04

Gene regulation

04-01/P

THE RNASE-L 3'UTR POSTTRANSCRIPTIONALLY REGULATES ITS EXPRESSION

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RNase-L is the terminal component of an interferon (IFN)-regulated RNA decay pathway that functions as a key mediator of antiviral and antiproliferative activities. The 2',5'-oligoadenylate synthetases required to activate RNase-L are transcriptionally induced by IFN; in contrast, RNase-L is expressed at low basal levels in most cell types and is not dramatically altered in response to antiproliferative stimuli. The mRNA encoding RNase-L mRNA contains a long 1.9kb 3'UTR, suggesting that this region may function to mediate rapid and transient changes in RNase-L expression. In fact, transfection of RNase-L and ß-globin constructs that contained or lacked the 3'UTR revealed a dramatic 3'UTR-dependent reduction of mRNA level. This regulation corresponded to a four-fold decrease in mRNA stability. Sequence analysis revealed several AU-rich elements (AREs) in the 3'UTR that may mediate this regulation. Deletion analysis and RNA immunoprecipitation determined that the ARE binding protein, HuR, interacted with a specific ARE to increase RNase-L expression. Furthermore, HuR transfection, or an increase in endogenous cytoplasmic HuR following UVC or heat shock stress, resulted in a 3'UTR-dependent increase in RNase-L expression. This study identifies a novel mechanism of RNase-L regulation in which the 3'UTR functions to maintain its mRNA at low basal levels in resting cells, but acts to increase RNase-L expression in conditions of cell stress. Analysis of the extent to which this regulation is disrupted in human malignancies may reveal a broader role for RNase-L in tumor suppression.

04-02/P

HYPOXIA DOWN-REGULATES INTERFERON GAMMA-INDUCED MIG/CXCL9 AND IP-10/CXCL10 GENE TRANSCRIPTION IN HUMAN TUMOR CELL LINE VIA INHIBITION OF RNA POLYMERASE II RECRUITMENT

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Interferon-γ (IFN-γ) is an important cytokine that protects the host against tumor growth and formation. IFNγ-inducible chemokine Mig/CXCL9 and IP-10/CXCL10 are chemoattractants for activated T cells and possess angiostatic activity. These chemokines are considered as important components for the anti-tumor activities of IFNγ. In the tumor microenvironment, most solid tumors develop regions of low oxygen tension called hypoxia, which is due to an imbalance in oxygen supply and consumption. This suggests that hypo xia may alter the response to IFN-γ and affect the gene expression of Mig and IP-10. Previously we demonstrated that hypoxia inhibited the IFN-γ induced Mig and IP-10 expression in human oral carcinoma and glioma cell lines. In the present study, we investigated the mechanism involved in the inhibition of Mig and IP-10 expression under hypoxia. Despite our expectations, hypoxia did not affect the levels of tyrosine-phosphorylated and nuclear-translocated STAT1 in human oral squamous carcinoma cell line HSC-2 cells. The DNA binding activity of STAT1 in the nuclear extracts from the cells cultured in the hypoxic condition was also comparable to that seen in the normoxic condition. Interestingly, ChIP assay demonstrated that although STAT1 was recruited to the Mig and IP-10 promotors in response to IFNγ, the recruitment of RNA polymerase II to the promoters was inhibited under hypoxia. To examine whether hypoxia inducible factor-1 (HIF-1α) was related to the inhibition of Mig and IP-10 expression, we used deferoxamine (DFX), an iron chelator, which stabilizes HIF-1α at normal oxygen conditions. The treatment of the cells with DFX lead to the inhibition of IFNγ-induced Mig and IP-10 mRNA expression under normoxia with a concomitant increase in HIF-1 protein expression. These data suggests that hypoxia-induced HIF-1α is involved in the inhibition of recruitment of RNA pol II to the Mig and IP-10 promoters. Our study suggested that the hypoxic condition in the tumor microenvironment may promote an immunosuppressive environment by reducing the expression of these IFNγ-induced chemokines.

04-03/P

ENDOGENOUS TYPE I INTERFERON IS FOUND IN THE BLOOD OF FOETUSES AFFECTED BY A FAMILIAL ENCEPHALOPATHY MIMICKING CONGENITAL VIRAL INFECTION

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Aicardi-Goutieres Syndrome (AGS) and a leucoencephalitis found in the Cree indian population of northern Canada are allelic autosomal recessive neurodegenerative disorders mimicking the sequelae of congenital infection. Newborns and infants with this syndrome suffer from an early onset encephalopathy with basal ganglia calcifications, myelin alteration and a chronic cerebrospinal fluid (CSF) lymphocytosis. One feature of these diseases is that newborns and infants have raised titres of interferon (IFN) alpha present in the CSF and peripheral blood for months or years. We report four cases of the antenatal diagnosis of AGS based on the detection and measurement of IFN-alpha in foetal blood. We show the presence of IFN-alpha in foetal blood in 3 cases from as early as the 27th week of gestation. In one case, the pregnancy was stopped and examination of the brain showed lesions characteristic of AGS. In two further foetuses, both newborns were affected and IFN-alpha was detected in CSF at birth at a titre higher than in serum taken in utero or at birth; thus suggesting that intrathecal IFN-alpha synthesis occurs in the brain during foetal life. In the 4th case without detectable IFN-alpha in foetal blood, the newborn was healthy.
These results show that detection of IFN-alpha in foetal blood can be a helpful adjunct to the diagnosis of AGS, and suggest that the developing brain is exposed to IFN-alpha during foetal life. Over-expression of IFN alpha in the brain of transgenic mice also leads to CNS damage similar to that observed in AGS. We have recently defined the molecular basis of AGS and Cree encephalitis. The relationship between the involved disrupted nuclease pathways and the production of IFN will be discussed.

