

## *Charting the transcriptional regulatory changes in mouse liver during fasting*

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## **Summary**

In a recent paper: **Goldstein I, Baek S, Presman DM, Paakinaho V, Swinstead EE, Hager GL. Transcription factor assisted loading and enhancer dynamics dictate the hepatic fasting response. *Genome Res.* 2017 Mar;27(3):427–39.**, the authors used a number of functional genomics approaches to explore the transcriptional regulatory dynamics that occur during hepatic fasting. They used chromatin landscape data to identify key fasting-related transcription factors, four of which were further investigated because they are known players of the fasting response: CEBPB (CCAAT enhancer binding-beta), CREB1 (cAMP responsive element binding protein I), GR (glucocorticoid receptor), and PPARA (peroxisome proliferator activated receptor alpha). The authors described two operating modules (GR-CREB1 and PPARA-GR) with synergistic effects driving gluconeogenesis through an assisted loading model and fatty acid oxidation/ketogenesis through a transcription factor cascade, respectively. Finally, using single-cell tracking, they confirmed that GR facilitates CREB1 binding to DNA.

## **General Background**

Upon food deprivation, the liver maintains metabolic homeostasis by first upregulating both glycogen mobilization and gluconeogenesis. During protracted fasting, glucose production shifts to fatty acid oxidation and ketogenesis. These metabolic responses are driven by multiple transcription factors that regulate the enzymes that catalyse key steps in the production of glucose. Regulators of the gluconeogenesis pathway include FOXO1 (Forkhead Box Protein 1), CEBPB, and GR, although to what extent CREB1 contributes to regulation of the fasting metabolic response remains controversial (1,2). In contrast, PPARA is a master regulator of fatty acid oxidation (1).

The regulatory dynamics of the hepatic response to fasting remain poorly understood. Where do transcription factors bind (enhancers and promoters)? How do they bind (regulation hierarchy and logic)? What are the genes they regulate?

Genome-wide technologies such as chromatin immunoprecipitation (ChIP-chip, ChIP-seq or ChIP-exo) and chromatin accessibility (Dnase-seq or ATAC-seq) can now quantitatively identify chromatin structure and transcription factor occupancy, and have been recently used to explore cistromes of fasting-related transcription factors in mouse liver. GR binding at GRE (Glucocorticoid receptor element) is associated with binding sites of other transcription factors like CEBPB in response to fasting (3,4). Interestingly, CEBPB was required for GR access and binding to DNA, leading to the model of assisted transcription factors loading (4). In contrast, CREB1 binding to DNA was associated with motifs for CEBPB, GR and PPARA, but CREB1 showed no occupancy changes upon fasting (5). It has been known for several decades that cooperation of hormone-related transcription factor plays a role in the metabolic response to fasting (6). For instance, CREB1 and GR binding sites are often closely located on the same element and it has been shown that co-binding of the two factors is facilitated by Foxa2 (7).

Despite an ever-increasing understanding of where in the genome fasting-related transcription factors bind, the mechanism of how these transcription factors interplay dynamically to control functional metabolic responses has been poorly understood.

### ***Who is faster during fasting?***

50 In a recent publication in Genome Research, Goldstrein et al. deciphered how fasting shapes chromatin and transcription factor binding reorganization in mouse liver. They showed that DNase I hypersensitivity sites with increased chromatin accessibility also display enriched H3K27ac (a histone mark associated with enhancer activity) upon fasting. These DNase I hypersensitivity sites seem to be enriched near activated genes during fasting, even  
55 if it is not clear if they are located in promoters or enhancers. They then inferred the footprint depth of putative transcription factor binding location, the flanking accessibility, and motif enrichment. Using this unbiased data, they identified the four main transcription factors known to be involved synergistically in the transcriptional response to fasting: GR, CREB1, CEBPB and PPARA.

60 The authors discovered two modules that operate sequentially after metabolic stimulation, initiated either by hormonal treatment of plated mouse primary hepatocytes or by food deprivation in mouse liver. CREB1 and GR represent the first module acting early during fasting by activating gluconeogenesis. During prolonged fasting, the GR-PPARA module eventually supplants CREB1-GR by stimulating fatty acid oxidation/ketogenesis gene  
65 expression, as has been seen previously in erythroid progenitor cells (8). The model proposed by the authors is that GR and PPARA may cooperate through a “transcription factor cascade” where GR progressively induces PPARA activity later during fasting, although definitive proof of this hypothesis will demand deeper mechanistic analysis.

70 The authors then dissected the molecular mechanisms involved in the synergistic cooperation between CREB1 and GR. First, the binding sites for both CREB1 and GR were mapped after both transcription factors were activated by simultaneous treatment of hepatocytes with glucagon (CREB1 activation) and corticosterone (GR activation). By comparing with single-activation conditions, the increased occupancy of a subset of CREB1-bound sites was found to be directly dependent on GR upregulation.

75 Using data from their previous study (4) they showed that corticosterone-increased CREB1 sites, as well as fasting-induced enhancers, are in an open conformation after GR activation in mouse liver. Altogether, these observations suggest an “assisted loading” model for transcription factors where GR helps in the opening of fasting-induced enhancers to promote CREB1 binding. The authors have previously shown such a model with C/EBPB  
80 increasing the chromatin accessibility at numerous GR binding sites in mouse liver (4). The last section of the article describes validating single-cell experiments that explored how CREB1 binds DNA with or without GR activation.

