(CBP) bind to the AL promoter at the CCAAT site. Mutations of the CCAAT site completely eliminated the binding. In the presence of mutant Htt, CBP was no longer bound to the AL promoter while C/EBPa remained on the CCAAT site. The binding of C/EBPa and CBP to the AL promoter was further verified by using a combination of EMSA and Western blot analyses. Moreover, The transcript and protein levels of C/EBPa in the liver of R6/2 mice were significantly lower than those in wildtype mice. Collectively, mutant Htt suppressed the functions of C/EBPa at multiple levels and might contribute to the disease progression of HD.

866.6

Defining MeCP2 repressor determinants

Michael Anthony Yarski[1], Harikrishnan KN[1], Emma K Baker[1], Assam El-Osta[2]. [1]Epigenetics, Baker Heart Research Institute, PO Box 6492 st Kilda road, Melbourne, 8008, Australia, [2]Epigenetics, Baker Heart Research Institute, PO Box 6492 St Kilda road, Melbourne, 8008, Australia

Among the activities that are dependent on methylation and histone deacetylation are the ATPase remodelling complexes that alter chromatin accessibility and transcriptional competence. Evidence suggests that methyl-CpG binding protein 2 (MeCP2)/methyl binding domain proteins (MBD) are involved in the recruitment of co-repressor complexes and the assembly of chromatin on methylated DNA. However, the precise mechanism of MeCP2 repression on methylated DNA in the context of chromatin has been a hotly debated topic. We have recently demonstrated that human Brahma (hBrm), a catalytic component of the SWI/SNF-related remodelling complex, associates with MeCP2 in vivo and is functionally linked with repression. These findings present a new paradigm for SWI/SNF function that is relevant to our understanding of MeCP2 mediated transcriptional repression. We present for the first time compelling physical and biochemical evidence for a previously undescribed role of cell-cycle specific regulators associated with repression. Funded by NHMRC.

866.7

Induction of transcription by occupational heavy metals through the metal-activated transcription factor 1 is regulated by a labile repressor. Superinduction of metallothionein I by cyclohexmide.

Yongyi Bi[1,3], Gary X. Lin[1], Lyndell Millecchia[2], Qiang Ma[1]. [1]Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, [2]Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, 1095 Willowdale Rd., Morgantown, WV, 26505, [3]Toxicology, Wuhan University School of Public Health, 185 Donghu Rd., Wuhan, 430071, China, People's Republic of

Induction of metallothioneins (MTs) through the metal-activated transcripton factor-1 (MTF-1) provides a model response for analyzing transcriptional gene regulation by heavy metals. We report inhibition of protein synthesis by cycloheximide (CHX) increases induction of Mt1 by ~5 fold. Characterization of superinduction reveals it is time and concentration-dependent of CHX, requires the presence of an Mt1 inducer, and occurs at a transcriptional level, suggesting a labile repressor in the control of Mt1 induction. Genetic analyses show that superinduction of Mt1 is mediated through MTF-1 and MRE-dependent transcription. Analyses by inductively coupled plasma emission spectroscopy and fluorescence imaging demonstrate that treatment with CHX alone or CHX plus an inducer does not increase the total zinc accumulation or the concentration of free zinc in cells. Moreover, superinduction is observed in cells cultured in a zinc-depleted medium, suggesting that superinduction does not involve elevation of intracellular zinc concentration. Finally, inhibition of the ubiquitin-26S proteasome mediated protein turnover by MG132 superinduces Mt1 similarly to CHX, implicating the 26S proteasome pathway in the superinduction of Mt1. We propose that a labile repressor negatively controls Mt1 induction through a 26S proteasome-dependent pathway.

