

A FATAI AP-1 dimer switch in hepatosteatosis

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Activator protein 1 (AP-1) is a dimeric transcription factor with important roles in specifying genetic and cellular programs. AP-1 refers to a collection of DNA-binding homo- and heterodimers formed by members of the JUN (c-Jun, JunB, and JunD) and FOS (c-Fos, FosB, Fra-1, and Fra-2) families, as well as ATF and MAF proteins. AP-1 dimers recognize similar and specific sequence elements in the promoters and enhancers of target genes. The pool of AP-1 dimers is determined by the protein levels of its individual components and through upstream kinases. Various combinations of AP-1 dimers co-exist, and together with dimer-specific variation in target DNA sequence affinity determine the genes that are positively or negatively regulated by AP-1 in a given cell type and biological context.

The liver is an essential metabolic organ, and understanding AP-1 function in the liver has been the focus of attention for many years. Gene inactivation and replacement experiments demonstrated an essential role of c-Jun in embryonic liver development and functional substitution of c-Jun by JunB or JunD.^{1,2} Furthermore, individual *Jun* and *Fos* genes appear dispensable for adult liver homeostasis, while having important and specific roles in the response of the liver to various stress conditions^{3–5} and in liver cancer.⁶ Importantly, the role of specific AP-1 dimers in adult liver function remained elusive.

The functions of AP-1 were investigated in lipid metabolism of the liver, a central organ in the pathogenesis of obesity-associated metabolic diseases.⁷ Deregulation of hepatic lipid metabolism can result in lipid accumulation within hepatocytes, a condition called “fatty

liver disease (FLD)” or hepatosteatosis. FLD is the most common liver disorder in industrialized countries and an important health concern. FLD often progresses to life-threatening conditions, such as liver inflammation (steatohepatitis), cirrhosis, and cancer. In the BXD population of inbred mouse strains, hepatic *fra-1* was found reduced under high-fat diet (HFD), indicating that AP-1 might be a modulator of metabolic liver diseases. We next explored the role of AP-1 proteins in hepatic lipid metabolism using a non-exhaustive collection of AP-1 mouse mutants. These included broad and liver-specific loss-of-function mutants of AP-1 as well as recently generated Doxycycline (Dox)-switchable liver-specific gain-of-function AP-1 monomer and dimer mutant mice. Strikingly, hepatocyte-restricted Fra-1 expression in Fra-1^{hep} mice prevented and even reverted HFD-induced FLD by suppressing the transcription of the nuclear receptor PPAR γ , a central regulator of lipid metabolism. Similarly, Fra-2^{hep} mice, expressing the closely related Fra-2 protein, also exhibited reduced PPAR γ expression and signaling, leading to decreased expression of genes involved in hepatic fatty acid uptake and lipid droplet formation and reduced HFD-induced FLD. In contrast, ectopic c-Fos expression in murine livers increased the expression of PPAR γ and PPAR γ target genes. Consistently, chromatin immunoprecipitation experiments using mouse livers and human liver cell lines indicated that all Jun and Fos proteins can bind the proximal *Pparg2* promoter. Notably, c-Fos and Fra-1/2 exerted antagonistic effects on transcription from a *Pparg2* promoter reporter in human cell lines.

An important implication of the combinatorial character of AP-1 is that functional redundancy between dimers could hinder the identification of AP-1 transcriptional targets in loss-of-function mouse models. Consistently, with the exception of *Jund*, genetic inactivation of individual *Jun* and *Fos* genes did not affect hepatic PPAR γ expression and HFD-induced FLD. Importantly, changes in the levels of one AP-1 component can lead to qualitative and quantitative changes in other AP-1 dimers, which can affect gene expression, leading to phenotypic alterations that might not be directly related to the manipulated AP-1 monomer. To clarify how Fos proteins, which act as obligate heterodimers with Jun proteins, can antagonistically modulate *Pparg2* expression, specific Fos-containing AP-1 dimers were expressed in Dox-switchable AP-1^{hep} mice. AP-1 monomers are joined by a flexible polypeptide tether to force specific pairing and insulate the forced dimer from other potential dimerizing partners. This “single-chain approach”,⁸ which is used for the first time in vivo, demonstrated that any Jun-c-Fos dimer, such as c-Jun-c-Fos, JunB-c-Fos, and JunD-c-Fos activated, whereas c-Jun-Fra-2 dimers inhibited PPAR γ expression and signaling in the liver (Fig. 1).

