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Topical Review

Cell Volume and Gene Expression

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Introduction

The multitude of physiological and pathophysiological mechanisms challenging cell volume constancy faces a comparable diversity of genes which are transcriptionally modified by cell swelling or cell shrinkage. The products of some of those genes serve cell volume regulation e.g., by cellular accumulation of osmolytes. Other genes encode for transcription factors and other signaling molecules, presumably linking cell volume to altered expression or function of proteins. A third group of genes protects proteins against the destabilizing effects of high ionic concentrations. The products of a number of genes, however, seem not to be related to cell volume regulation, although their transcript levels alter with changing cell volume. The expression of cell volume sensitive genes may be triggered not only by alterations of extracellular osmolarity but as well by other factors modifying cell volume. The change of cell volume induced by certain hormones for example may thus represent an important novel mechanism comparable to a second or third messenger triggering certain patterns of gene expression.

This article briefly reviews the molecular basis for regulation of mammalian gene expression in dependence of cell volume and highlights some of the genes, whose expression changes in response to cell volume alterations.

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Regulation of Cell Volume Constancy Is Challenged under Anisotonic and Isotonic Conditions

In mammalian tissues, most cells are exposed to extracellular fluid with well controlled osmolarity. A notable exception is the kidney medulla, where extracellular osmolarity may range from isotonicity to more than 1500 mOsm (Roy, Layton & Jamison, 1992) during water deprivation. But even at constant extracellular osmolarity, cell volume is subjected to continuous fluctuations caused by altered intracellular content of osmotically active substances due to transport of electrolytes or organic substrates across the cell membrane as well as degradation or formation of macromolecules like proteins and glycogen to or from their osmotically more active monomeric components. Via modulation of ion transport processes a wide variety of hormones has been shown to alter cell volume (Lang et al., 1997). Most importantly, insulin swells hepatocytes by activation of both Na^+/H^+ exchange and Na^+, K^+, $2Cl^-$ cotransport (Hallbrucker et al., 1991*a*) and glucagon shrinks hepatocytes, presumably by activation of ion channels (Hallbrucker et al., 1991*b*). The effects of these hormones on cell volume account for several of the effects on hepatocyte metabolism. Whether cell volume sensitive gene transcription participates in the metabolic effects of these hormones is not yet clear, but the identification of a serine/threonine protein kinase transcriptionally strongly modified by anisotonic and isotonic cell volume changes might provide a functional link between the cellular hydration state and

metabolic control by altered gene transcription (Waldegger et al., 1997).

