

Osmoadaptation of Mammalian Cells – An Orchestrated Network of Protective Genes

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Abstract: In mammals, the cells of the renal medulla are physiologically exposed to interstitial osmolalities several-fold higher than found in any other tissue. Nevertheless, these cells not only have the ability to survive in this harsh environment, but also to function normally, which is critical for maintenance of systemic electrolyte and fluid homeostasis. Over the last two decades, a substantial body of evidence has accumulated, indicating that sequential and well orchestrated genomic responses are required to provide tolerance to osmotic stress. This includes the enhanced expression and action of immediate-early genes, growth arrest and DNA damage inducible genes (GADDs), genes involved in cell cycle control and apoptosis, heat shock proteins, and ultimately that of genes involved in the intracellular accumulation of non-perturbing organic osmolytes. The present review summarizes the sequence of genomic responses conferring resistance against osmotic stress. In addition, the regulatory mechanisms mediating the coordinated genomic response to osmotic stress will be highlighted.

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INTRODUCTION

Osmolalities exceeding the physiological range of 280-300 mosm/kg H₂O have severe consequences as they induce cell shrinkage, oxidative stress, DNA damage, cell cycle delay, and ultimately apoptosis in the absence of protective mechanisms. Renal medulla-resident cells provide an excellent paradigm for the adaptation of mammalian cells to osmotic stress, because, depending on the hydration status, these cells are exposed physiologically to wide fluctuations in ambient tonicity. In situations of water shortage (i.e. systemic volume depletion), the urinary concentrating mechanism is activated rapidly, which entails generation of papillary interstitial osmolalities several times higher than of the systemic circulation. In contrast, during prolonged diuresis, papillary interstitial osmolalities may drop progressively to values close to that of the systemic plasma. In the absence of counterregulatory mechanisms, medullary cells would not be able to correct changes in cell volume caused by osmotically obliged movement of water across the cell membrane, driven by the transmembrane osmotic gradient.

Following a rise in extracellular tonicity, renal medullary cells shrink and activate membrane transport pathways mediating the net uptake of NaCl and, in consequence, recovery of cell volume by osmotically driven entry of water [1-3]. Concomitantly, most of the imported sodium ions are replaced rapidly by potassium due to increased activity of the Na⁺,K⁺-ATPase [2]. Although these manoeuvres return the cell volume to normal or nearly normal values, they are inevitably associated with severe disturbances of intracellular ion homeostasis.

CELLULAR EFFECTS OF ELEVATED NaCl

Rise in Cellular Ionic Strength

Following an acute rise in ambient tonicity, the intracellular concentration of monovalent inorganic electrolytes (particularly potassium and chloride) increases in renal medullary cells to maintain osmotic equilibrium with the extracellular space [4]. A prolonged rise in the intracellular concentration of inorganic electrolytes, i.e. the cellular ionic strength, however, may negatively affect the function and integrity of cellular macromolecules [5, 6]. Specifically, the activity of several enzymes is reduced and both protein and DNA synthesis are diminished [7, 8]. In the initial phase following the induction of osmotic stress, specific heat shock proteins (HSPs) are assumed to protect medullary cells from the adverse effects of high intracellular electrolyte concentration in view of their protein-stabilizing properties [9, 10]. Subsequently, excess inorganic electrolytes are replaced continuously by compatible organic osmolytes (see below) [11].

In addition to interference with protein structure and function, a persistent rise in cellular ionic strength is associated with increased incidence of DNA damage and apoptotic cell death [12]. Specifically, chromosome alterations, sister chromatid exchanges and DNA single- and double-strand breaks have been detected in cells exposed to osmotic stress [12, 13]. These alterations occur in the initial phase of hyper-tonicity and are specific for impermeant substances that exert osmotic stress, since solutes that equilibrate rapidly across the cell membrane, like glycerol or urea, fail to elicit these effects [12, 13].

Tonicity-Induced Oxidative Stress

Recent data indicate that renal medullary cells are exposed to substantial concentrations of reactive oxygen spe-

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cies, particularly in situations of systemic volume contraction with concomitant activation of the urinary concentrating mechanism and rising medullary interstitial NaCl concentrations. The major sites for intracellular ROS production are the mitochondria, which convert approximately 0.2-2% of the oxygen available to ROS, particularly superoxide anions [14, 15]. To a lesser extent, extra-mitochondrial oxidases, hydroxylases, and cytochrome P450 enzymes contribute to ROS formation [14]. In addition to their role as signalling molecules, excessive production of ROS may cause severe cell damage [14].