These data firmly establish AP-1 as a physiologically relevant regulator of PPAR γ signaling and FLD and open potential therapeutic avenues for metabolic liver disease. We discovered an antagonism between specific AP-1 dimers on the same target gene, leading to opposing functional consequences and a striking, health-relevant metabolic phenotype in vivo. Previous studies in other organs,

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Submitted: 02/28/2014; Accepted: 03/04/2014; Published Online: 03/12/2014

<http://dx.doi.org/10.4161/cc.28514>

Comment on: Hasenfuss SC, et al. Cell Metab 2014; 19:84–95; PMID:24411941; <http://dx.doi.org/10.1016/j.cmet.2013.11.018>

such as bone, suggested overlapping functions of c-Fos and Fra-1/2 proteins, but, to date, *Pparg* is the first gene shown to be antagonistically regulated by different Fos proteins. In addition, we identify a dimer-partner-dependent antagonistic effect of c-Jun on the *Pparg2* promoter, as c-Jun-c-Fos dimers increased, whereas c-Jun-Fra-2 dimers reduced PPAR γ 2 expression. Understanding how such similar protein complexes have opposite effects on the same target promoter and how signaling cues translate into the assembly of different AP-1 complexes is the next challenge. Such studies need to be extended to human FLD and associated pathologies. The recent revolutionary development of techniques to study gene expression and transcription factor binding on a genome-wide level will certainly help to solve the “mystery” of dimer-specific AP-1 functions in the liver and beyond.

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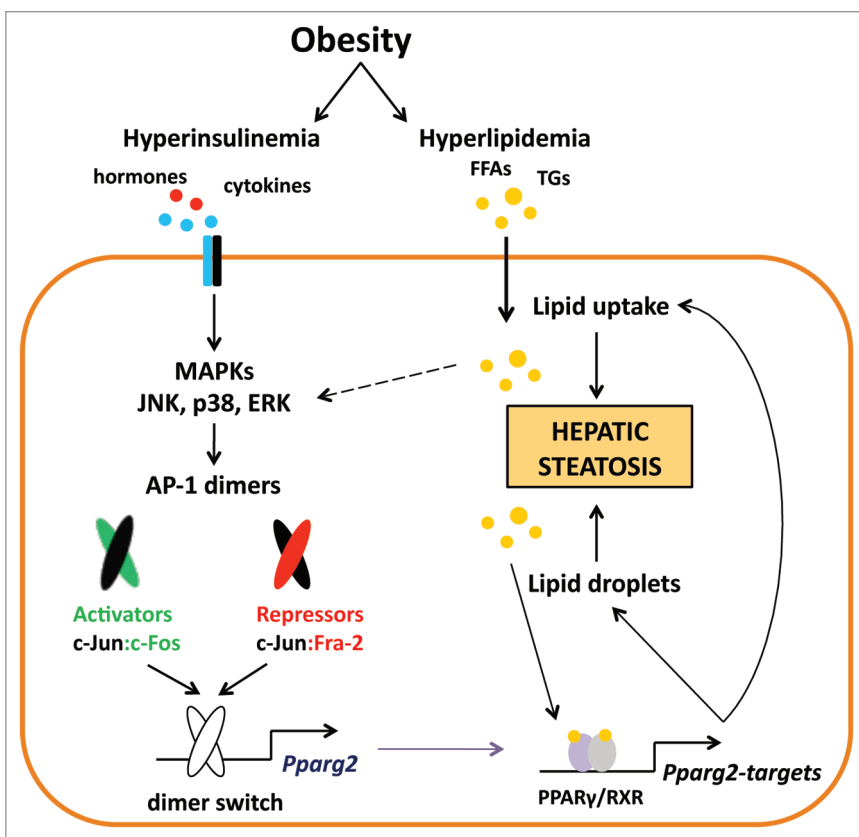


Figure 1. In diet-induced obesity, systemic changes in hormones, cytokines, and lipids activate multiple signaling pathways, thereby altering AP-1 composition in hepatocytes and favoring *Pparg2*-activating dimers. Increased PPAR γ and PPAR γ signaling increases expression of PPAR γ targets involved in lipid uptake and lipid droplet formation, resulting in lipid accumulation within hepatocytes and in FLD. FFA, free fatty acids; TG, triglycerides; RXR, retinoid X receptor.