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Regulation of the *myo*-inositol and betaine cotransporters by tonicity

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Regulation of the *myo*-inositol and betaine cotransporters by tonicity. Cells of the hypertonic renal medulla accumulate high concentrations of the non-perturbing osmolytes *myo*-inositol, betaine, and taurine, and are thereby protected from the perturbing effects of hypertonicity. Kidney-derived MDCK cells accumulate high levels of these three non-perturbing osmolytes when cultured in hypertonic medium and have been used to study their accumulation. The increase in the intracellular concentration of these non-perturbing osmolytes is the result of an increase in the abundance of the mRNA for the specific cotransporter for each osmolyte and the ensuing increase in the activity of the three specific sodium coupled transporters. The increased abundance of mRNA for the *myo*-inositol and the betaine cotransporters is driven by an increase in the rate of transcription of their genes. We have identified a 13 basepair cis-acting element in the 5′ flanking region of the gene for the betaine cotransporter. The element is an enhancer that mediates the transcriptional response to hypertonicity. The protein(s) that binds to the tonicity responsive element is much more active in hypertonic than in isotonic cells, and is in all likelihood the mediator of the transcriptional response to changes in tonicity.

In virtually all species, cells employ the same fundamental strategy in response to the stress of hypertonicity. Rather than suffer the perturbing effects of the elevation of cell electrolyte concentration required to osmotically balance extracellular hypertonicity, almost all bacteria, plants, and animal cells maintain their electrolyte concentration similar to that of cells in an isotonic medium by balancing the additional extracellular tonicity by accumulating certain small organic solutes that, in contrast to high concentrations of electrolytes, do not perturb the function of macromolecules. The characteristics and biologic distribution of non-perturbing or compatible osmolytes were summarized by Yancey and Somero and colleagues in 1982 in a seminal review [1]. The mechanism of perturbation or compatibility has been considered recently [2].

Because of the marked changes in tonicity that the renal medulla experiences as part of he mechanism of urinary dilution or urinary concentration, many renal physiologists have studied the accumulation and loss of compatible osmolytes by kidney cells. We have focused on compatible osmolytes accumulated by transport. Following the lead of the NIH group [3], we work with MDCK cells, derived from an unknown site in canine kidney. When cultured in a hypertonic medium, MDCK cells accumulate high concentrations of *myo*-inositol, betaine [3], and taurine [4] by increasing the activity of a specific sodium coupled transporter for each. The importance of the uptake of the three compatible osmolytes in hypertonic medium was evident when their uptake was prevented by removing them from the medium in which MDCK cells were cultured. Absence of the osmolytes had no discernible effect on the ability of MDCK cells to form colonies in isotonic medium. Colony formation was markedly impaired, however, in hypertonic medium unless at least one of the three osmolytes was present in the medium [4]. The experiment also demonstrates the inability of MDCK cells to synthesize osmoprotective amounts of any of those three compatible osmolytes. All three cotransporters are located in the basolateral plasma membrane of MDCK cells [5], which implies that uptake *in situ* is primarily from the interstitium rather than from tubule fluid, but that extrapolation from studies of cultured cells has not been tested in tubules.

Early studies indirectly testing for the level of mRNA for each cotransporter by comparing the expression of polyA$^+$RNA (mRNA) from isotonic versus hypertonic MDCK cells suggested that the increase in activity of each of the cotransporters was the result of an increase in the abundance of their mRNA [6–8]. To gain a more fundamental understanding of regulation by hypertonicity we used expression cloning in oocytes and used the cloned cDNAs to confirm that hypertonicity elevated the abundance of their mRNAs in MDCK cells [9–11]. Along the same line, the mRNA for the three cotransporters is expressed most abundantly in the hypertonic renal medulla [9–11]. Studies in mammals indicate that the accumulation of *myo*-inositol, betaine, and taurine is increased in hypertonic renal medulla [12–14]. We focused our subsequent efforts on the MDCK cell *myo*-inositol and betaine cotransporters whose activity goes up more than fivefold in cells shifted to hypertonic medium. The activity of the taurine cotransporter is high in MDCK cells in isotonic medium and increases by only 50% in cells in hypertonic medium [4].

Nuclear run-on assays established that the increase in mRNA abundance for the two cotransporters in hypertonic cells is the result of an increase in transcription of their genes, transcription and mRNA abundance having very similar time courses when cells are switched from isotonic to hypertonic medium or from hypertonic to isotonic medium [15, 16]. We have no evidence that tonicity affects the stability of the mRNA for either cotransporter. To understand the mechanism of the very specific increase in transcription of the cotransporter genes, we cloned the two genes and analyzed their structure [17; Rim, Preston, Takenaka, Handler, and Kwon, unpublished observations]. The canine betaine

cotransporter gene has a 13 basepair element 5′ to the first exon that conveys a hypertonicity inducible increase in transcription to its own or a heterologous promoter in MDCK cells. That same 13 basepair element binds a nuclear protein that is far more abundant in hypertonic than in isotonic MDCK cells and in several tests has all the characteristics of an enhancer element that mediates the transcriptional stimulation seen in hypertonic MDCK cells [18]. We have named that tonicity responsive element, TonE. Currently, we are cloning the cDNA for the nuclear protein that binds to the tonicity responsive element and searching for an element that performs the same function for the gene for the *myo*-inositol cotransporter.

Recently, we identified more acute, post-translational regulation of the *myo*-inositol and the betaine cotransporters. Both proteins include consensus sequences for phosphorylation by protein kinase A (PKA) and protein kinase C (PKC) [9, 10]. Activation of either kinase results in 30% inhibition of the activity of both cotransporters. The effects of the two kinases are not additive. Also, the degree of inhibition is not dependent on tonicity, that is, activity is inhibited by about 30% in hypertonic and in isotonic cells. The two cotransporters respond differently when PKC is down-regulated by prolonged exposure to an active phorbol ester. Activity of the *myo*-inositol cotransporter increases by 150% whereas the activity of the betaine cotransporter is not affected [19]. Understanding the molecular basis of the action of the protein kinases will require determination of the state of phosphorylation of the cotransporter proteins, a determination that requires the development of suitable antibodies. The effect of the kinases may be important in the renal medulla whose cells respond to a variety of hormonal signals by activation of one or both kinases.

An important question is how cells sense change in the tonicity of their environment. We have recently studied a line of cells derived from a human malignant melanoma [20]. The cancer cells exhibit frequent blebbing at their plasma membrane. They lack actin binding protein 280 (ABP280). Genetic correction of the defect in ABP280 by transfection with the cDNA for ABP280 corrects the abnormal blebbing. The uncorrected cells display a weak response after 24 hours of hypertonicity in that the activity of their *myo*-inositol cotransporter only doubles. The activity of the cotransporter in the corrected melanoma cells increases several-fold when they are shifted to hypertonic medium. Thus a single defect in the cytoskeleton markedly disturbs the cell's response to hypertonicity. The link between the cytoskeleton and the response remains to be determined.

Regulation of the accumulation of compatible osmolytes is complex. Accumulation is regulated by tonicity, the activity of protein kinases, and the availability of other compatible osmolytes [21].

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