04-04/ O

DYNAMIC REGULATION OF LONG-RANGE CHROMATIN CAPTURING VIA NFAT1

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Adaptive immunity plays the most active and central role in the host defense system, and is composed of two cell fates: T helper 1 (Th1) and 2 (Th2) cells. Since the unbalance of the cytokine repertoire from both cell types directly induces diseases such as autoimmunity and asthma, it is highly necessary and needed to determine the fundamental molecular basis of these cytokine gene expressions. Recent decades, scientists found that *cis*-regulatory regions such as Rad50 LCR (locus control regions) and VVa are important for the proper Th2 cytokine gene expression. Using a recently developed technique 3C (*chromosome conformation capture*), I first showed that transcription factor NFAT (nuclear factor of activated T cells) 1 plays the fundamental and essential role for the close cross-talk between these distally located *cis*-regulatory regions and a target gene promoter, *IL-4* promoter. In addition, I determined that the communication between these regions is dynamically changed by the stimulation and depended on the lineage of cell-types. Acute T cell simulation strongly induced a close localization of a *cis*-regulatory region RAD50 LCR and *IL-4* promoter in both uncommitted naïve CD4+ T and lineage-restricted Th2 cells. The communication between these regions was sensitive to CsA, an inhibitor of NFAT activation, and was totally canceled in uncommitted CD4+ T cells but not in Th2 cells from *NFAT1*-null mice. The communication between VVa and *IL-4* promoter was selectively induced only in Th2 cells but not in uncommitted naïve CD4+ T cells, and was sensitive to CsA. The dynamic cooperation between these regions was not detected from lineage-restricted Th1 cells. Finally, I clearly showed that ectopic expression of constitutively active form of NFAT1 can induce a close localization of Rad50 LCR and *IL-4* promoter in *in vitro* system.

04-05/P

BCL-6, STAT5, AND THE TYPE I IFN RESPONSE IN DAUDI B LYMPHOMA CELLS.

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The Daudi B Lymphoma cell line is very sensitive to Type I Interferons (IFNs) and has been used to study the antiproliferative mechanisms of alpha IFNs against tumor cells. In the Daudi cell line, STATs 1, 2, 3, 5, and 6 are activated within minutes to hours after IFN alpha (2b) 'stimulation'. Constitutive DNA binding of STATs 1, 3, or 5 are not observed in untreated Daudi cells although these are commonly observed in leukemias of various origins. Our model for regulation of gene expression in this and other systems is a mosaic of activation and/or upregulation of transactivators (i.e. STATs) and repressors (i.e. IFI-16), as well as the deactivation and/or down regulation of transactivators (i.e. c-Myc) and repressors (i.e. Bcl-6). In support of this model, we demonstrate direct substitution of the Bcl-6 repressor with complexes of activated STAT5 in nuclear extracts of Daudi cells, prepared within minutes of Type I IFN 'stimulation', on several cis regulatory elements from interferon stimulated genes. We further demonstrate sequence-specific and contextual requirements for these binding activities. Beyond these observations, we demonstrate the direct interaction of STATs 2 and 5 in solution, but not on DNA, within minutes to hours of IFN treatment. We hypothesize that activation and accumulation of STAT2 following activation of STAT5 results in the attenuation of STAT5 mediated transactivation by the formation of STAT2/5 heterodimers that do not bind DNA. This model is indirectly supported by the ultimate down regulation of genes known to be positively regulated by STAT5 in other systems, hours after IFN 'stimulation' in Daudi. The above observations and mechanistic concepts should be applicable to other cell types.

04-06/P

EXTENSIVE MODULATION OF MICRO RNA LEVELS BY TYPE I INTERFERON.

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Interferons (IFNs) are pleotropic cytokines which mobilize various levels of immune defenses against pathogens and neoplasias. We and others have examined in detail the IFN-stimulated gene profiles in human leukocytes. Micro RNAs are small 23-25 residue RNAs that can control gene product expression through altering translation efficiency and/or stability of multiple target mRNAs. We tested the general hypothesis that Type I IFNs can differentially regulate miRNAs, implying that the true changes in gene product expression in target cells stimulated with IFN includes coordinated regulation of miRNAs to influence the ultimate utility of mRNAs. We employed a new commercial glass slide array hybridization method to test ~250+ known human miRNAs in three cell lines, A549 lung fibroblasts, Daudi B lymphoma, and Huh 07 hepatoma cells, at different times after IFN alpha 2b treatment. Our results indicate that certain miRNAs are modulated with a consistent polarity in multiple cell lines. Our results also indicate the presence of, and IFN-mediated regulation of, novel human miRNAs which are homologous to those previously identified in lower organisms, including C. elegans. Predicted mRNA targets for these IFN-regulated miRNAs include IL-18, IRF1, IRF4, ISGF3g (IRF9), Jak2, NFkB1, PKR (double-stranded RNA-activated kinase), SOCS2, SOCS5, TANK, TGFB1, WARS (Tryptophnyl tRNA synthetase) and YARS (Tyrosyl tRNA synthetase). These results indicate that the Type I IFN response pathways in humans are more complicated than previously thought.

