23 CONTRIBUTION OF INTRAGENIC SEQUENCES TO CALCIUM REGULATION OF C-FOS TRANSCRIPTION
Claudia Lange and Hilmar Bading
Medical Research Council, Laboratory of Molecular Biology
Hills Road, Cambridge CB2 0HG, England
e-mail: cm1@mrc-lmb.cam.ac.uk

Induction of gene expression is one mechanism by which neurons transform short-lasting electrical events into long-lasting adaptive changes that may underlie learning and memory. Calcium (Ca++) influx through ligand- and/or voltage-gated ion channels is the trigger for electrical activity-dependent transcriptional responses, such as the induction of the c-fos gene. Analyses of the c-fos promoter led to the identification of Ca2+-responsive DNA regulatory elements that include the serum response element (SRE) and the cAMP response element (CRE). Either of these elements can mediate transcriptional induction in response to activation of Ca2+ signalling pathways when placed in front of a minimal c-fos gene, not responsive to Ca2+ signals. However, neither the SRE nor the CRE are able to mediate a robust Ca2+ dependent induction when inserted upstream of heterologous reporter genes such as the chloramphenicol acetyl transferase or luciferase gene. These findings suggest the existence of additional as yet unidentified control regions within the transcribed region of the c-fos gene that control the magnitude of Ca2+ dependent transcriptional activation. We provide evidence that a CRE-like sequence located in the first intron of the human c-fos gene plays a role in c-fos regulation by Ca2+ signals. This intragenic CRE-like sequence appears to be required for a full Ca2+ -activated, CRE-mediated transcription response but is dispensable for SRE-mediated, Ca2+ -activated transcription and for c-fos induction following growth factor receptor stimulation. These findings indicate that cooperation of specific upstream regulatory elements with intragenic sequences can determine the magnitude of transcriptional activation by Ca2+ signals.

24 Regulation of the p300/CREB Coactivators by the CDK Inhibitor p21WAF1/CIP1
Andrew W. Snowden, Lisa A. Anderson, Gill W. Webster and Neil D. Perkins
Department of Biochemistry, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, Scotland, U.K.

The activity of many promoter binding transcriptional activators is dependent upon interaction with the general co-activator proteins p300 and CBP. In addition to forming a bridge between these factors and the basal transcription complex, p300 and CBP also possess histone acetyl transferase (HAT) activity and interact with a number of other HAT proteins. Acetylation of nucleosomes disrupts chromatin structure and facilitates transcriptional activation. It has become apparent that the activity of p300 and CBP is also a regulated step, providing additional specificity to the function of many promoter binding transcription factors. Factors regulating p300/CBP activity include interactions with many viral oncproteins and modulation by signal transduction pathways.

p300 and CBP interact with many transcription factors involved in cell cycle regulation such as p53 and E2F. Here we demonstrate that the transcriptional activities of p300 and CBP themselves are modulated by cell cycle regulatory proteins: both p300 and CBP are transcriptionally activated when co-expressed with the Cyclin Dependent Kinase inhibitor p21WAF1/CIP1. Furthermore, we demonstrate that this effect is independent of the carboxy terminal HAT domain of p300/CBP and also a previously described CyclinE/Cdk2 complex that binds this region. Instead, p21 inducibility occurs as a result of the regulation of a transcriptional suppression domain within the amino terminal half of these proteins. Data will be presented mapping the regulatory domains involved. We will describe an important transcriptional regulatory mechanism with many implications for the control of gene expression at the G1 to S phase transition and during cell cycle arrest.

25 Regulation of NF-kB Transcriptional Activity by EGR-1
Neil R. Chapman and Neil D. Perkins
Department of Biochemistry, MSI/WTB Complex, University of Dundee, Dundee DD1 5EH, Scotland, U.K.