85 This study deciphered the global chromatin changes and the relationship between the key transcription factors involved in fasting in mouse liver, but the genomic and computational approaches have important limitations. For instance, DNase-seq footprinting works unevenly for different transcription factors. Validating DNase-seq results using similar techniques such as ATAC-seq would be useful. Further, *in silico* identification of transcription factor binding sites is always imperfect, since even a perfect transcription factor  
90 matrix does not directly show transcription factor binding much less functional binding. By combining the chromatin accessibility and H3K27ac occupancy, the authors generated a vast catalogue of *cis*-regulatory elements that are assigned as enhancers. It is not clear how they differentiate enhancers and promoters, both of which carry H3K27ac and can correspond to DNase I hypersensitivity sites.

95 Interestingly, the results of Goldstein et al contradict a prior study on how fasting  
impacts CREB1 occupancy, where the authors found an enrichment of CREB1 binding at  
fasted-induced enhancers during fasting versus feeding in the liver, a result discordant with a  
previous study (5). Although the experimental stimulation of CREB1 by glucagon and  
corticosterone in cell lines may not perfectly reflect the binding enrichment of CREB1 when  
100 found *in vivo*, Goldstein et al have confirmed key features of their *in vitro* results in liver.

Finally, the authors nicely showed that two transcription factor modules are governing  
two phases of the metabolic response to food deprivation. While transcription factor cascades  
seem to be widely documented models in hormonal responses, to our knowledge an assisted  
loading model has not been reported outside the liver field. This last model is reminiscent of  
105 pioneer transcription factors that increase chromatin accessibility for other transcription  
factors by binding to nucleosomes transiently or along with other non-pioneer transcription  
factors.

In sum, this study shows how an integrated analysis using the tools of functional  
genomics, when cleverly applied, can dissect how regulatory networks orchestrate metabolic  
110 responses to fasting.

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### Figure legend:

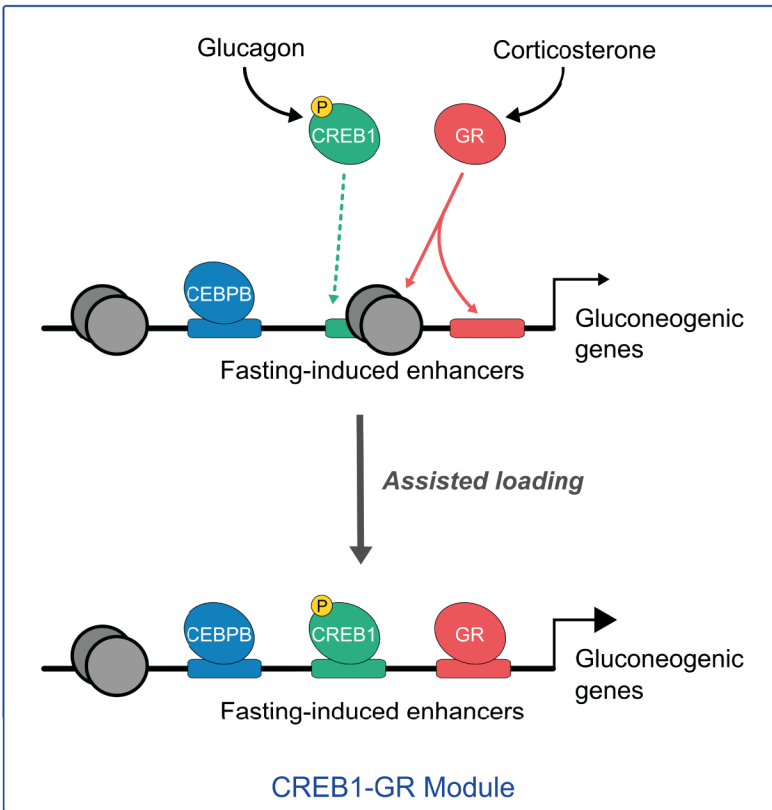
#### 140 **Two regulatory modules operate during fasting in the liver.**

The CREB1-GR and the GR-PPARA modules transcriptionally regulate the genes involved in  
gluconeogenesis and fatty acid oxidation/ketogenesis in the liver, respectively. **Left panel:**  
First, glucagon activates CREB1 by phosphorylation, and the binding of CREB1 to target

145 gene enhancers drives limited gluconeogenic gene expression; CEBPB constitutively binds  
gluconeogenic enhancers. Second, further secretion of corticosterone activates GR, which  
then facilitates the binding of CREB1 by assisted loading. The subsequent co-binding of  
CREB1 and GR leads to synergistically enhanced gluconeogenic gene expression. **Right**  
**panel:** Glucagon-activated GR can bind enhancers of the PPARA gene and drives its  
150 expression. Then, PPARA regulates downstream fatty acid oxidation/ketogenic gene  
expression by binding at fasting-induced enhancers. This successive activation of genes  
during late fasting follows a transcription factor cascade model.

CEBPB: CCAAT enhancer binding-beta; CREB1: cAMP responsive element binding protein  
I; GR: glucocorticoid receptor; PPARA: peroxisome proliferator activated receptor alpha;  
FAO: Fatty Acid Oxidation.

## GLUCONEOGENESIS



## FATTY ACID OXIDATION / KETOGENESIS