04-07/O

CONTEXT-SPECIFIC FUNCTIONAL EFFECTS OF GENETIC POLYMORPHISMS IN CYTOKINES AND THEIR RECEPTORS

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DNA sequence polymorphisms that alter the binding of specific transcription factors provide one of the most interesting models for fine-tuning of gene regulation. Interferon-γ (IFN-γ) is a key mediator of the host immune response, and the IFN-γ receptor 1 subunit (encoded by *IFNGR1*) is essential for IFN-γ binding and signalling. Rare disruptive mutations in *IFNGR1* cause extreme vulnerability to mycobacterial infection and are often fatal. Here we report a deletion/insertion polymorphism in the promoter region of *IFNGR1* that is only found in Africans and is associated with resistance to severe malaria in West Africa. We find that it has opposite effects on gene expression in different cellular contexts. The *IFNGR1*-470del allele acts to suppress binding of nuclear proteins to the *IFNGR1* promoter region in manner that is specific for cell type. In B-lymphocytes, the *IFNGR1*-470del allele suppresses the binding of a ~35 kDa nuclear protein and acts to increase reporter gene expression. In epithelial cells, the same allele acts to decrease gene expression and suppresses the binding of ~90 kDa STAT-1 and STAT-2 proteins. In T-lymphocytes, this allele causes only subtle differences in nuclear protein binding and has no significant effect on gene expression. Our findings suggest a mechanism by which a single genetic variant may cause a broad range of phenotypic consequences. We discuss how small nucleotide changes in a gene promoter may not have obvious and dramatic effects (e.g. loss of gene activity) but may fine tune promoter function, thus resulting in subtle functional differences that may be appreciated only in specific dynamic conditions.

04-08/P

POSTTRANSCRIPTIONAL REGULATION OF IL-10 AND IL-10 RECEPTOR EXPRESSION.

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IL-10 is an immunomodulatory cytokine that plays a pivotal role in the regulation of inflammatory processes and the pathogenesis of various diseases. Therefore, it is of critical importance to determine how the expression of IL-10 and IL-10 receptor chains is regulated. During the course of the inflammatory response, a balance between transcriptional induction and posttranscriptional control regulates the expression of genes encoding cytokines. We hypothesized that the expression of these molecules was substantially regulated by control of mRNA turnover. We used THP-1 cells, a human promonocytic leukemia cell line, to examine how the expression of IL-10 and IL-10 receptors is modulated by inflammatory stimuli such as bacterial LPS and IFN-γ. Treatment of THP-1 cells with LPS leads to a dramatic increase of IL-10 expression and an induction of mRNA encoding IL-10R1. IFN-γ regulates the expression of IL-10 and IL-10R1 mRNA differentially. We showed that LPS increases the half-life of IL-10 and IL-10R1 mRNA. However, IFN-γ opposes LPS-mediated stabilization of IL-10 mRNA, but prolongs the half-life of IL-10R1 mRNA. Since both IL-10 and IL-10R1 mRNA contain A+U-rich elements (AREs) in their 3'-untranslated region (3'-UTR) that are involved in posttranscriptional regulation of many cytokine mRNAs, we examined the role of mRNA control elements on the expression of IL-10 and IL-10R1 and their mRNAs. We have defined cis-acting elements, located in the 3'-UTR of IL-10 and IL-10R1 mRNA, that regulate the expression of IL-10 and IL-10R1. In addition, the interaction of AUF1, an mRNA destabilizing protein, with the 3'-UTR of IL-10 mRNA has been examined. Our data delineate how the levels of IL-10 and its receptor IL-10R1 are controlled by the immune system, by other cytokines and by exogenous components such as LPS.

04-09/O

POSTTRANSCRIPTIONAL REGULATION OF IFN-γ GENE EXPRESSION

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IFN-γ gene expression is regulated at multiple levels, including transcriptional initiation, mRNA processing, mRNA stability and translational control. Stabilization of IFN-γ mRNA has been noted for some time and occurs in response to different stimuli, including treatment of cells with the combination of IL-2 + IL-12. We have previously demonstrated that IL-12 treatment of a human NK cell line results in the accumulation of IFN-γ transcripts in the nuclear periphery and subsequent IL-2 treatment results in a rapid release of the mRNA into the cytoplasm, leading to rapid protein expression. Although p38 MAPK has been demonstrated to be involved in the stabilization of the IFN-γ mRNA in response to cytokine treatment, the biochemical mechanisms involved in the stabilization have not been fully elucidated. Analysis of the nucleotide sequence of the IFN-γ mRNA across species has revealed a highly conserved 100 bp sequence that is present in the 3' untranslated region. This region resembles, but is distinct, from ARE regions found in numerous cytokine genes. Based on this evolutionary conservation, we have utilized RNA affinity chromatography to identify a 35 kd nuclear protein that may specifically interact with this region. This protein has characteristics of an RNA binding protein, is expressed in NK cells, binds to the sense but not anti-sense region of the IFN-γ mRNA and its' nuclear accumulation appears to be IL-12 dependent. However as yet, no known function has been attributed to this protein. Furthermore we have developed a unique mouse model where this 100 bp region of the IFN-γ mRNA has been deleted, thus providing an in vivo model for analysis of this conserved element in the regulation of IFN-γ expression.

04-10/P

IL-17 PROMOTES CHEMOKINE MRNA EXPRESSION VIA MRNA STABILIZATION

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This study explores the mechanistic basis for the ability of IL-17 to promote expression of the mouse neutrophil chemoattractant CXCL1 or KC. IL-17 was only a weak stimulus of KC protein and mRNA expression in comparison to IL-1a. Interestingly, IL-17 could cooperate effectively with TNFa (also a modest stimulus when used alone) to induce KC levels equivalent to those seen with IL-1a. IL-1a has been previously shown to promote both transcription of the KC gene and stabilization of its mRNA while TNFa induces only transcription. Based on these findings we hypothesized that IL-17 acts posttranscriptionally to stabilize the KC mRNA induced in response to TNFa. The time course of KC mRNA expression in response to TNFa was very limited and transient while that seen following treatment with either IL-1a or the combination of TNFa and IL-17 was markedly prolonged. In actinomycin D chase experiments the mRNA generated in response to TNFa plus IL-17 had an increased half-life compared to that produced by TNFa alone and decayed with kinetics similar to those seen with IL-1a. Furthermore, both IL-1a and IL-17 prolonged the half-life of KC mRNA in cells stimulated with TNFa followed by actinomycin D. Thus IL-17, like IL-1a, immediately stabilized KC mRNA without the need for new transcription. Using a KC promoter-driven luciferase assay, IL-17 was found to be a poor transcriptional stimulus. Hence IL-1a acts as a stimulus at both levels of regulation while TNFa and IL-17 each provide only half the requirements for robust gene expression. The differential mechanisms employed by each of these cytokines provide a useful setting to identify signaling events that selectively couple with transcription versus mRNA stabilization and to determine the molecular basis for such selectivity.