Previous studies have demonstrated that a cooperative interaction between RelA (p65) and Sp1 is required for HIV promoter activity in vivo. This interaction was mediated by the DNA-binding domains of the respective proteins (the Rel Homology Domain (RHD) in RelA and the zinc fingers of Sp1) and required the correct juxtaposition of their respective DNA binding sites.

We have investigated whether NF-kB can also interact both physically and functionally with other zinc finger containing transcription factors. In this study we demonstrate that the EGR-1 transcription factor (also known as Krox-24 or Zf268), whose DNA-binding domain shares a high degree of homology with that of Sp1, also interacts with RelA. In vitro, this interaction is mediated by the RHD of RelA and, similar to Sp1, the zinc fingers of EGR-1.

In contrast, however, to the synergistic activation of the HIV-LTR seen with RelA and Sp1, coexpression of EGR-1 specifically represses RelA transcriptional activity in a dose-dependent manner. This repressive effect was not observed upon co-transfection of EGR-1 with a Gal4-VP16 fusion protein and also does not appear to result from coactivator mediated sequestration. Further data will be presented on the mechanism through which EGR-1 exerts its repressive effect on NF-kB.

EGR-1 has been shown to induce apoptosis through stimulation of Tumour Necrosis Factor α (TNFα) gene expression. Since TNFα is a potent inducer of NF-kB, which is known to have anti-apoptotic effects, these observations provide a mechanism through which EGR-1 induction of cell death might be enhanced.

26 The activity of FOG-1 is potentiated by its ability to contact GATA-1 through multiple zinc fingers.
Asha H. Fox, M. Holmes, J. Mackay and Merlin Crosley
Dept. Biochemistry, University of Sydney, NSW Australia 2006
GATA-1 and its cofactor, FOG-1, are multiple-zinc-finger transcription factors known to play critical roles in haematopoietic differentiation. The two proteins physically interact through direct finger-finger contacts. FOG contains 9 zinc fingers, 4 of which are Knappel-like CCHH fingers, and the remaining 5 have a more unusual CCHC configuration. Previously it has been shown that one of FOG’s CCHC fingers, finger 6, interacts with GATA-1. Here we demonstrate that the remaining CCHC type zinc fingers of FOG (with the exception of finger 7) also interact with GATA-1. Likewise, a second family member, FOG-2 and a related protein in Drosophila, U-Shaped, also contain several fingers that are capable of interacting with GATA-1.

We undertook an alanine scan of FOG finger 1 and identified the key residues involved in contacting GATA-1. These residues are conserved within all known GATA-interacting fingers of the FOG type, and when mapped onto the structure of a CCHC finger, form a discernible face which we believe is the surface to which the GATA RHDs bind. We are now in a position to assess the primary sequence of new zinc fingers, FOG-1, and also does not appear to result from coactivator mediated sequestration. Further data will be presented on the mechanism through which EGR-1 exerts its repressive effect on NF-kB.

EGR-1 has been shown to induce apoptosis through stimulation of Tumour Necrosis Factor α (TNFα) gene expression. Since TNFα is a potent inducer of NF-kB, which is known to have anti-apoptotic effects, these observations provide a mechanism through which EGR-1 induction of cell death might be enhanced.

We undertook an alanine scan of FOG finger 1 and identified the key residues involved in contacting GATA-1. These residues are conserved within all known GATA-interacting fingers of the FOG type, and when mapped onto the structure of a CCHC finger, form a discernible face which we believe is the surface to which the GATA RHDs bind. We are now in a position to assess the primary sequence of new zinc fingers, FOG-1, and also does not appear to result from coactivator mediated sequestration. Further data will be presented on the mechanism through which EGR-1 exerts its repressive effect on NF-kB.

EGR-1 has been shown to induce apoptosis through stimulation of Tumour Necrosis Factor α (TNFα) gene expression. Since TNFα is a potent inducer of NF-kB, which is known to have anti-apoptotic effects, these observations provide a mechanism through which EGR-1 induction of cell death might be enhanced.