

Article Addendum

Protein-protein interactions enable rapid adaptive response to osmotic stress in fish gills

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Abbreviations: FKBP-51, FK506 binding protein 51; GR, glucocorticoid receptor; TCTP, translationally controlled tumor protein; IGF, insulin like growth factor; IGF1BP, insulin like growth factor binding protein

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Cells respond to changes in osmolality with compensatory adaptations that re-establish ion homeostasis and repair disturbed aspects of cell structure and function. These physiologically complex processes can be separated into two functionally distinct cellular phases. The first phase operates to temporarily minimize cellular damage and stabilize critical cell functions necessary for survival. This phase is contingent upon the ability to generate a rapid adaptive response. For this reason, it occurs largely in the absence of de novo protein synthesis and instead relies upon modifying the activity of existing cellular proteins through protein-protein interactions and post-translational modifications. The second phase of the osmotic stress response is centered upon adjusting the expression of specific effector proteins required to re-establish cellular homeostasis. This phase is dependent on the completion of signal transduction events; as well the transcription and translation of target genes, and is therefore characterized by a significant temporal delay and not detected until several hours post exposure. Osmotic effector proteins central to the second phase, such as ion transporting proteins and organic osmolyte generating enzymes, have been studied in considerable detail. However, knowledge surrounding the first phase of the osmotic stress response is limited. This article focuses on recent insights into the players and interactions governing the first phase of the osmotic stress response with specific emphasis on protein-protein interactions.

Introduction

Osmolality is a pervasive abiotic factor that has a strong influence on cellular and organismal function. The effects of osmotic stress can be severe, damaging cellular macromolecules, disturbing cell

volume, altering intracellular macromolecular density and modifying stringent intracellular inorganic ion concentrations.¹ Consequently, cells have evolved mechanisms to retain critical cell functions and re-establish cellular homeostasis in response to changes in osmolality. These physiological processes are highly complex and extend across all levels of biological organization from molecules to behavior.² At the molecular level, the osmotic stress response appears to occur in two distinct phases.^{1,3,4} Because adaptive processes like cell volume regulation and ion transport are not instantaneous, the first phase is aimed at temporarily increasing tolerance limits towards osmotic stress. This phase is predicated by speed, and as a result is characterized by changes in the activity of existing cellular proteins via protein-protein interactions and post-translational modifications.⁵ This first phase affords time for a subsequent response that involves the de novo synthesis of a separate set of osmotic stress specific effector proteins that work synergistically to re-establish cellular homeostasis.^{1,3,4} The completion of this second phase is dependent upon osmosensing and signal transduction events, as well as protein synthesis. Therefore, this phase involves a considerable time requirement, and changes in the abundance of effector proteins are not generally detected until 12–18 hours of osmotic stress exposure (Fig. 1).^{5,6} The function of effector proteins during osmotic stress, such as ion transporters and organic osmolyte generating enzymes, has been studied in great detail.⁷ However, our knowledge of the molecular mechanisms governing the first phase of the osmotic stress response is much more uncertain. In the proceeding sections we discuss recent developments surrounding the identity and expression of proteins involved in the first phase of the osmotic stress response in fish, with emphasis placed on protein-protein interactions that may drive early adaptive responses prior to changes in gene expression.

FK506-Binding Protein 51

FK506-binding protein 51 (FKBP-51) is a molecular chaperone comprising one component of a heterocomplex of proteins maintaining the glucocorticoid receptor (GR) in a constitutively inactive state.^{8,9} Recent data suggest that FKBP-51 may be an important component of early adaptive responses to osmotic stress.⁵ During osmotic stress, unfolded proteins may recruit the chaperone activity of FKBP-51 away from the GR, relieving its inhibitory functions and

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promoting GR ligand binding and subsequent signaling events. Such a scenario is especially appealing given the prominent role for GR-mediated processes during osmoregulation. Cortisol, the major corticosteroid in fish, is considered a master regulator of gill chloride cell differentiation and function during osmoregulation.¹⁰ The physiological actions of cortisol are exerted through the glucocorticoid receptor, which acts as a ligand-dependent transcription factor to control the expression of specific genes.¹¹ These actions are relatively rapid and take approximately 30 to 60 minutes. FKBP-51 mRNA is rapidly upregulated beginning at 2 hours post exposure during hyper-osmotic stress in fish gills.⁵ Given the rapid actions of the activated GR, it is plausible that FKBP-51 was recruited and the GR activated by cortisol during the first 60 minutes of hyper-osmotic stress. GR activity may become subsequently repressed as FKBP-51 mRNA levels increase through hour 12 (Fig. 1). This mechanism of GR regulation is consistent with current knowledge regarding the regulation of the GR by chaperones,¹² and reminiscent of heat shock factor regulation, an environmental stress-induced transcription factor responsible for the rapid synthesis of heat shock proteins.¹³

Translationally Controlled Tumor Protein

Translationally controlled tumor protein (TCTP) is a highly conserved, transcriptionally-regulated protein widely expressed in eukaryotic cells, and implicated in a variety of cellular processes.¹⁴ Interestingly, the protein structure of TCTP in yeast reveals homology with a family of small chaperone proteins.¹⁵ Recent data have shown that TCTP interacts with the cytoplasmic domain of the catalytic α -subunit of Na⁺, K⁺-ATPase, acting as a repressor of activity.¹⁶ In a mechanism similar to that described for FKBP-51, protein damage incurred by osmotic stress may recruit TCTP away from the Na⁺, K⁺-ATPase, relieving inhibition and promoting Na⁺, K⁺-ATPase dependent osmoregulatory processes (Fig. 1).⁵

