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REDOX REGULATION OF GLUCOCORTICOID-MEDIATED GENE EXPRESSION WITH REFERENCE TO THE ROLE OF THIOREDOXIN Y. Makino, H. Tanaka, K. Okamoto, and I. Makino. Second Department of Internal Medicine, Asahikawa Medical College, Asahikawa 078, Japan

The glucocorticoid receptor (GR), as ligand-inducible transcription factors, consists of a central DNA binding domain (DBD), a ligand binding domain (LBD), and several transcription activation functions, and ca. 20 cysteine residues are concentrated in the DBD and LBD. Recently, it becomes apparent that a variety of redox modificators including cellular thioredoxin (TRX) is involved in transcriptional processes, however, the interplay between the GR and TRX in situ has not yet been documented. We aimed to elucidate the effect of cellular redox status and TRX on the GR-mediated gene expression. Either antisense TRX expression or cellular treatment with hydrogen peroxide negatively modulates GR function and decreases glucocorticoid-inducible gene expression. Impaired cellular response to glucocorticoids was rescued by overexpression of TRX. Moreover, using domain deletion mutant GRs, we revealed that not only the LBD, but the DBD of the GR also might be a direct target of TRX in vivo, which was further supported by electrophoretic mobility shift assays. In summary, we here present evidence showing that cellular redox state and TRX levels are important determinants of cellular sensitivity to glucocorticoids, and propose that cross-talk between redox signals and hormonal signals may be one of essential mechanisms for cellular homeostasis.

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USE OF RECOMBINANT ADENOVIRUS VECTORS AND GREEN FLUORESCENT PROTEIN AS REPORTER GENE FOR PROMOTER ANALYSIS Chris Strock, Marco Cavagna, David Lewis and Giuseppe Inesi. Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore MD, USA

Enhanced Green Fluorescent Protein (EGFP) cDNA (Clontech) was subcloned into the pΔE1sp1A (Microbix System) plasmid, under control of the cmv promoter, or of the cTnT muscle specific promoter (Mar et al., JCB 107, 573, 1988). The resulting constructs were used directly for liposome mediated transfections of fibroblasts or myocytes in culture. Alternatively they were used as shuttle vectors for cotransfection of HEK293 cells in conjunction with the replication defective viral plasmid pJM17 (Microbix Systems) in order to obtain recombinant adenovirus vectors for gene transfer into cultured cells. The strong fluorescence provided a very convenient signal to follow the progress of EGFP expression by direct microscopic observation of the cells in culture, and by spectrofluorometry of detached cells. The transfection efficiency was 5 to 8% with liposomes, and 80-90% with adenovirus vectors. The cTnT promoter proved to be absolutely muscle specific, and its -268 segment was 6-8 times weaker than the cmv promoter. It was demonstrated that the reporter EGFP gene was not influenced by adenovirus cryptic promoters (Supported by NIH grant PO1HL-27867).

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Identification and Characterization of Multiple Osmotic Response Sequences in the Human Aldose Reductase Gene. Ben, C.B. Ko and Stephen, S.M. Chung. Institute of Molecular Biology, The University of Hong Kong.

Aldose reductase (AR) has been implicated in osmoregulation in the kidney because it reduces glucose to sorbitol, which can serve as an osmolite. Under hyperosmotic stress, transcription of this gene is induced to increase the enzyme level. This mode of osmotic regulation of AR gene expression has been observed in a number of nonrenal cells as well, suggesting that this is a common response to hyperosmotic stress. We have identified a 132-base pair sequence 1 kilobase pairs upstream of the transcription start site of the human AR gene that enhances the transcription activity of the AR promoter as well as that of the SV40 promoter when the cells are under hyperosmotic stress. Within this 132-base pair sequence, there are three sequences that resemble TonE, the tonicity response element of the canine betaine transporter gene, and the osmotic response element of the rabbit AR gene, suggesting that the mechanism of osmotic regulation of gene expression in these animals is similar. These three sequences are designated as OreA, OreB, and OreC respectively. Analysis of the mouse AR gene also revealed that these three sequences are highly conserved between the mouse and human. Results from site-directed mutagenesis and gel mobility shift assays suggested that the OreC is the most important element for the osmotic response and cooperative interaction among the three elements in the human AR gene is essential for their enhancer function. The human aldose reductase gene osmotic response elements are the first osmotic response elements characterized in human.