The increased abundance of carbonylated proteins is regarded as evidence for extensive oxidative stress within renal medullary cells both *in vivo* and *in vitro* [16]. Protein carbamylation is recognized as a measure for severely oxidatively damaged proteins and is associated with a variety of disorders associated with excessive local oxidative stress [17, 18]. Although the mechanism of protein carbamylation during osmotic stress is incompletely understood, it is likely that ROS rather than NaCl per se mediate this effect, since both NaCl and raffinose increase the carbonyl content of cellular proteins and both solutes induce ROS in renal medullary cells [16]. Accumulation of carbonylated proteins may affect cellular function negatively either by loss of catalytic and/or structural integrity, or by interruption of regulatory pathways [19].

In medullary thick ascending limb cells, generation of ROS is linked to the stimulation of the Na⁺,K⁺-ATPase and the Na⁺/H⁺ exchanger in response to osmotic stress, whereas inhibition of the Na⁺,K⁺-ATPase by ouabain abolishes ROS production [20, 21]. Thus, increased ATP demand to maintain secondary and/or primary active transmembrane transport processes with elevated mitochondrial respiration may represent the primary mechanism for ROS production in renal medullary cells in response to osmotic stress. In addition, angiotensin II promotes the formation of superoxide *via* NADPH oxidase [22] and a process involving PKC in pericytes of descending vasa recta [23].

TONICITY-RESPONSIVE SIGNAL TRANSDUCTION

Although the genetic response to osmotic stress has been studied extensively in eukaryotic cells over the last two decades, to date the precise sequence of intracellular signalling events leading to osmotolerance has been identified only in yeast. In these cells, two membrane-resident osmosensors, SLN1 and sho-1, have been identified. These transduce an increase in ambient tonicity *via* several steps into activation of the mitogen-activated protein kinase (MAPK) HOG1, a homologue of mammalian p38. Activated HOG1 in turn drives the expression of enzymes involved in the intracellular accumulation of glycerol and trehalose, both of which represent the major organic osmolytes in these cells [24-26]. In mammalian cells a membrane-bound osmosensor has not yet been identified. Nevertheless, osmotic stress entails a series of intracellular events that culminate in transcriptional activation of a *trans*-acting protein, known as tonicity-responsive enhancer binding protein (TonEBP), that stimulates the expression of various genes conferring osmoresistance and ensuring proper function of the renal medulla during antidiuresis (see below). TonEBP has been cloned inde-

pendently by several groups; the corresponding *cis*-acting sequence in the 5'-regions of osmoprotective genes was identified some years ago [27-29]. The latter is known as tonicity-responsive enhancer (TonE), which is essential for induction of osmosensitive genes in response to hyperosmolar stress. Evidence that TonE confers osmotic inducibility of these genes has been provided by several experimental approaches, demonstrating that both single-base mutations or deletion of TonE elements blunt the osmotic induction of TonE-driven reporter constructs [24]. The following tonicity-responsive genes have been identified as TonEBP target genes: aldose reductase (AR), the betaine γ -amino-butyrate transporter (BGT-1), the sodium *myo*-inositol transporter (SMIT), the taurine transporter (TauT), heat shock protein 70 (HSP70), and aquaporin (AQP)-2 [29-33]. Indeed, mice with targeted disruption of TonEBP show severe and progressive atrophy of the renal medulla in parallel with reduced expression of osmoprotective genes [34]. Although the importance of TonEBP for renal medullary cells has been demonstrated convincingly, other tonicity-inducible genes essential for the integrity and function of the renal medulla appear to be regulated independently from TonEBP (e.g. Na⁺,K⁺-ATPase, Cox-2).

INDUCTION OF OSMOSENSITIVE GENES

Immediate-Early Genes

Immediate-early genes (IEGs) are activated rapidly and transiently in response to a broad variety of cellular stimuli, including osmotic stress. The induction of IEGs is far earlier than that of genes involved in osmolyte accumulation, suggesting important functions under conditions in which organic osmolytes have not been accumulated in sufficient quantities.

Well characterized is the transiently increased expression of Fos and Jun proteins in response to osmotic stress in different tissues. Fos and Jun interact with each other in the heterodimeric AP-1 protein complex. AP-1 is a transcription factor that stimulates gene expression by binding to its consensus sequence, the AP-1 binding site, which is localized in the promoter region of target genes. In supraoptic nucleus neurons, Fos and Jun proteins appear within 30 min after the onset of osmotic stress, peak after 90-120 min, and disappear almost completely within 240 min [35]. Also induced by hyperosmolarity in supraoptic nucleus neurons is the early growth response protein-1 (EGR-1), also known as NGFI-A [36]. In renal cells, the transcription rate of EGR-1 is increased by hyperosmotic urea as well as by hypotonic conditions [37]. EGR-1 is a zinc-finger-containing transcription factor, activating the transcription of various downstream genes, encoding growth factors, structural proteins, adhesion molecules or other transcription factors [38].

