L 244 TRANSCRIPTIONAL REGULATION OF THE HUMAN CFTR PROMOTER BY SP1 AND A NEGATIVE ELEMENT. Ting-Chung Suen and Lap-Chee Tsui. Department of Genetics, The Hospital for Sick Children and University of Toronto, Ontario, M5G 1X8, Canada.

Cystic fibrosis (CF) is a frequent autosomal recessive genetic disease in Caucasians caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Major advances have been made towards developing gene therapy protocol in animal models and patients. However, much less is known about how CFTR transcription is regulated. The proximal sequence of the CFTR promoter is G-C-rich and contains multiple consensus and potential Sp1 binding sites. In this report, we show by electrophoretic mobility shift assay (EMSA) that several protein-DNA complexes could be detected with a DNA fragment previously shown to have basal promoter activity (-214 to -96). The pattern of these shifted-bands and the result of competition assay with an Sp1 oligonucleotide suggest that Sp1 or its related proteins are involved. Methylation interference assay located the major binding site to a 10-bp sequence (GTGGGTGGAG) at -130, similar to the previously defined GT box. Functional stimulation was observed when an oligonucleotide containing this GT box was cloned upstream to a heterologous thymidine kinase promoter. Binding of purified Sp1 protein to the sequence was also detected by EMSA. Thus, Sp1 and/or other related transcription factors appeared to play a positive role in CFTR gene regulation. In addition, we have further localized the previously identified negative regulatory element to a 60-bp region (-335 to -277). We now show that this 60-bp DNA fragment could suppress the CFTR promoter even in the presence of multiple GC-boxes or the enhancer from SV40. Interestingly, cross-competition in EMSA was observed between the negative element and the basal promoter fragment (-214 to -96). Moreover, an EMSA complex was detected with a 30-bp fragment (-308 to -279) which contained the 3'-half of the negative element and the complex could be competed away by an oligonucleotide containing an Sp1 site. This result suggests an interaction between the putative protein factor binding to this region and Sp1. Thus, the regulation of the CFTR promoter is highly complex and further analysis of the regulatory elements is in progress.

L 245 **INDUCTION OF THE BRAIN CREATINE KINASE PROMOTER BY THE ESTROGEN RECEPTOR IN THE ABSENCE OF AN ERE MAPS TO A REGION THAT CONTAINS A BINDING SITE FOR MADS DOMAIN PROTEINS.** Drew A. Sukovich, Ranjan Mukherjee, and Pamela A. Benfield, Cardiovascular Molecular Biology, DuPont Merck Pharmaceutical Company, Wilmington DE 19880.

The estrogen receptor (ER) typically activates gene transcription by binding to estrogen responsive elements (ERE's). The brain creatine kinase gene (BCK) promoter is responsive to estrogen but contains no consensus ERE. To investigate the mechanism of estrogen induction, we introduced the estrogen receptor into HeLa, primary cardiomyocytes, and primary fibroblast cells with 195 bp of BCK promoter linked to a chloramphenicol acetyltransferase (CAT) reporter gene. A 10-fold and 5-fold stimulation of CAT activity was observed in HeLa and primary fibroblast cells, respectively. This stimulation is not sensitive to cyclohexamide and is blocked by estrogen antagonists. No stimulation of CAT activity was observed in the primary cardiomyocytes. This is in contrast to a CAT reporter construct containing the vitellogenin gene ERE linked to the TK promoter, which exhibited activation by estrogen in all three cell types. Deletion mutants of the ER demonstrate that the DNA binding and ligand binding domains of the ER are required for this induction although point mutations in the DNA binding domain which abolish DNA binding are still capable of estrogen induction. This suggests that ER binding to DNA is not required for estrogen induction. Analysis of 5' deletion and linker scan mutations indicates sequences between -45 and -75 including a TA-rich and CCAAT sequence to be crucial for stimulation of the BCK promoter by the ER. Furthermore, BCK promoter sequences (-37 to -195) confers estrogen inducibility when linked to the heterlogous β-globin promoter and mutations in the TA-rich sequence severly decreases the inducibility. We have previously shown that this TA-rich sequence binds a protein complex TARP (TA-rich binding protein). We propose a possible mechanism whereby ER stimulates transcription of the BCK gene by interacting with TARP.

L 246 IN VIVO ANALYSIS OF THE HUMAN TATA BINDING PROTEIN: SURFACES REQUIRED FOR ACTIVATED TRANSCRIPTION IN HUMAN CELLS. William P. Tansey and Winship Herr, Cold Spring Harbor Laboratory, 1 Bungtown Rd., PO Box 100, Cold Spring Harbor, NY 11724.

Activated transcription initiation results from the interaction of a DNA-bound activator protein with one or more of the general transcription factors (GTFs), assembled in a preinitiation complex around the TATA motif of RNA polymerase II-transcribed genes. One likely target for such interactions is the TATA binding protein (TBP), which is the first of the GTFs to enter the preinitiation complex, and has been shown to interact directly with a number of transcriptional activators in vitro.

We are interested in determining the role of TBP in activated transcription in human cells, by identifying the regions of TBP that are required to respond to various different classes of transcriptional activators in vivo. Adapting the altered specificity TBP assay (developed by Strubin and Struhl *Cell* ***68:721-730***) for use in HeLa cells, we have shown that transient coexpression of altered specificity human TBP can restore wild-type levels of activated transcription on a mutant promoter in which the canonical TATAAA motif had been changed to TGTAAA. We have used this assay to test the response of a number of mutant TBP molecules to multiple activators carrying the GAL4 DNA binding domain and activation domains belonging to the acidic, proline-rich, and glutamine-rich classes.

We have demonstrated that the conserved carboxyl-terminal domain of human TBP is sufficient to respond to all activator types tested. Yeast TBP, which is over 70 % identical in this region, responds similarly to most, but interestingly not all, of the activators used in this study. Additionally, we have found that double and triple point mutations in clustered regions on the surface of TBP have little effect on its response to most activators, with the exception of the acidic activation domain of VP16. Together, these results suggest that different regions of TBP may be involved in mediating the effects of different activators, and that, in general, multiple contacts across the surface of TBP are required for its function.

L 247 A DOMINANT MUTATION AFFECTS THE ACTIVATION POTENTIAL OF CERTAIN TRANSCRIPTION ACTIVATORS IN YEAST

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A genetic screening designed to isolate genes that communicate the effect of transcription activators to the basic transcription machinery was undertaken based on the observation that overproduction of the GCN4 protein has a deleterious effect on the growth of yeast cells. Generating genetic backgrounds where this effect would be alleviated should lead to the identification of molecules that participate in the transcriptional activation process at least by GCN4. Four unlinked mutations afr1,2,3,4, were obtained that upon GCN4 overproduction allowed for enhanced growth rate of yeast cells relatively to wild-type. Analysis of the properties of strains bearing the afr1 dominant mutation revealed complete immunity to toxic levels of GCN4 which was accompanied with reduction of the GCN4 activation function. In addition such strains exhibit impaired GAL4, HAP2/3/4, yAP1 and CAD1 activity *in vivo* while leaving a LexA-VP16 chimeric activator unaffected. We conclude that afr1 is a pleotropic mutation affecting specifically the activation potential of a number of transcription factors in yeast.