04-12/O

IRF-8 IS INDISPENSABLE FOR THE EXPRESSION OF PML: ROLE IN MYELOID CELL DIFFERENTIATION AND SUPPRESSION OF CML

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IRF-8, previously known as ICSBP, is a myeloid cell essential transcription factor induced by IFNγ. Accordingly, IRF-8$^{-/-}$ mice exhibit defects in the maturation of myeloid progenitor cells towards macrophages and develop Chronic Myelogenous Leukemia (CML). In this presentation we demonstrate that IRF-8 is a key factor in the induced expression of isoform I of the Promyelocytic Leukemia (PML) gene in activated macrophages. IRF-8 effectively regulates the expression of PML by interacting with two other myeloid cells essential transcription factors; IRF-1 and PU.1. PML is a tumor suppressor gene that serves as a scaffold protein for nuclear bodies (NBs). Being a key player in myeloid cells differentiation, IRF-8 mRNA levels are down regulated in peripheral blood cells of CML patients and recovers to normal levels, comparable to healthy donors, in remised patients treated with IFN-α or Gleevec. Using quantitative RT-PCR analysis we show that PML expression follows the same pattern in CML patients; very low in blood samples of primary diagnosed patients and return to normal level upon remission. Our results shed new light on two major IRF-8 dependent regulatory events that are delegated, in part, by the target gene, PML. It assigns an important role for PML in myeloid cells differentiation not only towards the granulocyte lineage but also towards the monocyte lineage. PML therefore serves as an integrator of incoming signals: retinoic acid that will result in granulopoiesis or IFN-γ, delegated by IRF-8, that will drive monopoiesis. It further suggests that the CML tumor suppressor activity of IRF-8 is mediated by PML. Reduced IRF-8 expression results in low PML transcripts that eventually lead to genomic instability and the subsequent development of aggressive leukemia. Our data therefore suggests that PML functions as a tumor suppressor gene in CML.

04-13/O

FUNCTIONAL GENOMICS AND THE INNATE CYTOKINE/ INTERFERON RESPONSE: CAN WE SAVE THE WORLD FROM THE NEXT VIRAL PANDEMIC?

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Functional genomics approaches including microarrays, proteomics, and bioinformatics offer a unique opportunity to study virus-host interactions and the host defense and cytokine responses at a global level. We are utilizing microarray DNA oligonucleotide slides containing unique human, macaque, and mouse genes for our in vitro and in vivo studies. It will be critical to examine not only mRNA expression patterns, but also physical levels of cellular proteins, which are altered by virus infection. For our proteomics analysis we are utilizing the Accurate Mass and Time Tags (AMT) approaches and mass spectrometry to identify and compare complex mixtures of proteins; we are employing the TAP vector system and pull-downs to perform targeted proteomics. To obtain a complete picture of viral pathogenesis and the host response at the level of transcription and translation, we are attempting to place these inevitably complex genetic changes in the context of multifactor clinical outcomes and pathology. Utilizing these approaches we will present data on the global and interferon cytokine host responses using animal and human models together with in vitro models of pandemic influenza virus, hepatitis C virus, HIV, Ebola, and SARS virus infections. Further, we will present results from a total proteome analysis of interferon treated human liver cells and HIV infected cells. We are also developing high throughput approaches to integrate global RNA and protein expression data. A longer range goal is to develop a "Virus Compendium" which would enable us to compile a centralized data base to catalogue events in cells infected by all mammalian viruses. Identification of new common and unique host pathways impacted by viruses should ultimately reveal novel therapeutic targets, improved much needed antiviral therapies, and viral vaccines.

04-16/P

INTERFERON-BETA MEDIATED INHIBITION OF INTERLEUKIN-8 GENE EXPRESSION

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Interleukin-8 (IL-8) is a member of the CXC chemokine family and was first identified for its chemotactic properties toward leukocytes. IL-8 also plays a role in a number of pathological states, and has been shown to be upregulated in various cancers, including the primary brain tumor glioblastoma. IL-8 expression is induced rapidly by a number of pro-inflammatory cytokines through the NF-κB family of transcription factors. It has long been known that interferons (IFNs) can inhibit IL-8 expression, although the mechanism by which IFNs exert inhibitory effects is unknown. Type I IFNs activate the interferon-stimulated gene factor-3 complex (ISGF3) comprised of STAT1, STAT2 and IRF-9, which binds to interferon stimulated response elements (ISRE) within the promoters of interferon responsive genes and activates transcription. IFNs are able to inhibit IL-8 expression, even though no ISRE element has yet been found within the IL-8 promoter. We previously demonstrated that IFN-β inhibits PMA-induced IL-8 expression at the transcriptional level. As well, IFN-β treatment results in decreased binding of NF-κB p65 and RNA polymerase II, and decreased acetylation of histones 3 and 4 to the IL-8 promoter in chromatin immunoprecipitation assays when compared with PMA treatment alone. In this study, we have utilized a number of ISGF3 mutant cell lines to dissect the role of each member of the ISGF3 signaling complex in IFN-β mediated inhibition. Our results indicate that STAT1, STAT2 and IRF-9 are all required for the inhibition of IL-8 by IFN-β. Additionally, the dimerization domain of STAT1 is required, but the transactivation domains of STAT1 and STAT2 are not required. In all, these data reveal the specific requirements of ISGF3 cmponents in the IFN-β-mediated inhibition of IL-8.

04-17/O

GENETIC RECODING BY RNA EDITING: REGULATION AND FUNCTION OF THE INTERFERON-INDUCIBLE A-TO-I DSRNA DEAMINASE.