866.8

Identification and characterization of putative downstream targets of Hoxa2 in the murine central nervous system

Xiaoyu Yan[1], Zeynep Akin[1], Robert Holt[2], Adil Nazarali[1]. [1]College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, S7N 5C9, Canada, [2]Genome Sciences Centre, BC Cancer Research Centre, 675 West 10th Avenue, Vancouver, British Columbia, V5Z 1L3, Canada

Chromatin immunoprecipitation (ChIP) technique has been employed to isolate new downstream targets of Hoxa2 from E18 mouse spinal cord and hindbrain tissue. Several new DNA fragments were isolated and a search of sequence databases for the target sequences revealed that one sequence belongs to a novel murine homolog of the human *dual specificity tyrosine phosphorylation regulated kinase 4* (*Dyrk4*) *gene* and another one matches a sequence to the *high temperature requirement factor 3 (HtrA3) gene*. The expression patterns of murine *Dyrk4 and HtrA3* during embryonic development were analyzed in wild-type and Hoxa2$^{-/-}$ mice using RT-PCR. Transcripts for both *Dyrk4* and *HtrA3* were detected in the developing embryo and the adult CNS. Interestingly, HtrA3 expression within the hindbrain and spinal cord of Hoxa2$^{-/-}$ mice were higher than in wild-type mice. We have employed electrophoretic mobility shift assays (EMSA) to determine the Hoxa2 binding specificity to the *Dyrk4* and *HtrA3* target sequences and revealed a putative binding site for Hoxa2. The *in vivo* regulatory activities of Hoxa2 on these sequences were examined in the transient transfection and luciferase assay system. All of the evidences suggest that Hoxa2 may be involved in the regulation of *Dyrk4* and *HtrA3* transcription and impact signaling molecules in the developing CNS (Supported by NSERC).

866.9

Nucleolin is involved in Interferon Regulatory Factor-2 dependent transcriptional activation

Atsuko Masumi[1], Hidesuke Fukazawa[2], Kazunari Yamaguchi[3]. [1]Safety Research on Biologics, National Institute of infectious Diseases, Musashimurayama-shi, Gakuen,4-7-1Â¬, Tokyo, 208-0011, Japan, [2]Bioactive Molecules, [3]National Institute of Infectious Diseases, Shinjuku-ku, Toyama, 1-23-1, Tokyo, 162-8640, Japan, [3]Safety Reserch on Biologics, National Institute of Infectious Diseases, Musashimurayama-shi, Gakuen,4-7-1, Tokyo, 208-0011, Japan

We have previously shown that Interferon regulatory factor-2 (IRF-2) is acetylated in a cell growth-dependent manner, which enables it to contribute to the transcription of cell growth-regulated promoters. To clarify the function of the acetylation of IRF-2, we investigated the proteins that associate with acetylated IRF-2. Transfection of p300/CBP-associated factor (PCAF) enhanced acetylation of IRF-2 in 293T cells. In cells transfected with both IRF-2 and PCAF, IRF-2 associated with endogenous nucleolin, in contrast, little association was observed when IRF-2 was transfected with a PCAF HAT deletion mutant. In a pull-down experiment using stable transfectants, acetylation defective mutant IRF-2 (IRF-2K75R) recruited nucleolin to much lower degree than that of wild type IRF-2, suggesting that nucleolin preferentially associates with acetylated IRF-2. Confocal analysis indicated that IRF-2 colocalized with nucleolin in the perinucleolar region. Nucleolin in the presence of PCAF enhanced IRF-2-dependent H4 promoter activity in NIH3T3 cells. Affinity DNA binding analysis with H4 promoter DNA indicated that nucleolin associated with IRF-2 in growing NIH3T3 cells, but not in growth-arrested counterparts. We conclude that nucleolin is recruited to acetylated IRF-2, contributing to gene regulation crucial for the control of cell growth.

