The Distal Location of the Iron Responsive Region of the Hepcidin Promoter

Jaroslav Truksa*
Pauline Lee*
Hongfan Peng*
Jonathan Flanagan†
Ernest Beutler*

*Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA

†Department of Hematology, St. Jude Children's Research Hospital, 332 N Lauderdale St, D-5055, Mail Stop 310, Memphis, TN 38105, USA

Corresponding author: Ernest Beutler, Department of Molecular and Experimental Medicine, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037, USA, e-mail: beutler@scripps.edu
ABSTRACT

The response of hepcidin transcription to iron has been repeatedly documented in living mice, but it is difficult to demonstrate the response in ex vivo systems. We have hydrodynamically transfected mice with plasmid constructs comprised of a murine hepcidin 1 promoter and fragments of the promoter fused to a firefly luciferase reporter. This method enabled us to quantitate the response of the hepcidin promoter to short term feeding of a high iron diet to mice that have been maintained on an iron-deficient diet. We show that the region of the promoter between 1,600 bp and 1,800 bp upstream from the start of translation is essential for the response to iron. The promoter region between -260 and -1600 bp is not essential for the iron responsiveness of hepcidin promoter. The iron-responsive region that we have mapped is the same region required for the in vitro response of HepG2 cells to stimulation with bone morphogenetic proteins and differs from the LPS/IL-6 responsive area.
The regulation of hepcidin expression is of major interest because the mature 25 amino acid hepcidin peptide is a central regulator of iron homeostasis. Dysregulation of hepcidin transcription is the cause of most forms of hemochromatosis and may play an important role in the anemia of chronic inflammation\(^1\). Hepcidin transcription is upregulated by iron, by the cytokines IL6, IL1\(\alpha\), IL1\(\beta\), and by the bone morphogenetic proteins (BMPs). It is downregulated by anemia and by hypoxia. Until now, the basis of hepcidin gene expression in response to iron has been elusive because a robust response is only observed in intact animals. Recent studies by Lin et al. show that there is a limited response even in primary hepatocytes in vitro\(^2\), provided that the iron-transferrin stimulus is applied within 2 hours of harvesting the cells. This response is much less than that observed in vivo and, in any case, is not suitable for promoter mapping.

We have now devised a means of studying the in vivo response of the hepcidin promoter to iron. Details of the methodology have been published previously\(^3\). In brief, we hydrodynamically transfected mice with constructs containing fragments of the hepcidin promoter fused to the firefly luciferase (\(luc\)) reporter. To elicit a response to iron, mice were maintained for at least two weeks on an iron poor diet containing only 2-5 ppm of iron, as suggested by Rivera et al \(^4\) and then fed a diet containing 2x10\(^4\) ppm iron for 24 hours. Luciferase expression driven by the different hepcidin promoter regions was measured in the intact animals using an IVIS Live image instrument (Xenogen, Hopkinton, MA).

The results of our studies are shown in the figure. It is apparent that there is a region of the promoter between 1,600 and 1,800 kb upstream from the start of translation that is required for the response to iron. This region, together with the first 260 bp of the promoter is sufficient to
provide a near-maximal response to iron stimulation. Interestingly, this is the same region required for the *in vitro* response of HepG2 cells to stimulation with BMP-4 or BMP-9 \(^5\).

Previous investigations of the cytokine responsiveness of hepcidin promoter constructs have focused on regions <1,000 bp upstream from the start of translation. These studies have demonstrated that IL6 stimulation is mediated by STAT3 activation and subsequent hepcidin promoter binding by STAT3 in the proximal 150 bp region \(^6,7\). There are many potential transcription factor binding sites in the iron regulatory region, and the technology that we have developed to map the *in vivo* iron response area is much more cumbersome than that using cells transfected with reporter constructs. However, the present studies show clearly that the *in vivo* response to short term iron loading resides in an area far distant from inflammatory cytokine response elements, and helps to focus our attention on a part of the hepcidin promoter (>1,600 bp) which has, up to now, not been explored. The region between -260 and -1,600 bp from the start of translation is not needed for the iron response.

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Authorship Contribution Statement

Dr. Truksa aided in design of the experiment(s) and performed the studies with the assistance of Ms. Peng. Dr. Lee aided Dr. Truksa in the design of studies. Dr. Flanagan carried out some of the experiments. Dr. Beutler conceived the experimental approach and provided overall supervision.

The authors declare no competing financial interests.
Figure Legend

Figure 1. Location of the iron responsive element in vivo: Mice receiving an iron-deficient diet (2-5 ppm) were hydrodynamically transfected with a pGL3 reporter plasmid containing the firefly luciferase gene (luc) under the control of various lengths of the murine hepcidin 1 (Hamp1) promoter. After three days, the basal level of bioluminescence was determined and mice were divided into two groups; one received a high iron diet (2x10^4 ppm), the second group remained on the iron-deficient diet (2-5 ppm). After 24 hours, the mice were anesthetized and re-injected with luciferin and the bioluminescence was re-measured (Day 4). The Day 4 bioluminescence is expressed as fold change over baseline Day 3 bioluminescence. Because the reporter is delivered by hydrodynamic transfecion that results in transient expression, the actual levels of expressed reporter decreased with time. As a result, without stimulation the Day 4 bioluminescence is about 1/3 of the Day 3 bioluminescence. Thus the fold change Day 4 bioluminescence over Day 3 baseline bioluminescence in mice on an iron-deficient diet is less than 1.

The number of base pairs (bp) upstream of the start of translation is given for each promoter construct. The construct designated 260bp+(1,600 to 1,800 bp) contains the first 260 bp and the portion of the promoter between 1,600 and 1,800 bb after the start of translation. The number of animals in each group on iron-deficient and high iron diet was equivalent and is shown in the brackets and the error bars represent 1 standard error of the mean.
Reference List


Figure 1
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