

***Cis* and *trans* regulation of hepcidin expression by Upstream Stimulatory Factor**

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Hepcidin is the presumed negative regulator of systemic iron levels; its expression is induced in iron-overload, infection, inflammation and by cytokines but is suppressed in hypoxia and anaemia. Although the gene is exquisitely sensitive to changes in iron status *in vivo*, its mRNA is devoid of prototypical iron-response elements and it is therefore not obvious how it may be regulated by iron flux. The multiplicity of effectors of its expression also suggests that the transcriptional circuitry controlling the gene may be very complex indeed. We show here that members of the basic helix-loop-helix leucine zipper (bHLH-ZIP) family of transcriptional regulators control hepcidin expression. The Upstream Stimulatory Factor USF2, previously linked to hepcidin through gene ablation in inbred mice, exerts a polar or *cis*-acting effect while USF1 acts in *trans* to control hepcidin expression. In addition, c-Myc and Max synergize to control the expression of this hormone, supporting previous findings for the role of this couple in regulating iron metabolism. Transcriptional activation by both USF1/USF2 and c-Myc/Max heterodimers occurs through E-boxes within the promoter. Site-directed

mutagenesis of these elements rendered the promoter unresponsive to USF1/USF2 or c-Myc/Max. Dominant-negative USF1 and USF2 mutants reciprocally attenuated promoter transactivation by the cognate wild-type isoforms. Promoter occupancy by the transcription factors was confirmed by mobility shift and chromatin immunoprecipitation assays. Taken together, these members of the bHLH-ZIP family of transcriptional regulators may synergistically subserve an important role in iron metabolism as well as other pathways in which hepcidin may be involved.