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Signaling and Molecular Mechanism of TonEBP/NFAT5 Transcriptional Activator

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TonEBP is a transcriptional activator of the Rel family. Activated by hypertonicity, TonEBP plays a major role in stimulation of genes in the renal medulla. Studies using cultured cells revealed that TonEBP responds to changes in ambient tonicity in a bi-directional manner : it is stimulated by an increase in tonicity while inhibited by a decreased in tonicity. The tonicity-dependent regulation of TonEBP involves multiple pathways. Stimulation of TonEBP by hypertonicity is mediated by increased TonEBP abundance due to induction, nuclear translocation, increased transactivation, and phosphorylation. Inhibition of TonEBP by hypotonicity is mediated by decreased TonEBP abundance, cytoplasmic translocation, and decreased transactivation.

One key question has been how TonEBP senses the changes in ambient tonicity. Recent studies suggest that changes in cellular ionic strength rather than cell volume dictate the activity of TonEBP. On the other hand, increased cell water content overrides the effects of high ionic strength. When both cellular ionic strength and water content are increased, the activity of TonEBP is decreased probably because the cytoplasm is diluted resulting lower concentration of protein. Thus, ionic strength and protein concentration are major signals that regulate TonEBP.

Nucleocytoplasmic trafficking of TonEBP is exquisitely regulated by ambient tonicity. There is a monopartitie nuclear localization signal (NLS) located N-terminal to the Rel-homology domain in TonEBP. The activity of the NLS is tonicity-responsive. When the NLS is mutated, TonEBP becomes constitutively cytoplasmic regardless of ambient tonicity. On the other hand, when the NLS of TonEBP is fused to a cytoplasmic protein, nuclear localization of the fusion protein becomes tonicity-dependent like TonEBP. The leptomycin B-sensitive nuclear export signal is not present in TonEBP. It appears that the nucleocytoplasmic trafficking of TonEBP is controlled by the tonicity-responsive NLS.

Unlike other members of the Rel-family, TonEBP has multiple domains involved in transactivation, i.e., activation of transcription. The three activation domains (AD's) of TonEBP do not include the conserved glutamine repeats. One of them, AD1, was specific to the c-isoform of TonEBP indicating that alternative splicing influences transactivation. Only AD2 is activated by hypertonicity. In addition, there are two modulation domains (MD1 and MD2) that potentiate the activity of the AD's. Only MD1 is stimulated by hypertonicity. All the AD's and MD's act in synergy leading to a tremendous, tonicity-sensitive transactivation.

TonEBP is a phosphoprotein in basal conditions. When cells are switched to hypertonic medium, phosphorylation of TonEBP increases over the course of several hours. The hypertonicity-induced phosphorylation is not seen in mutant TonEBP molecules incapable of DNA binding due to mutations in the DNA binding sites or dimerization interface. In other words, DNA binding is required for the hypertonicity-induced phosphory-

lation.

Since hypertonicity causes double strand DNA breaks, the role of DNA-dependent protein kianse (DNA-PK) and ataxia telangiectasia mutated (ATM) are examined. Emerging data indicate that DNA-PK and ATM are activated in response to hypertonicity, as expected. In cells deficient in either DNA-PK or ATM, activation of TonEBP in response to hypertonicity is significantly reduced. Survival of the deficient cells is compromised in hypertonic conditions demonstrating the protective role of TonEBP. Interestingly, phosphorylation of TonEBP is also blunted in response to hypertonicity in the deficient cells. It appears that TonEB DNA-PK and ATM phosphorylate TonEBP as DNA-PK is capable of phosphorylating TonEBP *in vitro*.

In summary, double strand DNA breaks are important signals generated by hypertonicity. Activated by the double strand DNA breaks, DNA-PK and ATM directly phosphorylate and stimulate TonEBP. How phosphorylation of TonEBP leads to its activation is unknown.