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Adenosine deaminase that acts on RNA (ADAR1) catalyzes the C-6 deamination of adenosine (A) to generate inosine (I) in viral, cellular and synthetic RNAs with double-stranded (ds) character. Because I is recognized as G instead of A, A-to-I editing thereby leads to genetic recoding. ADAR1 is interferon (IFN) and virus inducible. We have isolated and characterized cDNA and genomic clones of ADAR1, both human and mouse. The ~7-kb cDNA of ADAR1 specifies a long ORF that includes three copies of the dsRNA-binding domain (R1, R2, R3), two copies of the Z-DNA/Z-RNA binding domain (Za, Zb), and within the C-terminal region the deaminase catalytic domain. The dsRNA-binding and deaminase catalytic activities of ADAR1 were established by mutational analyses and functional biochemical assays. Two-sized forms of the A-to-I RNA editing enzyme were characterized: the IFN inducible ~150-kDa protein present in both the cytoplasm and nucleus, and a constitutively expressed N-terminally truncated ~110-kDa protein that is predominantly if not exclusively nuclear. Alternative promoters, one IFN-inducible and two others constitutively active, were identified that drive expression of alternative exon 1-containing transcripts that encode the IFN-inducible ~150-kDa and constitutively expressed ~110-kDa ADAR1 enzymes. Analysis of tissues from mice revealed tissue selectivity of ADAR1 transcript expression, and differential expression in uninfected and infected animals. ADAR1 deaminase efficiently edited double-stranded RNA and pre-mRNAs for the glutamate receptor GluR-B and the serotonin receptor 5-HT2cR. Vaccinia virus E3L protein and adenovirus VA1 RNA efficiently antagonized ADAR enzymatic activity. The role of ADAR1 during the replication of selected DNA and RNA animal viruses was assessed. (*Supported in part by NIAID, NIH).*

04-18/P

TOLL-LIKE RECEPTOR-DEPENDENT AND -INDEPENDENT *VIPERIN* GENE EXPRESSION AND COUNTER-REGULATION BY PRDI-BF1/BLIMP1

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Here we identify Viperin as a highly inducible gene in response to lipopolysaccharide (LPS), synthetic double stranded RNA (poly I:C) or Sendai virus (SV) infection. The only known function of Viperin relates to its ability to inhibit Human Cytomegalovirus replication. Few data are available on the regulation of this gene. *In silico* analysis of the promoter identified two IFN-stimulated response elements (ISRE), which in other genes bind IRF3 or the IFN-stimulated gene factor-3 (ISGF3) complex. LPS and poly I:C induce very high levels of Viperin in wild type cells but not in cells deficient in TRIF, TBK1, IRF3 or the type I IFNa/bR. Sendai virus-induced Viperin gene expression was mediated independently of TLR signaling by Retinoic acid-inducible gene (RIG-I) and *m*itochondrial *a*nti-*v*iral *s*ignaling (MAVS). Virus induced Viperin expression was not attenuated in macrophages deficient in either TBK1 or IKKe alone. Moreover, IRF3-deficient but not IFNa/bR-deficient macrophages still induced Viperin in response to SV. Promoter reporter studies combined with DNA-immunoprecipitation assays identified the ISGF3 complex as the key regulator of Viperin gene expression. Moreover, positive regulatory domain I-binding factor 1 (PRDI-BF1, also called Blimp1) binds the ISRE sites and competes with ISGF3 binding in a virus inducible manner to inhibit Viperin transcription. Collectively, these studies identify Viperin as a tightly regulated ISGF3 target gene, which is counter-regulated by PRDI-BF1.

04-19/P

NF- κB AND IFN REGULATORY FACTOR FAMILY TRANSCRIPTION FACTORS REGULATE CCL19 GENE EXPRESSION IN HUMAN DENDRITIC CELLS

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CCL19/MIP-3β chemokine plays an important role in regulating DC traffic and recruitment of naïve T cells to the vicinity of activated DCs. CCL19 is also produced by activated DCs at later phases of the maturation process and thus it has a central role in DC biology. In the present study, we have analyzed the regulation of CCL19 gene expression in human monocyte-derived DCs. DCs infected with *Salmonella enterica* or Sendai virus produced CCL19 at late times of infection. Treatment of DCs with MAP kinase, NFAT, or NF-κB inhibitors suggested that CCL19 gene expression was at least partly regulated by NF-κB. Computer analysis of the CCL19 promoter also identified two putative NF-κB binding sites and one interferon stimulated response element (ISRE). Transcription factor binding experiments demonstrated that *Salmonella* and Sendai virus infection increased the binding of p50, p52, p65, and RelB to both CCL19 promoter NF-κB elements. Interestingly, *Salmonella* and Sendai virus infection also increased the binding of multiple IRFs, as well as STAT1 and STAT2, to the putative ISRE site. Enhanced binding of IRF1, IRF3, IRF7, and IRF9, but not that of IRF5, to CCL19 promoter ISRE site was detected in *Salmonella* or Sendai virus-infected cell extracts. The role of NF-κB and various IRF family members in transcriptional control of CCL19 gene was further studied in transfection experiments using CCL19 promoter luciferase reporter construct. The expression of NF-κB p50/p65 or p52/RelB dimers readily activated CCL19 promoter. The expression of IRF1, IRF3, or IRF7 proteins was also able to activate the promoter in the presence of Sendai virus infection. CCL19 promoter constructs, in which NF-κB and/or ISRE sites were mutated, were only weakly activated. In conclusion, our experiments reveal that the expression of CCL19 gene is regulated by a combined action of several members of NF-κB, IRF, and STAT family transcription factors.