Osmotic Cell Shrinkage Stimulates Expression of Osmolyte Transporters

Anisotonic exposure is a major stress for cells of the inner renal medulla. Renal cell lines like Madin-Darby canine kidney (MDCK) and PAP-HT25 have been widely used to study osmotic regulation in mammalian cells. The adaptive response to an abrupt hyperosmotic stress starts with an acute influx of salts and water within seconds in order to reestablish the cellular volume (called regulatory volume increase, RVI). Ion transport across the cell membrane is the most efficient and rapid means of altering intracellular osmolarity. The major ion transport systems participating in the RVI are the Na^+, K^+, $2Cl^-$ cotransporter (Geck & Pfeiffer, 1985) and the Na^+/H^+ exchanger (Grinstein, Clarke & Rothstein, 1983). The synchronous activation of the Na^+/K^+ ATPase prevents an intracellular accumulation of Na^+, which is replaced with K^+. Simultaneously, to avoid cellular electrolyte loss, K^+ channels and Cl^- channels are inhibited by cell shrinkage (Lang, Busch & Völkl, 1998). Although these fast and very potent mechanisms reestablish the original cellular volume within minutes after hypertonic exposure, they face the intracellular milieu with the difficulties of high ion concentrations, which may lead to disturbances in structure and function of macromolecules including proteins (Yancey et al., 1982). To escape this dilemma, cells accumulate — in exchange for the excessive electrolytes — osmotically active organic solutes, which create osmolarity without compromising other cell functions and therefore are called ''compatible osmolytes'' (Yancey et al., 1982). The major compatible osmolytes in mammalian tissues include the polyalcohols sorbitol and inositol, the methylamines glycerophosphorylcholine and betaine, a variety of amino acids and the amino acid derivative taurine (Lang et al., 1997). As compared to RVI accomplished by ions, accumulation of osmolytes is a slow process often taking hours to days. The osmolyte accumulation is mediated by the transcriptional activation of genes encoding for proteins such as aldose reductase catalyzing the hydration of glucose to sorbitol, and Na^+- or Na^+, Cl^--coupled cotransporter for betaine, inositol, amino acids and taurine, that are directly involved in the metabolism and transport of osmolytes (Ferraris et al., 1994; Garcia-Perez et al., 1989; Smardo, Burg & Garcia-Perez, 1992; Uchida et al., 1993; Yamauchi et al., 1994*a;* Yamauchi et al., 1996). The transcriptional regulation of these genes is more part of a specific genetic program, responding to alterations of extracellular osmolarity and hence alterations of cell volume, than part of an unspecific cellular stress response, particularly since other stress factors like heat stress do not induce the respective genes (Sheikh-Hamad et al., 1994). The signaling mechanisms which direct the primary extracellular hyperosmolar stimulus to the transcription machinery of the nucleus are not yet completely understood. Increasing the extracellular osmolarity by membrane impermeable solutes leads, due to a netto movement of water passively following the osmotic gradient out of the cells, to cell shrinkage accompanied by an increase in intracellular potassium and chloride concentration. Due to the potent RVI mechanisms cellular volume more or less returns back within minutes to the original value at the expense of the intracellular osmolarity, which remains elevated throughout the persistence of the hypertonic exposure. This initial rise in intracellular electrolyte concentrations (synonymous with intracellular ionic strength) seems to be an important triggering signal for induction of the genes responsible for organic osmolyte accumulation (Uchida et al., 1989; Smardo et al., 1992). During hyperosmotic challenge the function of intracellular proteins may be compromised by both, decreased protein stability due to the destabilizing effects of salts, and increased protein concentration due to cell shrinkage (macromolecular crowding) (Zimmermann & Minton, 1993; Garner & Burg, 1994; Parker, Dunham & Minton, 1995). Urea, similar to electrolytes, potently perturbs protein structure and function, whereas methylamines such as trimethylamine oxide and betaine effectively stabilize protein structure and thus counteract the destabilizing effects of salts and urea (Yancey et al., 1982; Lin & Timasheff, 1994). It is not yet clear, whether intracellular ionic strength by itself or via alteration of macromolecular crowding exerts its effects on gene transcription, and how gene transcription is modulated by these parameters.

Positive Response Elements Control Adaptive Gene Transcription

To clarify complex transcriptional regulatory mechanisms it is often easier not to start at the beginning of the signaling cascade but to put the cart before the horse and to start with the end of the signaling cascade, which — in case of transcriptional regulation — usually is contained in the gene flanking regions, especially the 5′-flanking region. The sine qua non of this approach is the cloning of the respective genes, which usually is performed using cDNA sequences derived from the messenger RNA of the cells under investigation. Known genes in osmolyte regulation include the genes encoding for the aldose reductase (Ferraris et al., 1994), sodium/*myo*-inositol cotransporter (SMIT) (Kwon et al., 1992), betaine transporter (BGT1, for betaine/GABA transporter) (Yamauchi et al., 1992), taurine transporter (NCT, for Na^+- and Cl^--dependent taurine transporter) (Uchida et al., 1992),

and ROSIT, a renal osmotic stress induced Na^+-Cl^--organic solute cotransporter (Wassermann et al., 1994). Sequence information obtained by cloning of these genes enabled the identification of genomic clones, which then allowed the analysis of the 5′-flanking regions adjacent to the structural gene.