866.10

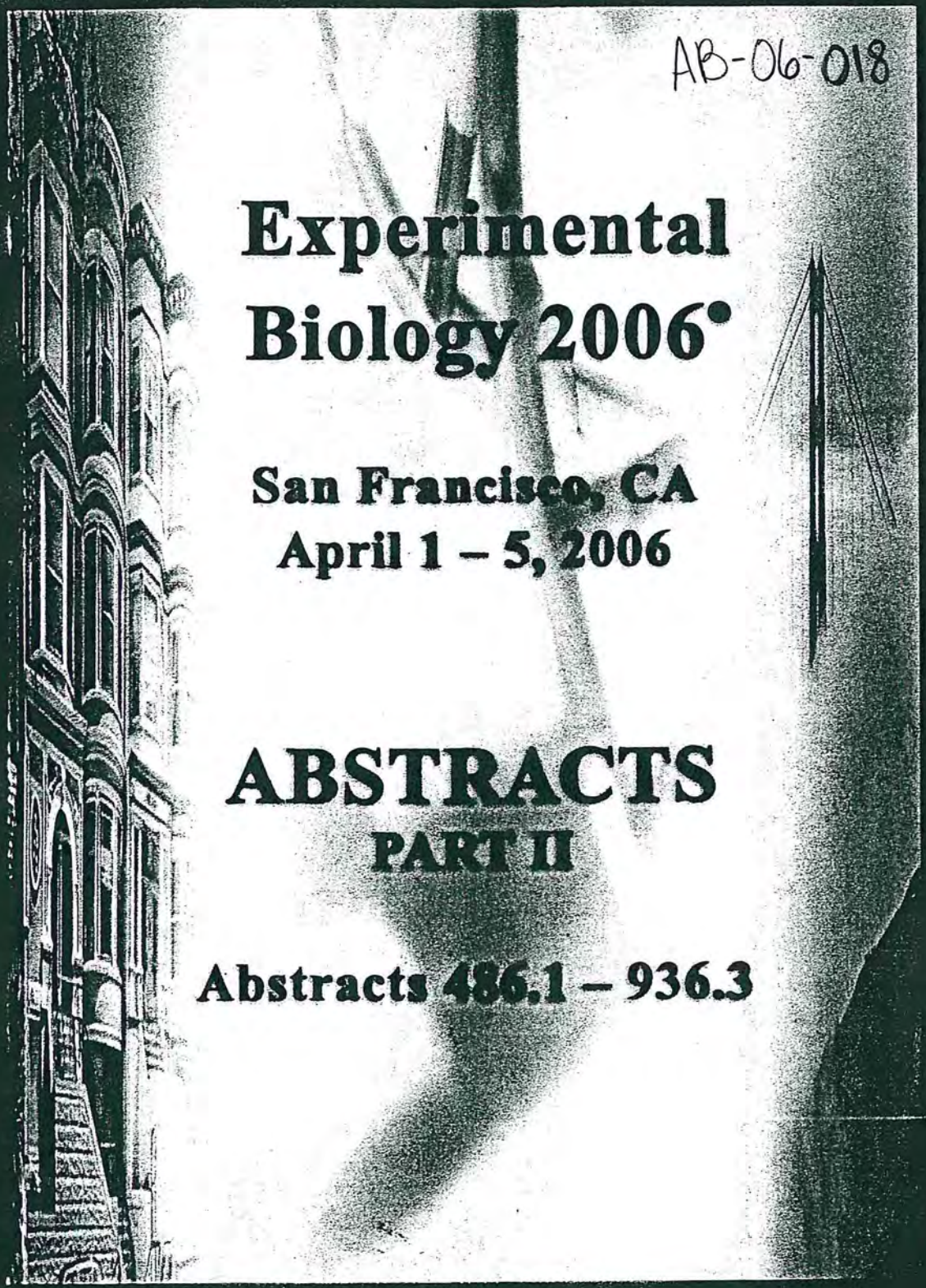
Collaboration between SRF, Sp1, YB-1, and Pur proteins in reprogrammed cardiomyocytes during transplant-associated cardiac fibrosis

Aiwen Zhang[1], Jason J. David[1], Charles G. Orosz[2], Arthur R. Strauch[1]. [1]Physiology & Cell Biology, [2]Surgery, Ohio State University, Davis Heart & Lung Research Institute, 473 West 12th Avenue, Room 535, Columbus, OH, 43210

Chronic fibrosis is a maladaption to cardiac transplant. Proximity effects between scar formation in murine cardiac grafts and cardiomyocyte (CMC) stress were manifested by re-activation of the fetal smooth muscle alpha-actin (SMA) gene. While nuclear Sp1 and Smad2/3 mediated activation in myofibroblasts, re-expression of SMA in CMCs

The FASEB Journal

The New Biology: Reports, Reviews, and FJ Express



AB-06-018

Experimental Biology 2006•

San Francisco, CA
April 1 – 5, 2006

ABSTRACTS

PART II

Abstracts 486.1 – 936.3

March 7, 2006
Vol. 20, No. 5