04-20/O

IDENTIFICATION OF STAT1 AS A CYTOPLASMIC REGULATOR OF GENE TRANSCRIPTION AND APOPTOSIS

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The transcription factor STAT1 has both inducible and constitutive gene regulatory activities. For the inducible transcription the importance of nucleocytoplasmic cycling has been well established. On the contrary, for the constitutive functions of STAT1 the necessity of permanent nucleocytoplasmic shuttling has not been demonstrated yet. Here, we present a STAT1 mutant protein that retained the inducible nuclear import, but that was devoid specifically of constitutive nucleocytoplasmic shuttling. Surprisingly, the constitutive induction or repression of a number of STAT1-dependent genes continued unaltered. Moreover, also the TNFα-dependent induction of apoptosis was not influenced by the lack of nuclear localization of unphosphorylated STAT1. While cytoplasmic STAT1 did not trigger apoptosis via NFκB suppression or cytochrome c release from the mitochondrium, our data stress the crucial role of caspase-1 expression for the execution of apoptosis. Notably, the low steady-state levels of caspase-1 mRNA in cells lacking STAT1 indeed reflected reduced gene transcription, since it was found that the decay of caspase-1 mRNA was not accelerated by the absence of STAT1. The ectopic expression of caspase-1 in STAT1-deficient cells completely rescued the apoptosis phenotype, whereas the inhibition of caspase-1 in STAT1 expressing cells rendered these cells resistant to TNFα-induced apoptosis. These results indicated that STAT1 was dispensable for the apoptotic program, yet STAT1 conferred sensitivity to apoptosis through the efficient transcription of the caspase-1 gene. As this was seen irrespective of STAT1 nuclear import, we concluded that STAT1 could elicit transcriptional responses independent of promoter binding from a cytoplasmic location.

04-21/P

PATHOGENIC AND COMMENSAL BACTERIA ACTIVATE DISTINCT TRANSCRIPTION FACTOR PATTERN IN HUMAN DENDRITIC CELLS

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In the present work we have analyzed the activation mechanisms of monocyte-derived dendritic cells (DCs) by two Gram-positive bacteria. Stimulation of DCs with pathogenic *Streptococcus pyogenes* resulted in enhanced expression of co-stimulatory molecules and the production of Th1 type proinflammatory cytokines and chemokines. In contrast, DCs stimulated with nonpathogenic *Lactobacillus rhamnosus* showed only a moderate expression of co-stimulatory molecules and, most importantly, produced only minute amounts of cytokines such as TNF-α and CCL20.

Next we analyzed which transcription factor pathways are activated by these bacteria. As a model gene we used CXCL10 chemokine, which is regulated by NF-κB and ISRE binding transcription factors. Furthermore, only *S. pyogenes* stimulation induced efficient CXCL10 production in DCs. Oligonucleotide affinity binding experiments revealed that *S. pyogenes* and *L. rhamnosus* similarly activated classical (p50/p65) and alternative (p52/RelB) NF-κB pathways demonstrating that both bacteria are able to interact with DCs and stimulate NF-κB activation. However, a completely different IRF and STAT transcription factor activation pattern was observed between *S. pyogenes* and *L. rhamnosus*. While *S. pyogenes* induced IRF1, IRF4, IRF7, IRF8, IRF9, STAT1, and STAT2 DNA binding to CXCL10 ISRE element, *L. rhamnosus* stimulation only resulted in enhanced IRF4 DNA binding.

Our results show that both pathogenic *S. pyogenes* and nonpathogenic *L. rhamnosus* are able to active NF-κB pathway in DCs. However, efficient DC maturation and cytokine production requires a broader transcription factor activation that is taking place during the interaction of *S. pyogenes* with DCs.

04-22/P

ROLE OF STAT1 AND IRF1 IN INTERFERON INDUCED TRANSCRIPTION

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We analysed the interplay and the individual roles of signal transducer and activator of transcription1 (STAT1) and interferon (IFN) regulatory factor1 (IRF1) in transcriptional regulation of the inducible nitric oxide synthase (iNOS) gene that is activated during infection of macrophages with *Listeria monocytogenes*, and of the guanylate-binding protein2 (GBP2) that responds predominantly to IFN-γ. The iNOS promoter contains binding sites for NFκB, STAT1 and IRF1. STAT1, activated by IFN-β produced during infection, caused the subsequent production of IRF1, whereas NFκB was activated by *Listeria* independently of IFN-β. iNOS was highly expressed following the combined action of IFN-β and other *Listeria*-derived signals, whereas either signal alone caused only weak induction. STAT1 was crucial for iNOS expression as shown in STAT1-deficient macrophages. The synergistic induction of iNOS expression correlated with the ordered activation of NFκB, IRF1 and STAT1 and their recruitment to the iNOS promoter, as analysed by chromatin immunoprecipitation (ChIP). The data suggest functional, but not physical interaction of STAT1/IRF1 and NFκB in iNOS promoter activation during bacterial infection.
The interplay of STAT1 and IRF1 in the induction of GBP2 by IFN-γ was examined in gene-targeted fibroblasts. These studies showed that both transcription factors are required for GBP2 mRNA expression. ChIP analysis demonstrated that STAT1 associated with the GBP2 promoter independently of IRF1. Histone 4 acetylation and recruitment of the transcriptional coactivator CBP were dependent on STAT1, but independent of IRF1. In contrast, IRF1 associated with and recruited RNA Pol II to the GBP2 promoter. These studies demonstrate a coordinated, but functionally diverse action of STAT1 and IRF1 in IFN-γ-induced gene expression.

04-23/P

NFκB NEGATIVELY REGULATES INTERFERON-INDUCED GENE EXPRESSION AND ANTI-INFLUENZA ACTIVITY.