Expression of IEGs is probably mediated by tonicity-activated MAPK pathways [39] and can be considered as the first phase of the genomic response to exposure to osmotic stress. IEGs often encode transcription factors, which in turn activate the transcription of other genes, also called late-response genes. Therefore, the expression of IEGs is a prerequisite for the activation of late-response genes (such as genes involved in osmolyte accumulation or HSP genes), that protect the cell from deleterious effects of hypertonicity.

Genes Involved in DNA Damage Repair, Growth Arrest and Apoptosis

As mentioned above, an increase in cellular ionic strength is associated with DNA damage. DNA double-strand breaks are detected by the ataxia telangiectasia-mutated (ATM) kinase, which in turn activates other proteins. Accordingly, ATM contributes to full activation of TonEBP, although ATM alone is not sufficient for this process [40-42]. As a consequence of DNA damage due to increased intracellular ionic strength, renal medullary cells activate mechanisms leading to cell cycle delay and growth arrest, thus allowing repair of damaged DNA and preservation of genomic stability [43]. Increased expression and activation of the tumor suppressor gene product p53 by ATM kinase and p38 kinase mediates G₁ and G₂/M arrest, thereby possibly protecting medullary cells from apoptosis after a rise in ambient tonicity and allowing repair of damaged DNA [40, 44, 45]. Prevention of the increased p53 expression, and therefore prevention of growth arrest under hypertonic conditions promotes subsequent cell death during the S-phase [46]. The p53-induced growth arrest observed after the onset of osmotic stress persists for several hours, whereas when adaptation to hypertonicity is complete, cultured cells proliferate indefinitely [47], although at significantly lower rates than under isotonic conditions.

Hypertonicity also increases the copy number of the growth arrest and DNA damage (GADD)-induced proteins 45 and 153 [48]. GADD45 exists in three isoforms, α , β and γ [49]. In mouse inner medullary collecting duct cells, GADD45 is up-regulated by moderate osmotic stress (540 mosm/kg) and contributes to cell cycle arrest at the G₂/M cell-cycle checkpoint. During severe osmotic stress (620 mosm/kg), however, GADD45 γ enhances apoptosis while GADD45 α and β inhibit later stages of apoptosis [50]. These results indicate that GADD45, besides p53, plays a crucial role in the regulation of the balance between cell survival and apoptosis during osmotic stress.

Other DNA-damaging processes also cause transient growth arrest allowing activation of DNA repair mechanisms such as the meiotic recombination (Mre)11 complex [51]. Literature-data are inconsistent with respect to repair of NaCl-induced DNA breaks. Dmitrieva *et al.* have reported, that under conditions of elevated NaCl concentrations, Mre11 exits the nucleus, thus leaving the DNA unrepaired, even after completion of osmoadaptation of the cell and resume of proliferation [52, 53]. The latter studies suggested that checkpoint kinase (CHK) 1, involved in initiation of cell-cycle arrest, and histone H2AX, which is required for successful DNA repair, are also inhibited by high NaCl. However, in contrast, Sheen *et al.* have observed that Mre11 remains in the nucleus following an increase in tonicity and that also H2AX was induced, resulting in an intact cellular response to tonicity-induced DNA damage [54]. The reasons for these inconsistencies are not clear.

Heat Shock Proteins

The induction of heat shock proteins (HSPs) is one of the most conserved mechanisms protecting mammalian cells from various cellular stress conditions, including osmotic stress. HSPs are a group of proteins highly conserved from

bacteria to mammalian cells. Cell stress entails the accumulation of misfolded or aggregated proteins, while HSPs act as molecular chaperones, which assist other proteins to fold correct and assemble correctly. HSPs are also involved in protein transport and degradation of misfolded proteins, and interfere with the apoptotic signaling cascade [55]. According to their molecular weight, the HSPs are classified into several families (for a review see [56]). In mammalian cells, particularly members of the small HSP- (sHSP), HSP70- and HSP110-family are up-regulated in response to osmotic stress. It is well established that induction in response to heat stress occurs by binding of the heat shock transcription factor (HSF) to a heat-shock element (HSE) in the promoter region of HSP genes. Although osmotic stress also activates the transcription factor HSF-1, this event seems not to contribute substantially to increased mRNA-levels of HSP genes [57]. Instead, transcriptional activation by TonEBP plays a crucial role in HSP expression during hyperosmolality.

