Uroguanylin Gene Expression in Response to a Hypertonic Challenge in HT29-18-N2 Cells Sekhar Padmanabhan. Mitchell Cohen, and Elizabeth Mann Division of Gastroenterology & Nutrition, Cincinnati Children's Hospital Medical Center

Guanylate cyclase activating peptide uroguanylin (UGN) mRNA is significantly increased in HT29-18-N2 (human intestinal) cells in response to a hypertonic challenge. 5' upstream deletion analysis suggests that the UGN promoter elements that respond to hypertonicity are located between 291 and 135 base pairs upstream of the transcription start site. This sequence contains several putative hypertonicity response elements including consensus NFATC sequences. To determine the principal promoter sequence that responds to hypertonicity, oligonucleotides containing potential transcription factor binding sites were made. Nuclear extract from salt treated HT-29-18-N2 cells show increased binding to one of these oligonucleotides and competition assays using unlabeled oligonucleotides demonstrate that the nuclear extract binds preferentially to the proposed NFATC site. Mutations were introduced into the nuclear extract binding site area; however, luciferase expression of the mutated UGN promoter constructs was increased in response to hypertonic challenge. This suggests the presence of additional hypertonic response elements.