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Interferons (IFNs) are antiviral cytokines that selectively regulate gene expression through several signaling pathways including nuclear factor κB (NFκB). To investigate the specific role of NFκB in IFN signaling, we performed gene expression profiling after IFN treatment of embryonic fibroblasts derived from normal mice or mice with targeted deletion in NFκB p50 and p65 genes. Interestingly, several antiviral and immunomodulatory genes were induced higher by IFN in NFκB knockout cells. Chromatin immunoprecipitation (ChIP) experiments demonstrated that NFκB was basally bound to the promoters of these genes, while IFN treatment resulted in the recruitment of STAT1 and STAT2 to these promoters. However, in NFκB knockout cells IFN induced STAT binding as well as the binding of the IFN regulatory factor-1 (IRF1) to the ISG promoters. IRF1 binding closely correlated with enhanced gene induction. Moreover, NFκB suppressed both antiviral and immunomodulatory actions of IFN against influenza virus. Our results identify a novel negative regulatory role of NFκB in IFN-induced gene expression and biological activities, and suggest that modulating NFκB activity may provide a new avenue for enhancing the IFN's therapeutic effectiveness.

04-24/P

ROLE OF TRANSFORMING GROWTH FACTOR- BETA IN CYTOGLOBIN-STIMULATED COLLAGEN I ALPHA I EXPRESSION

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Cytoglobin is an oxygen-binding protein whose function is unknown. It is augmented in fibrotic rat liver and in nephropathy. We have previously localized cytoglobin in fibroblasts and hepatic stellate cells, both of which are responsible for the deposition of extracellular matrix (ECM). This is in line with the hypothesis that cytoglobin plays a role in the metabolism of ECM components. Collagen I and III are the predominant ECM molecules in the fibrotic septa. It is reasonable to speculate that cytoglobin is involved in the metabolism of collagens. Using an acute carbon tetrachloride-induced toxicity model, we report the simultaneous up-regulation of cytoglobin and collagen I α(I) mRNA 24 hours after a single dose of CCl_4 treatment, by 2.5 folds and 5 folds respectively, while the expression of collagen III α(I) chain mRNA remained unchanged at this time-point. Transforming growth factor-ß (TGF-β) has been reported to increase the expression of collagen I α(I) mRNA in NIH-3T3 cell line over-expressing cytoglobin, thus implicating the possibility of TGB-β-dependent control of collagen I α(I) transcription by cytoglobin. We have investigated the control of expression of cytoglobin and thus collagen I α(I) by TGF-β in HSC-T6, an immortalized rat hepatic stellate cell line, and the results will be presented.

04-25/O

ICER/CREM – MEDIATED TRANSCRIPTIONAL ATTENU ATION OF IL-2 AND ITS ROLE IN SUPPRESSION BY REGULATORY T CELLS

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Inducible cAMP early repressor/cAMP response element modulator (ICER/CREM) transcription factors play critical roles in transcriptional attenuation of interleukin-2 (IL-2). To investigate further their role in T cell suppression we examined ICER/CREM expression in $CD4^+CD25^+$ T (T_R) cell suppression assays. ICER/CREM accumulated in suppression assays in the presence of natural T_R cells suppressing IL-2 transcription. However, in mock assays, in the absence of T_R cells, ICER/CREM failed to express permitting transcription of IL-2. Moreover, naïve $CD4^+CD25^-$ ($CD25^-$) responder T cells retrovirally transduced with Foxp3 can induce the accumulation of ICER/CREM in suppression assays and replace natural T_R cells. Importantly, ICER/CREM expression is induced in activated responder T cells early upon incubation in T_R assays and correlates with a sharp decrease in the number of IL-2 expressing cells. Our results show that abrogation of T_R-mediated suppression by blockade of CTLA-4 or activation of GITR leads to the increase in IL-2 transcription and reduction of ICER/CREM. Furthermore, ICER/CREM fails to accumulate in T_R assays in the presence of activated mature dendritic cells (mDCs) and is accompanied by abrogation of T_R-mediated suppression leading to abundant IL-2 expression. We conclude that ICER/CREM is a critical component of T_R-mediated inhibitory function that effects transcriptional attenuation of IL-2 production in $CD25^-$ responder T cells.

04-26/P

DYNAMIC AND TRANSIENT CHROMATIN REMODEL-ING OF THE MACROPHAGE IL-10 GENE LOCUS DURING TRANSCRIPTION

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To gain insight into the molecular mechanism(s) whereby macrophages produce large amounts of IL-10, we analyzed IL-10 gene expression and temporally correlated it with modifications to chromatin associated with the IL-10 promoter by using a chromatin immunoprecipitation (ChIP) assays together with quantitative real-time PCR (qRT-PCR). In resting cells, which make essentially no cytokines, the IL-10 promoter is associated with histones containing little or no detectable modifications. Macrophages stimulated in the presence of immune complexes begin to produce high levels of IL-10 pre-mRNA transcripts within minutes of stimulation. Coincident with this tran-

scription was a rapid and dynamic phosphorylation of histone H3 at specific sites in the IL-10 promoter. This alteration appeared to be specific for the IL-10 promoter but not other genes such as IL-12p40. The magnitude of the phosphorylation of H3 along the proximal promoter region of IL-10 gene was correlated with that of micrococcal nuclease and DNase I enzyme accessibility. Both phosphorylation of histone H3 and enzyme accessibility occurred within a short period of time. Importantly, the remodeling of chromatin permitted the recruitment of transcription factors such as Sp1 to its corresponding site on the promoter region. Of note, blocking the activation of ERK prevented histone phosphorylation and transcription factor binding to the IL-10 promoter. In contrast to histone phosphorylation, the peak of histone acetylation at this promoter did not occur until after transcription had peaked. Inhibition of histone deactylase (HDAC) did not alter IL-10 gene expression, suggesting that phosphorylation but not acetylation was the proximal event responsible for IL-10 transcription. Our findings reveal a rapid and well-orchestrated series of events in which ERK activation causes a rapid and transient phosphorylation of histone H3 at specific regions of the IL-10 promoter, resulting in a transient exposure of the IL-10 promoter to the transcription factors that bind there. This exposure is essential for the efficient induction of IL-10 gene expression in macrophages.