HSP27 belongs to the family of sHSPs. In renal cells, HSP27 expression is increased by osmotic stress and contributes to protection of renal medullary cells from high urea concentrations [58]. The distribution of HSP27 in the kidney follows the corticomedullary osmotic gradient, with low abundance in the cortex and high amounts in the inner medulla, that are elevated even further during antidiuresis [58]. The molecular mechanisms regulating HSP27 expression in response to osmotic stress are largely unknown. However, hyper- and hypoosmolarity induces phosphorylation of HSP27 *via* the p38 MAPK pathway, and subsequent capping of actin by HSP27, thereby promoting actin polymerization and stabilization of the actin cytoskeleton [59].

Another member of the sHSP family is α B-crystallin, which is also induced during osmotic stress. It shows homology to HSP25, is a molecular chaperone [60], and is one of the major structural proteins of the ocular lens of vertebrates. In human retinal epithelial cells and in kidney cells, expression of α B-crystallin increases in response to hypertonicity [61-63]. Furthermore, overexpression of α B-crystallin in HEK293 cells enhances cell survival following osmotic stress [63]. The increased expression of α B-crystallin apparently does not involve TonEBP [32], while the JNK kinase pathway seems to be involved in the up-regulation during hypertonicity [63].

Proteins of the HSP70 family may be expressed both constitutively and may be inducible by osmotic stress. In mice and human, there are two major HSP70 isoforms, of which only one is induced by osmotic stress [32, 64]. Tonicity-inducible HSP70 genes in different species contain TonE sites in their 5' -flanking region, suggesting that the increased transcription during osmotic stress is mediated by TonEBP [32]. In addition to its function as a molecular chaperone, stress-inducible HSP70 inhibits apoptosis by direct interaction with Apaf-1, thus preventing the recruitment of caspase-9 to the apoptosome complex [65, 66]. In the isoosmotic renal cortex, the stress-inducible HSP70 is barely detectable, while in the hypertonic renal medulla, the protein is highly abundant [67] and is regulated according to the diuretic state [67, 68]. By virtue of its chaperoning functions, HSP70 protects other proteins from destabilization, and counteracts protein aggregation [55]. These properties are consistent

with the role of HSP70 in protection of renal cells against high urea concentrations [69].

The osmotic stress protein (OSP) 94 is a member of the HSP110 family [70], and is a molecular chaperone *in vitro* [71]. Expression of OSP94 in the kidney correlates with the corticomedullary osmotic gradient and the highest abundance is observed in the inner medulla [70]. During antidiuresis, the already high expression in the inner medulla is elevated even further [72]; indeed, recent data imply that OSP94 is one of the most strongly induced genes following tonicity stress in the proteome of mouse inner medullary collecting duct (mIMCD) cells [73]. In OSP94-deficient mice, 12% of the animals develop hydronephrosis [74] and are preferentially susceptible to osmotic stress. Kojima *et al.* have detected a TonE-like element in the 5'-flanking region of the gene, to which TonEBP probably binds and upregulates the transcription in response to osmotic stress [75].

Genes Involved in Osmolyte Accumulation

As mentioned above, a rise in ambient tonicity results in initially increased cellular ionic strength, accompanied by adverse effects on cellular macromolecules. To overcome these adverse effects, cells have evolved mechanisms to replace excess inorganic ions by a group of organic osmolytes that preserve the osmotic balance but do not interfere with macromolecular structure and function. Consistent with their role in osmoadaptation and their neutral effect on cell metabolism, these compounds are termed compatible organic osmolytes. The best characterized organic osmolytes in mammalian cells are the polyols sorbitol and *myo*-inositol, the trimethylamines betaine and glycerophosphocholine (GPC), and free amino acids like taurine. Exposure of cells to osmotic stress activates various mechanisms that enable intracellular accumulation of these compounds.

The polyol sorbitol is produced from glucose, catalyzed by the enzyme aldose reductase (AR). AR abundance increases substantially following osmotic stress, reflecting enhanced transcription of the gene [76]. In the 5'-flanking region of the AR gene, several TonE-sites are present, to which TonEBP binds under hyperosmotic conditions and enhances gene transcription [27, 29, 77]. The tonicity-induced expression of AR is implicated in serious complications in patients with diabetes. The high level of accumulated sorbitol is involved in the development of the characteristic diabetic cataract in lens cells and the pathogenesis of diabetic nephropathy and neuropathy. However, clinical trials using AR inhibitors to ameliorate these complications have failed to date (for a review, see [78]).