Insulin like Growth Factor Binding Proteins

Exogenous application of IGF-1 increases salinity tolerance in rainbow trout, Atlantic salmon, killifish and brown trout.^{17,18} The majority of IGFs in extracellular fluids are found complexed with specific high affinity binding proteins (IGFBPs). IGFBPs have been suggested to mediate the efflux of IGFs from the vascular space to the cell surface, thereby modulating interactions between ligands and receptors, and to prevent proteolytic degradation of IGFs, prolonging their half-lives.¹⁹ The rapid induction of IGFBP-1 mRNA in hyper-osmotically stressed gill tissue is consistent with a

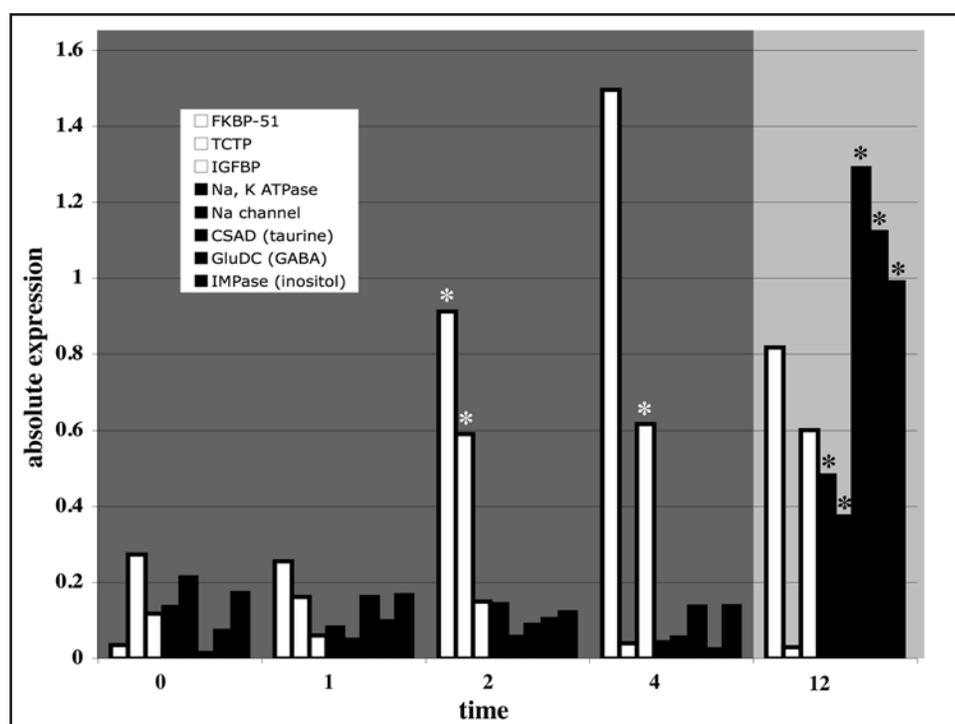


Figure 1. Expression profiles of osmotically-regulated genes illustrating the two-phase osmotic stress response (shaded areas). Genes mediating protein-protein interactions (open bars; FKBP-51, TCTP, IGFBP from left to right) and putatively involved in the first phase of the osmotic stress response (darkly shaded area) are significantly differentially expressed earlier than effector proteins (filled bars; Na⁺, K⁺ ATPase alpha subunit, Na⁺ channel IX alpha subunit, organic osmolyte catalyzing enzymes: cysteine sulfinic acid decarboxylase (taurine), glutamate decarboxylase (GABA) and inositol monophosphatase (inositol) (from left to right)) involved in the second phase (lightly shaded area). Because the completion of the second phase is dependent upon osmosensing and signal transduction events, as well as de novo protein synthesis, changes in the abundance of effector proteins are not detected until 12 hours of osmotic stress exposure. Protein-protein interactions, which enable rapid adaptive responses in the absence of changes in gene expression, may therefore underlie the first phase of the osmotic stress response. Expression and significance values were extracted from Evans and Somero,⁵ and are illustrated as the absolute value of the log fold change. Open asterisks denote earliest time point of significant expression for phase 1 genes. Closed asterisks denote earliest time point of significant expression during of phase 2 effector proteins.

role in early osmoregulatory events (Fig. 1).⁵ Given the prominent role for IGFs in osmoregulatory processes, it is likely that IGFBPs serve to efficiently transduce IGF driven signaling events.

Concluding Remarks

At the molecular level, achieving ion homeostasis during osmotic stress is contingent upon the cell's ability to recognize and quantify environmental osmolality and arrange an appropriate response. Integral are the coordinated activities of osmosensors, which activate appropriate signal transduction pathways; signal transducers, which relay molecular messages to specific target molecules; and effectors, which work in concert to actively restore homeostasis.² Effector mechanisms involved in osmotic acclimation of a great many fish species have been identified and characterized in detail. In contrast, only modest attention has been directed towards identifying the molecular osmosensing and signal transduction events leading up to the activation of these effector proteins. However, recent efforts have begun to shed light on the identity and expression of molecules that function during the earliest osmoregulatory events. These data have shown that multiple major signaling pathways work in concert

to modify diverse effectors, and that these pathways operate within a framework of regulatory proteins such as FKBP-51, TCTP and IGFBPs.⁵ These regulatory proteins likely operate independent of de novo protein synthesis and may enable the earliest adaptive responses to osmotic stress.

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