04-27/P

NFATC1 AUTOREGULATION: A CRITICAL STEP FOR T CELL FATE DETERMINATION

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NFATc transcription factors appeared in evolution with the emergence of lymphocytes in jawed fish and play decisive roles in the development of the adaptive immune system. In peripheral T cells NFATc1 and NFATc2 are the most prominent NFAT factors which overlap in their function but differ remarkably in the mode of expression. Whereas NFATc2 synthesis is constitutive, the expression of NFATc1/ αA, the most prominent NFATc1 isoform in peripheral T cells, is strongly induced and maintained upon T cell activation by positive autoregulation. The six NFATc1 isoforms in T cells differ in their N- (α, ß) and C-terminal (A, B and C) peptides. Due to the activity of the inducible promoter P1 and an inducible proximal polyA site, T cell receptor (TCR) and co-receptors signals lead to a 20 fold induction of the short isoform NFATc1/ αA RNA. NFATc1/ αA lacks the C-terminal sequences of 245 aa. They constitute a second transactivation domain in NFATc1/C. Similar to NFATc2, NFATc1/C – but not NFATc1/ αA – supports the "Activation Induced Cell Death" of T effector cells. Persistent signals are necessary for NFATc1/ αA expression: whereas cyclosporin A treatment of EL-4 T cells does not block NFATc2 expression, it abolishes any NFATc1/ αA protein within 1-2 days. Both *nfatc1* promoters represent DNase I hypersensitive chromatin sites and DNA methylation islands. In most lymphoid cells, P1 and P2 DNAs are hypomethylated, while in human lymphomas with defects in immunoreceptor signaling, DNAs of both promoters are hypermethylated. While the P2 promoter is constitutively active in murine DN thymocytes and resting peripheral T cells, P1 activity is strongly induced and maintained by positive NFAT autoregulation in T effector cells. Here, we present details of NFATc1 autoregulation and a mathematical model of molecular network controlling NFATc1 expression which helps to understand NFATc1 function in effector T cells.

Reference

Chuvpilo S, *et al*. *Immunity* 2006; 16: 881-95.

04-28/P

UP-REGULATION OF RASGEF1B GENE EXPRESSION IN NIH 3T3-LIKE FIBROBLASTS LACKING THE DOUBLE-STRANDED RNA-DEPENDENT PROTEIN KINASE PKR

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The double-stranded RNA-dependent protein kinase PKR regulates cellular responses through modulation of activity of a number of cellular components. In addition to its role in translational control, PKR also contributes in regulation of gene transcription. In this work we have used spontaneously immortalized mouse embryonic fibroblasts to determine the role of PKR in regulating the expression of RasGEF1b, a novel Ras-associated guanine exchange factor whose expression is induced in response to inflammatory stimuli including Toll-like receptor TLR2 agonists, LPS and poly-IC. We demonstrate that expression of RasGEF1b is significantly increased upon transfection-mediated delivery of poly-IC in fibroblasts. Intriguilly, in untreated control PKR-null, but not wild-type fibroblasts, the expression of RasGEF1b is found dramatically increased. Yet, a significant decrease in its expression is observed upon poly-IC treatment of PKR-null cells. To gain insights on the mechanisms underlying these events, we have cloned the putative promoter region of RasGEF1b gene in a luciferase-based plasmid reporter. It appears that the regulatory effects of PKR on RasGEF1b expression are mediated at transcriptional levels, as the promoter activity in PKR-null cells is at least 32-fold higher than in wild-type cells. We are currently restoring PKR function in PKR-null fibroblasts to determine its role as a mediator of negative regulation in transcriptional activation. Further studies on the gain-of-function of RasGEF1b in cells may help us to better understand the increased susceptibility of Ras-transformed cells and PKR deficient cells to some virus.

04-29/P

PROMOTER ORGANIZATION OF THE INTERFERON-A GENES DIFFERENTIALLY AFFECTS VIRUS-INDUCED EXPRESSION AND RESPONSIVENESS TO TBK1 AND IKKε

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Virus-induced expression of interferon (IFN)-A genes is regulated by two members of the IFN regulatory factor family, IRF-3 and IRF-7, which are activated by phosphorylation during viral infection by the IKK-related serine/threonine kinases TBK1 and IKKε. In this study, we demonstrate that three IRF-binding sites located in the virus responsive element mediate the transcriptional activation of the IFN-A4 promoter by IRF-3. The precise arrangement of these IRF-elements is required for synergistic activation of the IFN-A4 promoter following NDV infection or activation by TBK1 or IKKε. The ordered assembly of IRF-3 multimers on the promoter also determines cooperative recruitment of IRF-3 and CBP, and differential virus-induced expression of IFN-A4 gene promoter compared to IFN-A11. Naturally occurring nucleotide substitutions disrupt two of the IRF-elements in the IFN-A11 gene promoter, leading to a dramatic decrease in IRF-3 and CBP recruitment and in IRF-3-dependent transcription. Transcription of the IFN-A4 promoter by IRF-7 is mediated by two IRF-elements; promoter mutants that carry a reversed IRF-element retain the ability to respond to IKKε or TBK1 expression in the presence of IRF-7, but lose the capacity to respond to virus or kinase-induced IRF-3. Interestingly, IKKε or TBK1 stimulates the IRF-7-mediated transcription of IFN-A11, although at a lesser extent compared to IFN-A4. Our data indicate that virus-induced expression of IFN-A genes is dictated by the organization of IRF-elements within the IFN-A promoters and that the differential IFN-A gene expression, based on the IRF-3 responsiveness, is partially compensated in the presence of IRF-7 when both factors are activated by IKKε or TBK1. Sequence analyses revealed a similar organization of IRF-sites in human IFN-A gene promoters.