Under hyperosmotic conditions, *myo*-inositol is transported from the extracellular space into the cytosol by the sodium-*myo*-inositol transporter SMIT. The transport of 1 *myo*-inositol molecule is energized by the symport of 2 sodium ions. SMIT probably possesses 14 transmembrane domains and belongs to the family of the Na⁺/glucose cotransporters. Expression of SMIT is stimulated under hypertonic conditions by TonEBP binding to the promoter region of the gene [29]. In the isoosmotic renal cortex, only low amounts of SMIT are expressed, while in the hyperosmotic renal medulla, expression is several-times higher, particularly in cells of the thick ascending limb of Henle's loop (TALH) [79] and

in collecting duct cells [80]. Expression of SMIT in the renal medulla is also significantly increased during antidiuresis [81]. Pharmacological inhibition of SMIT activity results in severe injury of the tubular cells of the TALH, emphasizing the crucial role of *myo*-inositol accumulation during osmotic stress [82].

The methylamine betaine is accumulated in the cytosol by two different mechanisms. First, under hyperosmotic conditions, TonEBP enhances transcription of the gene encoding the betaine-GABA-transporter (BGT)-1 [29, 30], a 614-amino acid protein [83]. BGT-1 transports betaine from the extracellular space into the cell along with 3 sodium- and 1 or 2 chloride ions [83]. The normal serum betaine concentration is 20 - 144 μM, however, in renal medullary cells, it may be concentrated up to 50 mM [84]. A second mechanism by which betaine can be accumulated under hypertonic conditions, is the production of betaine aldehyde from choline, catalyzed by choline dehydrogenase, and the subsequent conversion to betaine by betaine aldehyde dehydrogenase [85]. However, in renal medullary cells the betaine uptake from the extracellular space by BGT-1 is the most important mechanism [86].

Glycerophosphorylcholine (GPC) in the cell is derived from phosphatidylcholine, which is deacylated by phospholipases A₁ and A₂ and lysophospholipase [87]. The degradation of GPC is catalyzed by GPC:choline phosphodiesterase. Hyperosmotic concentrations of NaCl or urea decrease the enzymatic activity of GPC:choline phosphodiesterase, thereby resulting in cellular accumulation of GPC [87]. In addition to this mechanism, mouse inner medullary collecting duct cells can synthesize GPC from phosphatidylcholine *via* neuropathy target esterase (NTE), a phospholipase B that is up-regulated by osmotic stress, probably *via* TonEBP [88].

Transport system A is a Na⁺-dependent transporter for neutral amino acids like glycine or alanine that may serve as compatible osmolytes. Three system-A transporters are known to date, ATA1-3. ATA2 is induced by hypertonicity [89, 90], directly or indirectly dependent on TonEBP [91]. Another free amino acid accumulated during osmotic stress is taurine [92]. Taurine is transported from the extracellular space into the cell *via* the taurine transporter (TauT) [93]. TauT is upregulated at the functional level by increased activity, and at the transcriptional level during hypertonic stress [93-95]. A TonE-site has been identified in the promoter region of TauT [31], indicating that taurine accumulation also depends on hypertonicity-induced TonEBP activation.

Other Proteins Induced by Osmotic Stress

The Na⁺,K⁺-ATPase exchanges 3 sodium ions from the intracellular space for 2 potassium ions at the cost of 1 ATP. Functional Na⁺,K⁺-ATPase is essential for maintenance of potassium and sodium gradients across the cell membrane, the latter being essential for sodium-coupled solute and osmolyte transport in renal medullary cells [96]. The Na⁺,K⁺-ATPase is composed of an α-subunit which contains the catalytic activity and an additional β-subunit without enzymatic properties, but which is required for the expression of the functionally active enzyme [97, 98]. Recent evidence indicates that in the kidney *in vivo*, an additional γ-subunit,

expressed exclusively in the outer and inner medulla, modulates Na^+, K^+ -ATPase affinity for ATP, sodium, and potassium [99-102], thereby possibly adjusting Na^+, K^+ -ATPase activity to metabolic demands in cells exposed to osmotic stress. Although increased abundance of all three subunits has been reported in response to tonicity stress [103, 104], the γ -subunit appears to contribute decisively to osmotolerance of renal medullary cells. Specifically, the γ -subunit is induced at both the transcriptional and translational level, and is subsequently incorporated into the basolateral membrane in medullary collecting duct cells exposed to acute or chronic hypertonic stress [105]. This process is apparently not dependent on TonEBP activation, but requires activation of JNK for induction of the γ -subunit at the transcriptional level, while increased expression at the translational level involves PI3K [106].