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THE STABILTY AND TURNOVER OF THE MALTOSE TRANSPORTER OF YEAST: EPITOPE TAGGING OF THE *MAL61* GENE PRODUCT. P. W. Dalgleish, P. G. Meaden and J. C. Slaughter. International Centre for Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland, UK

The regulated uptake of wort sugars (especially maltose) by yeast is a prime determinant of beer fermentation progress. Under some nutrient-limited conditions the ability to take up sugars declines markedly so fermentation is restrained or even ceases. In order to gain a better understanding of the molecular mechanisms involved in control of maltose uptake in yeast a method for the detection and measurement of the maltose transporter in yeast cells has been developed. The maltose transporter of yeast has been tagged with a short epitope at its C-terminal end by incorporating the DNA sequence encoding the epitope into the maltose transporter gene by PCR mutagenesis. Antibodies to the epitope are commercially available. It has been demonstrated that the addition of the epitope has no effect on the functional activity of the protein. The modified protein is abundantly expressed and localised to the cell membrane. The relationship between the amount of the transporter in the cell membrane and its activity under fermentation conditions is being explored. PWD acknowledge award of a BBSRC CASE studentship supported by Whitbread plc.

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C/EBPα CAN SUBSTITUTE FOR CREB IN MEDIATING cAMP RESPONSIVENESS W.J. Roesler, P.J. McFie and E.A. Park*. University of Saskatchewan, Saskatoon, SK, Canada and *University of Tennessee-Memphis, Memphis, TN, USA

Transcription of the gene encoding phosphoenolpyruvate carboxykinase (PEPCK) is strongly stimulated by cAMP in liver, and is mediated by a complex cAMP response unit consisting of a binding site for a CREB/ATF protein, three binding sites for a C/EBP protein, and an AP-1 binding site. Various experimental approaches, including the use of GAL4 fusion proteins, have indicated that CREB and C/EBPα are participants in this cAMP response. The cAMP response element (CRE) in this promoter is unique in that it binds both CREB and C/EBP with high affinity in vitro. Replacing the CRE with a binding site for GAL4 drastically reduces cAMP responsiveness, which can be reconstituted by expression of a GAL4-CREB fusion protein. Surprisingly, the cAMP responsiveness of this CRE/GAL4-switched promoter can also be reconstituted by expression of a GAL4-C/EBPα fusion protein i.e. in the absence of a CREB molecule bound to the promoter. In further support of this hypothesis, we show that a chimeric promoter containing four C/EBP and one AP-1 binding sites, rather than the three C/EBP: one CREB: one AP-1 binding site architecture of the native PEPCK promoter, is also cAMP responsive. We speculate that C/EBPα recruits the same limiting co-activator to the transcriptional apparatus as does CREB. (This work was supported by the Medical Research Council of Canada (WJR) and the National Institutes of Health (EAP)).

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Identification of AP-1 DNA: Protein Inhibitors Donna Ferguson, Carolyn Nouhan, and Jeffrey D. Scholten. Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company

Several compounds have been identified which block Hela nuclear extract protein from binding to the AP-1 consensus DNA sequence TGAGTCA. These compounds show a clear dose response curve in an electrophoretic mobility shift assay (EMSA). Furthermore, this inbibitory activity has been demonstrated to have effects on transcription in HEK293 cells against the amyloid precursor protein promoter using luciferase as a reporter gene. Data looking at specificity of this transcriptional effect will be presented.