what the stimulus might be.

Furthermore, although cells in the renal medulla, having accumulated organic osmolytes, survive and seem healthy, they still contain lesions that in other settings would be regarded as pathological, such as increased DNA strand breaks and oxidation of proteins and DNA bases (2). During hyperosmolality, however, these seemingly pathological lesions provide a benefit by signaling the osmoprotective activation of TonEBP/OREBP. The situation is not limited to mammalian cells. Such lesions are also present in marine (23) and terrestrial (24) invertebrates that are adapted to hyperosmolality. Finally, considering that accumulation of organic osmolytes is an almost universal response to hyperosmolality, it is striking that the recent studies that are reviewed above show so little conservation of the mechanisms that are involved.

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