Identification of ORE, the Osmotic Response Element, in the Aldose Reductase Promoter

Hypertonicity increases the sorbitol level in different cell types by increasing the activity as well as the amount of aldose reductase, the enzyme that catalyzes hydration of glucose to sorbitol (Garcia-Perez & Burg, 1991*a*). The rise in enzyme amount is caused by an increase in the level of aldose reductase mRNA. Since abundance of specific mRNAs reflects a steady state in mRNA transcription and -degradation, both, increased transcription or impaired degradation, determine the expression level of the respective mRNAs. In case of aldose reductase the impaired degradation was excluded as cause for the tonicity induced increase in aldose reductase mRNA by nuclear run on experiments (Smardo et al., 1992). The gene encoding for the aldose reductase was cloned from several different species, including man (Graham et al., 1991*a*), rat (Graham et al., 1991*b*) and rabbit (Ferraris et al., 1994). Analysis of the 5′-flanking region proximal to the CAP site, the transcription starting point, revealed common promoter elements such as a TATA box, a CCAAT box, and a GA-rich region found in a wide variety of genes contributing basal promoter activity (Wang, Bohren & Gabbay, 1993; Ferraris et al., 1994), but which do not — either alone or in concerted action — confer transcriptional regulation in response to hyperosmotic challenges. Combining a larger upstream fragment (about 3200 base pairs) of the 5′-flanking region with a reporter gene resulted in a strong osmotic regulation of the reporter gene product (Ferraris et al., 1994). After progressively subdividing this 3.2 kilo base pair region into discrete fragments in reporter gene constructs, a minimal sequence element of 11 base pairs located at position 1105–1094 upstream of the transcription initiation site was defined, which was able to confer hyperosmotic response on the reporter gene independent of other putative cis elements — the minimal essential osmotic response element (ORE) (Ferraris et al., 1996). An electrophoretic mobility shift assay with a 17 base pair DNA fragment containing the ORE suggested the binding of a hyperosmotically induced trans-activating factor to the ORE (Ferraris et al., 1996). The isolation of this trans-activating factor should elucidate the basic mechanisms for regulation of gene expression by hyperosmotic stress.

TonE and ORE Reveal a Considerable Degree of Similarity

The knowledge on the DNA- and amino acid sequence of the betaine transporter BGT1 allowed the integration of this protein — together with the taurine transporter NCT — into the superfamily of sodium- and chloride-coupled neurotransmitter transporters (Schloss, Puschel & Betz, 1994). The binding stochiometry of two sodium and one chloride ion together with one substrate molecule provides the electrochemical potential for accumulation of high intracellular substrate levels. BGT1 differs from the three other known isoforms of GABA transporters by its unique affinity to betaine. The affinity to GABA of BGT1 is by far lower than that of the other isoforms.

Exposure of MDCK cells to increased extracellular osmolarity is followed by a somewhat delayed increase in mRNA levels for BGT1, starting after a few hours and reaching the maximum at about 16 hours, preceding the changes in transport activity by about 3–4 hours. Analysis of the complete canine BGT1 gene sequence revealed a complex arrangement of the 5′-end caused by variations in the 5′-untranslated region. These variations do not affect the open reading frame of the gene, but they are associated with three independent promoters driving the expression of a complex mixture of mRNAs. Transcription from all three promoters again is similarly regulated by hypertonicity (Takenaka et al., 1995). Step-by-step deletions of 5′-flanking sequences in reporter gene constructs directed the interest to a 10 base pair DNA element between nucleotides 60 and 51 upstream of the transcription initiation point, which acted like an enhancer activated by hypertonicity — the tonicity-responsive enhancer element TonE (Takenaka et al., 1994). As expected for an enhancer the function of TonE is independent of orientation and concatamerization dramatically potentiates its function. The existence of a trans-activating factor in form of a TonE-binding protein (TonEBP), a putative transcription factor, was suggested by data from electrophoretic mobility shift assays using nuclear extracts from MDCK cells and DNA containing the TonE sequence. The identity of TonEBP remains to be determined. TonE seems to mediate osmotic regulation of transcription even in the complex regulatory networks of living animals, since transgenic mice harboring 2.4 kb of 5′-flanking region of the BGT1 gene fused to a reporter gene express this reporter gene in their renal medulla in dependence of concentrative efforts of the kidneys (Kaneko et al., 1995).

