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Molecular pathways that control Inflammation- and HJV- mediated hepatic Hepcidin expression

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Common disorders of iron metabolism like hereditary hemochromatosis or the anemias of inflammation are caused by inappropriate expression of the hepatic iron-regulatory hormone Hepcidin. Thus, the precise regulation of Hepcidin is essential to maintain body iron homeostasis: Hepcidin deficiency induces iron overload and Hepcidin excess results in anemia. Hepcidin expression is modulated in the liver in response to body iron stores, hypoxia, inflammatory and infectious stimuli. In addition the hemochromatosis proteins (HH) HFE, TfR2 and HJV positively control its expression. It is therefore crucial to understand the signalling pathways that regulate Hepcidin expression as well as their integration to assure appropriate Hepcidin levels.

This study investigates the regulatory cis-acting elements in the Hepcidin promoter and trans-acting factors required for Hepcidin activation in response to inflammatory signals and the hemochromatosis protein hemojuvelin. To assess how the inflammatory cytokine IL-6 activates Hepcidin mRNA expression Luciferase reporter vectors containing the 942 bp upstream of the transcription start site of the human Hepcidin promoter or different truncations/mutations thereof were transfected into the hepatocyte cell line Huh7. We show that inflammatory stimuli (exogenously administered IL-6 or conditioned medium from the monocyte/macrophage cell line THP-1) require a STAT-3 binding motif located at position -64/-72 of the Hepcidin promoter. The same STAT binding site is further required for high basal-level hepcidin mRNA expression and siRNA-mediated RNA knock down of STAT-3 strongly reduces endogenous Hepcidin mRNA expression. These results identify a missing

link in the acute-phase activation of hepcidin and establish STAT-3 as a key effector of baseline Hepcidin expression and during inflammatory conditions.

Mutations in the gene HJV (also known as hemojuvelin or RGMC) cause severe iron overload and are associated with low Hepcidin expression. Recent results indicate that HJV is a BMP coreceptor and that the decreased Hepcidin mRNA expression due to HJV dysfunction is a result of an impaired BMP signalling ability. Here, I used the same cell-based assay system to unravel how the HH protein HJV activates Hepcidin transcription. I have identified a critical BMP-responsive element (BMP-RE) at position -84/-79 of the Hepcidin promoter. I show that this element mediates the HJV-dependent high basal Hepcidin mRNA expression under control conditions. Unexpectedly, the mutation of the same BMP-RE element also severely impairs Hepcidin activation in response to IL-6 even in the presence of the intact STAT-3 binding motif. My data suggest that the IL-6 response requires at least two independent promoter elements and that a single promoter element is important for both, the IL-6 and the HJV/BMP-mediated activation of the hepcidin promoter. Therefore, the conserved BMP-RE at position -84/-79bp of the human Hepcidin promoter seems to play a critical role in integrating two Hepcidin activating stimuli, the inflammatory and iron-dependent response of the Hepcidin promoter. These findings constitute important steps on the way to understand the molecular basis of common iron metabolism disorders that in future may enable highly specific pharmacological interference.