Cyclooxygenase (Cox) is a key regulatory enzyme in the synthesis of prostaglandins from arachidonic acid. There are at least two Cox isoforms. While Cox-1 is expressed constitutively in various mammalian tissues, Cox-2 is induced by various cellular stresses, including osmotic stress [107]. Cox-2 is up-regulated by osmotic stress in cells of the mammalian liver, lung, kidney [107-109]. Several studies have suggested that the majority of renal medullary PGE_2 originates from Cox-2 [109], and contributes to protection of medullary cells from hypoxia by vasodilatory effects on descending vasa recta [110], and by stimulating the expression of osmoprotective genes like HSP70, SMIT and AR [111]. The importance of tonicity-induced Cox-2 expression in the kidney is illustrated by the well-known adverse effects of non-steroidal anti-inflammatory drugs, used in the therapy of joint pain and fever by virtue of their potent inhibition of prostaglandin synthesis. Their long-term use is limited by renal side effects, probably due to the reduced cytoprotection in the absence of PGE_2 . The tonicity-induced up-regulation of Cox-2 is probably not mediated by TonEBP [112]. Instead, as described above, hypertonicity induces the generation of ROS [113], leading to transactivation of the EGF receptor [114] and activation of MAP kinase pathways [115], which subsequently stimulate Cox-2 expression.

AQPs are a family of water channels, ubiquitous from bacteria to mammals. All aquaporins share a common tetrameric structure, with each subunit containing 6 transmembrane domains. In the mammalian kidney, the hormone vasopressin stimulates the translocation of AQP2 from the cytoplasm to the apical cell membrane, thus increasing water reabsorption in collecting duct cells. In cultured kidney cells, expression of the AQP2 gene is increased by osmotic stress, which is, at least in part, dependent on a TonE in the promoter region of the gene [33, 116]. Possible additional sites upstream from TonE also participate in the hypertonicity-induced up-regulation of AQP2 [117]. AQP1, also induced by hypertonicity in cultured kidney cells [118], lacks a TonE, but another cis-acting element conferring osmosensitivity has been identified in the promoter and termed "hypertonicity-responsive element" (HRE), however, the corresponding trans-acting factors are still unidentified [119, 120]. Hyperosmotic stress also increases expression of AQP3 in human keratinocytes [121] and MDCK cells [122], that of AQP5 in mouse lung epithelial cells [123] and that of AQP4 and AQP9 in rat astrocytes [124]. In all of these stud-

ies, elevated AQP expression was induced only by functionally membrane-impermeant solutes like NaCl, which establish an osmotic trans-membrane gradient; in contrast, hyperosmolality generated by membrane-permeant solutes like glycerol or urea failed to induce AQP expression.

As mentioned above, the major mechanism regulating water reabsorption in collecting duct principle cells in antidiuresis is the shuttling of AQP2 between the cytoplasm and the apical cell membrane [125]. The physiological role of enhanced expression of AQP2 by osmotic stress in medullary collecting duct cells may be related to the maintenance of increased levels of AQP2 protein, thereby contributing to urine concentration during prolonged antidiuresis. Accordingly, osmotic stress-induced AQP2 expression in the kidney may be more important for the function of the whole organ rather than for cell protection against the deleterious effects of hyperosmolality. The precise role of elevated AQP expression in response to osmotic stress in other tissues remains to be determined. Hill *et al.* have proposed a model in which AQPs can function as osmosensors and can initiate signaling pathways within the cell [126].

CONCLUDING REMARKS

Following exposure to osmotic stress, cells establish a timely well-orchestrated network of genetic responses that allow cell survival and function under these potentially harmful conditions. Early events include the activation of MAP kinase pathway(s), resulting in the expression of IEGs like Fos and Jun, which in turn promote expression of other cytoprotective genes (Fig. 1). The rapid water efflux and cell shrinkage due to hypertonic conditions is counteracted by activation of ion transport pathways that mediate the import of particularly sodium and chloride ions, and subsequent exchange of sodium for potassium by the Na^+, K^+ -ATPase, resulting in elevated intracellular electrolyte concentrations and the passive influx of water to restore cell volume. NaCl-induced DNA double-strand breaks activate a p53-mediated cell-cycle arrest, thus allowing DNA repair or, in the case of irreversible damage, induce apoptosis. Later events, often mediated by the transcriptional activator TonEBP, include expression of genes involved in accumulation of organic osmolytes to replace excess electrolytes, the expression of HSP, which assist other proteins to prevent misfolding or aggregation, and enhanced expression of Cox-2 and increased PGE_2 -synthesis (Fig. 2). These later events are observed particularly in renal medullary cells, which are indeed the only mammalian cells exposed routinely to substantial hypertonic stress. The cooperation of the systems described above confers upon these cells the remarkable ability to tolerate the extreme conditions present in the medulla of the concentrating mammalian kidney well, whilst dysfunction of one or more of these systems may entail renal medullary damage, as observed in various pathological situations.

