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Parallel session – IFN regulation of viral pathogenesis

O029

Chromatin exchange in interferon induced transcription

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Introduction. Type I interferons (IFNs) activate the JAK/STAT pathway and stimulate transcription from many IFN stimulated genes (ISGs). This process likely involves destabilization and reorganization of chromatin. However, chromatin events associated with ISG induction is poorly understood. To gain insight into a link between transcription and chromatin regulation, we studied whether IFN stimulation causes exchange of histones relevant to epigenetic regulation.

Methods. Because the histone H3.3 is implicated in transcription coupled chromatin change, we constructed NIH3T3 cells expressing GFP- H3.3 and examined H3.3 incorporation into ISGs by ChIP in parallel with ISG transcription and transcription factor recruitment.

Results. IFN stimulation led to rapid recruitment of RNA polymerase II and BRD4, an acetyl-histone binding factor to the ISG. This was followed by recruitment of the elongation factor P-TEFb, the pausing complex NELF/DSIF and SPT6. Along with these events, IFN stimulation caused rapid H3.3 accumulation in the ISGs. H3.3 accumulation was greater in the coding region and the gene end than in the promoter region where virtually no H3.3 incorporation was detected. Analysis with a BRD4 specific inhibitorJQ1 showed that H3.3 incorporation depended on BRD4 recruitment and ISG elongation. However, H3.3 incorporation into ISGs continued past ISG elongation, leaving the H3.3 mark on ISGs for at least two cycles of cell division. The mutant GFP-H3.3K36R was not incorporated into ISGs, indicating that methylation of K36 is requreid for H3.3 incorporation. Finally, H3.3 incorporation was also observed in another activation model, indicating the generality of transcription-induced histone exchange.

Conclusion. We show that IFN stimulation triggers rapid and extensive H3.3 incorporation into ISGs, which is presumably associated with expulsion of the preexisting H3 (H3.1/H3.2). This event required active ISG elongation, suggesting that passage of RNA polymerase II through the ISG gene body destabilizes the architecture of the RNA-DNA-nucleosome, necessitating reconstruction of nucleosomes composed of H3.3. Based on the remarkable persistence of the H3.3 mark left on the ISGs long after transcription, we suggest that transcription-induced H3.3 deposition represents an epigenetic mark linked to transcriptional memory.

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O030

Evasion of the OAS-RNase L pathway by murine coronavirus ns2 protein is required for viral replication and hepatitis

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Introduction. The 2′,5′-oligoadenylate synthetase-ribonuclease L (OAS-RNase L) system is a potent IFN induced antiviral pathway. Following infection, IFNs induce a group of OAS genes whose products are activated by viral double-stranded RNA. OAS uses ATP to generate 2′,5′-linked oligoadenylates (2-5A). 2-5A binds to and activates the ubiquitous cellular endoribonuclease RNase L causing cleavages of single stranded regions of both viral and cellular RNA thus inhibiting viral replication. In addition, detection of the newly generated short RNAs by cellular pattern recognition receptors, MDA5 and RIG-I, further enhances IFN production and the ensuing antiviral activities. The intracellular concentration of 2-5A is believed to be the primary factor controlling RNase L activation. The liver contains abundant innate immune cells, which provide the first line of defense against pathogens. However, the factors that determine whether a virus can bypass this defense to access and infect the liver parenchyma are not well understood. The murine coronavirus, mouse hepatitis virus (MHV), strain A59, infection of mice provides a model for virus induced hepatitis. The MHV accessory protein, ns2, antagonizes the type I IFN response in macrophages and promotes the induction of hepatitis. Here we will describe how the ns2 protein facilitates the development of viral hepatitis by blocking OAS-RNase L pathway.

Methods. Bone marrow macrophages (BMM) from wild type (wt) and RNase L−/− mice were infected with A59 and ns2 mutant MHV. Viral titers were determined by plaque assays. RNase L activity was monitored by rRNA integrity in RNA chips. Intracellular levels of 2-5A were measured using RNase L activation assays. Effects of ns2 on 2-5A levels in cells were determined by transfecting ns2 or mutant ns2 cDNAs into HEK-293T cells. Recombinant ns2 and mutant ns2H126R proteins were purified and incubated with 2-5A in vitro and the 2-5A breakdown products were measured by HPLC. Hepatitis was determined by histology following inoculation A59 or ns2 mutant MHV into wt and RNase L−/− mice.

Results. We found evidence for a new molecular mechanism of subversion of the RNase L pathway in macrophages that regulates acute hepatitis during MHV infection. Coronavirus ns2 belongs to the LigT-like protein family, within the 2H phosphoesterase superfamily, some of which possess cyclophosphodiesterase activity (CPD). We have found that ns2 is not a CPD, but instead is a 2′,5′-phosphodiesterase (PDE) that cleaves, and thus eliminates 2-5A, the activator of RNase L. We observed that ns2 blocks the IFN inducible OAS-RNase L pathway to facilitate hepatitis development. Ns2 prevents activation of RNase L and consequently limits viral RNA degradation. An ns2 mutant virus was unable to replicate in the liver or induce hepatitis in wt mice, but was highly pathogenic in RNase L−/− mice. Thus, RNase L is a critical cellular factor for protection against viral infection of the liver and the resulting hepatitis.

Conclusion. MHV accessory protein ns2 is a 2′,5′-PDE which degrades 2-5A and limits RNase L activation thus facilitating virus-induced hepatitis in mice.

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O031 TLR3 and RIG-I sensing of HCV infection by hepatocytes leads to interferon-independent CXCL10 induction

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Introduction. Chronic hepatitis C is characterized by a persistent hepatic inflammatory response and the recruitment of immune effector cells to the liver by pro-inflammatory chemokines. The chemokine CXCL10 is induced by HCV infection *in vitro* and *in vivo*, and is correlated with the outcome of Interferon (IFN)-based therapies. Therefore, we investigated how sensing of HCV infection by the pathogen recognition receptors (PRRs) TLR3 and Retinoic Acid Inducible Gene 1 (RIG-I) led to expression of CXCL10 in hepatocytes.

Methods. Primary human hepatocytes were infected *in vitro* with the HCV clone JFH-1. CXCL10 production was measured via real-time RT-PCR, Luminex Bead Array, and immunofluorescence. Type I and type III interferon induction was measured

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