The related function of TonE and ORE is reflected by a considerable degree of sequence similarity between the TonE and ORE sequences. Of the 12 base pairs in ORE, 7 are identical to TonE. It remains to be explored, if the same trans-acting factors bind to and activate ORE and TonE or if there exists a family of closely related

transcription factors involved in regulation of gene expression dependent on osmolarity.

Cis Acting Elements Have Not Yet Been Defined for the Inositol Transporter

The inositol accumulation in renal medullary cells exposed to hypertonicity is mediated by transcriptional activation of the gene encoding the sodium dependent *myo*-inositol cotransporter SMIT (Yamauchi et al., 1994*b*), which was originally cloned from MDCK cells via expression cloning (Kwon et al., 1992). Based on amino acid sequence, it belongs to a family that includes Na^+-dependent transporters for glucose (Hediger et al., 1987) and nucleosides (Pajor & Wright, 1992). The time course of transcriptional activation in response to hypertonicity resembles the activation of BGT1 transcription in cell culture experiments (Handler & Kwon, 1993; Handler & Kwon, 1996), and both, the mRNA levels for betaine and *myo*-inositol transporter return to their basal levels within eight hours after shifting the cells to isotonic medium (Yamauchi et al., 1993). Interestingly, a remarkable faster activation has been described in vivo in rat thick ascending limb of Henle's loop and macula densa cells (Yamauchi et al., 1995) and in rat brain (Minami et al., 1996), where SMIT mRNA levels increased markedly in the first hour after raising the extracellular osmolarity. Examination of SMIT genomic clones was not yet crowned with success in defining cis acting elements responsible for this kind of transcriptional regulation (Burg, Kwon & Kültz, 1996).

Filling the Gap Between Onset Of Osmotic Stress and Transcriptional Activation of Osmolyte Transporters — the Immediate Early Gene and Heat Shock Response

The onset of osmolyte accumulation in response to hyperosmotic stress due to increased mRNA transcription for the respective transport molecules takes several hours to days (Garcia-Perez & Burg, 1991*b*) and therefore has to be considered a late genetic response preceded by the increased expression of primary response genes, whose products may be involved in signaling for osmoregulation, in direct osmoprotection or in some other less well-understood aspects of the adaptation. Immediate early genes belong to the very heterogeneous group of primary response genes whose common denominator is their exceptionally fast transcriptional activation within minutes after onset of the stimulus, which is mediated via preexisting, constitutively expressed signaling cascades and therefore is independent of de novo protein synthesis (Greenberg, Hermanowski & Ziff, 1986; Waldegger et al., 1997). The conjunction with a particularly short half-life of the immediate early gene transcripts allows an efficient control of their mRNA steady state levels in dependence of a variety of stimuli, including mitogens (Kruijer et al., 1984; Simmons et al., 1992; Webster et al., 1993), phorbol esters (Bukh et al., 1990; Greenberg & Ziff, 1984; Kruijer et al., 1984), hormones (Webster et al., 1993), as well as physical stimuli like light (Sharp & Sagar, 1994) and heat shock (Andrews et al., 1987). The products of several immediate early genes are known or putative transcription factors, including the nuclear proto-oncogenes c-*fos*, c-*rel*, c-*myc* (Johnson & McKnight, 1989) and the zinc-finger protein *Egr*-I (Christy, Lau & Nathans, 1988), the cytoskeletal proteins actin and tropomyosin, transmembrane proteins including the glucose transporter [reviewed in (Herschman, 1991)] or signaling molecules such as rhoB, a member of the *ras* superfamily of GTP-binding proteins (Jahner & Hunter, 1991), and the immediate early gene kinases snk, sgk and h-sgk (Simmons et al., 1992; Webster et al., 1993; Waldegger et al., 1997).

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