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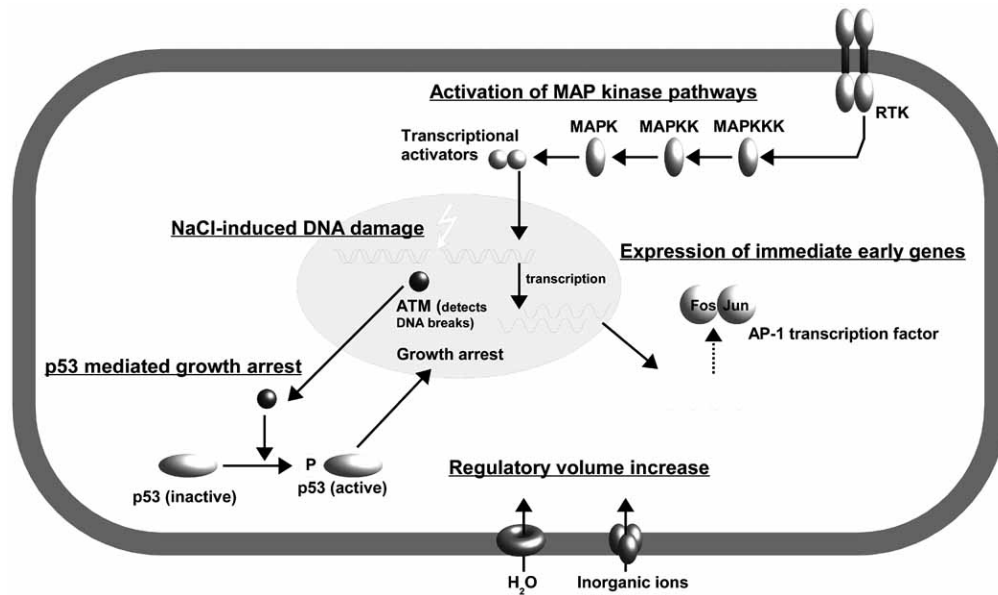


Fig. (1A). Early events in cells exposed to osmotic stress.

Hypertonic stress causes rapid water efflux and subsequent cell shrinkage. Transport of inorganic ions (Na⁺, Cl⁻) into the cell leads to passive water influx and restoration of cell volume, a mechanism termed regulatory volume increase. High [NaCl] cause DNA double-strand breaks, that are detected by the ataxia telangiectasia mutated (ATM) kinase. ATM activates p53, which in turn induces growth arrest by activation of the G1 checkpoint, thus allowing activation of repair mechanisms or induction of apoptosis. Osmotic stress activates MAP kinase pathways, probably mediated by one or more receptor tyrosine kinases, resulting in transcriptional activation of immediate early genes like Fos and Jun. These proteins assemble to the AP-1 transcription factor, which participates in expression of various cytoprotective genes.

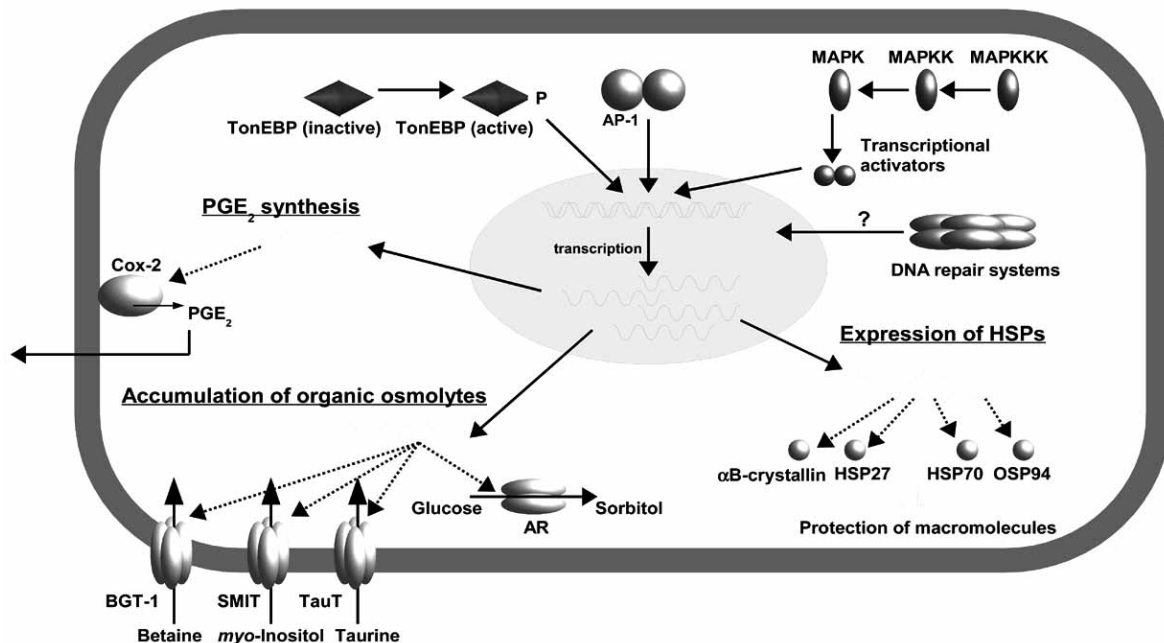


Fig. (1B). Late events in cells exposed to osmotic stress.

Upon phosphorylation, tonicity-enhancer binding protein (TonEBP) enters the nucleus and stimulates the transcription of several osmoprotective genes, amongst them the osmolyte accumulating genes aldose reductase (AR), betaine-GABA transporter (BGT)-1, sodium-*myo*-inositol transporter (SMIT) and taurine transporter (TauT). The corresponding proteins mediate accumulation of compatible organic osmolytes that can replace excess electrolytes. TonEBP-mediated expression of heat shock proteins contributes to protection of proteins against misfolding and aggregation due to increased intracellular ionic strength. Tonicity-induced expression of Cox-2 is probably independent of TonEBP. Synthesis of Prostaglandin E₂ (PGE₂) protects the cell by vasodilatory effects and by stimulating expression of several osmoprotective genes. Recent data suggest, that DNA repair systems like the meiotic recombination (Mre) 11 complex may enter the nucleus and repairs NaCl-induced DNA double-strand breaks.

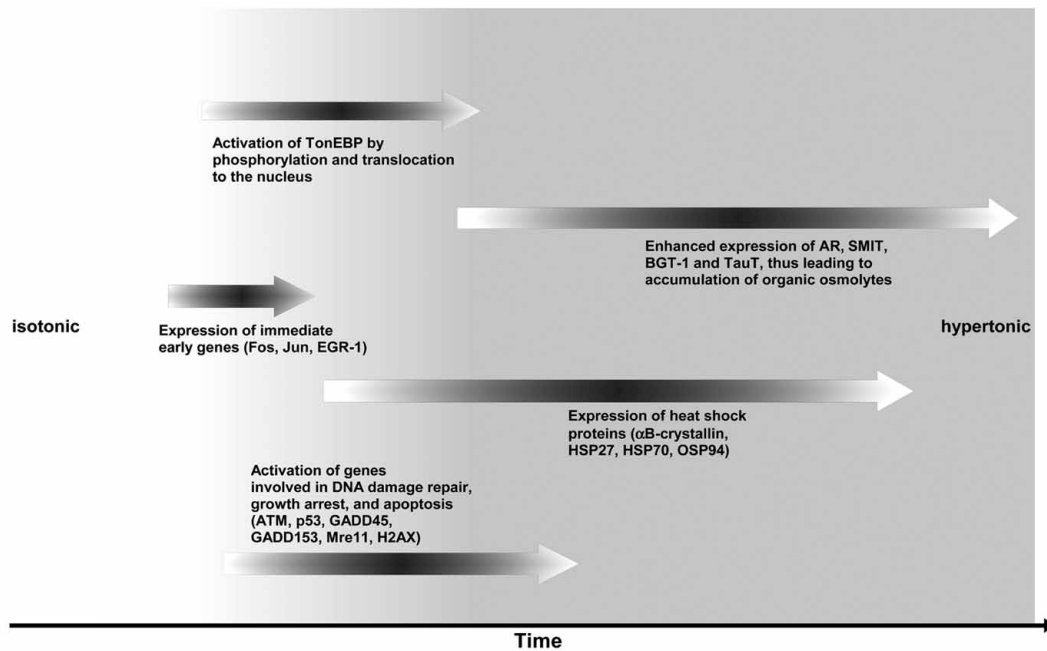


Fig. (2). Schematic representation of the time-course of activation of important osmoprotective mechanisms after an increase in ambient tonicity.

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