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DNA-PK: Relaying the Insulin Signal to USF in Lipogenesis

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To meet the constant energy requirement in the face of highly variable food supply, mammals employ intricate and precise mechanisms for energy storage. When total energy intake is in excess of energy expenditure such as after a meal, excess carbohydrates are converted to fatty acids (de novo lipogenesis). Excess fatty acids are then converted to triacylglycerol to be stored in adipose tissue and released as oxidative fuels for other tissues during times of energy need such as fasting and exercise. In sustaining the balance between energy excess and energy deficiency, the process of lipogenesis is tightly controlled by nutritional and hormonal conditions¹. Thus, enzymes involved in fatty acid and fat synthesis are tightly and coordinately regulated during fasting/feeding: Activities of these enzymes are very low in fasting due to the increase in glucagon/cAMP levels. Conversely, in the fed condition, especially after a high carbohydrate meal, activities of these enzymes drastically increase as blood glucose and insulin levels rise¹. Fatty acid synthase (FAS), a central lipogenic enzyme, plays a crucial role in de novo lipogenesis by catalyzing all of the seven reactions involved in fatty acid synthesis. While FAS is not known to be regulated by allosteric effectors or by covalent modification, its transcription is exquisitely regulated by fasting/feeding and by insulin¹. The FAS promoter thus provides an excellent model system to dissect the transcriptional activation of lipogenesis by feeding/insulin.

In early studies of insulin regulation of the FAS promoter, we found that Upstream Stimulatory Factor (USF) binding to the -65 E-box is required for transcriptional activation by insulin^{2,3,4}. The critical role of USF in the activation of the FAS promoter by insulin was further verified by overexpressing dominant negative or wild type forms of USF^{2,3}. The induction of FAS by fasting/feeding was significantly impaired in USF knockout mice⁵. Functional analysis and chromatin immunoprecipitation (ChIP) in mice transgenic for various 5' deletions and mutations of the FAS promoter-CAT reporter gene showed that USF binding to the -65 E-box is required for feeding/insulin-mediated FAS promoter activation in vivo⁶. Notably, USF binding was detected in both fasted and fed states. On the FAS promoter, USF recruits another transcription factor SREBP-1, whose level increases upon insulin treatment via the PI3K pathway⁷, to bind the -150 SRE and mediate insulin/feeding responsiveness⁸. Although early studies of ectopically expressed SREBP-1 in cultured cells has been shown to bind the -65 E-box⁹, the functional analysis and chromatin immunoprecipitation (ChIP) in mice transgenic for various 5' deletions and mutations of the FAS promoter-CAT reporter gene clearly showed SREBP-1 binds the -150 SRE, but not -65 E-box to activate the FAS promoter during feeding/insulin treatment in vivo⁶. Although SREBP-1c binding to the -150 SRE is critical for the feeding/insulin response, SREBP-1c itself cannot bind the SRE without being recruited by USF, which is constitutively bound to the -65 E-box^{4,6,10}. Many lipogenic promoters contain a closely spaced E-box and SRE in the proximal promoter region, and a similar mechanism for activation of several lipogenic genes has been documented previously⁴. Possibly, the SREBP-1c promoter is also regulated by USF and SREBP-1c in response to feeding/insulin.

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Thus, USF, along with SREBP-1c, plays a critical role in mediating the transcriptional activation of lipogenesis in response to feeding/insulin.

Studies have shown that LXR may play a role in the transcriptional regulation of lipogenesis by activating SREBP-1c transcription¹¹. LXR has also been reported to directly regulate the FAS promoter in cultured cells¹². A carbohydrate response element (ChoRE) where ChREBP can bind has also been reported to be present far upstream of the FAS promoter region¹³. Nevertheless, FAS promoter-reporter transgenic mice studies showed that the FAS promoter that contains both an E-box and SRE, but lacks a LXRE or ChoRE, is sufficient for high level activation of the FAS promoter during fasting/feeding suggesting that binding of LXR or ChREBP may not be critical in vivo⁶. Regardless, questions remain in understanding the FAS promoter activation involving USF and SREBP. Apart from HAT/HDAC, are other coactivators required for activation? What chromatin remodeling machinery and mediators are recruited to the FAS promoter? Are there common mechanisms to explain the transcriptional regulation of other coordinately regulated lipogenic genes? Is chromatin folding involved in sharing transcription machineries among lipogenic gene promoters? Further studies are necessary to understand the details of the transcriptional activation of lipogenic genes.

While many metabolic effects of insulin are mediated through protein phosphorylation via the well characterized PI3K cascade which activates PKB/Akt, insulin can also exert metabolic effects through dephosphorylation catalyzed mainly by PP1¹⁴. Regardless, USF is bound to the E-box on the FAS promoter in both fasted and fed states and neither USF expression nor post-translational modification have been shown to be altered by insulin. Although it is suggested that USF mediates the insulin response of lipogenic gene promoters, the precise mechanism of how USF responds to insulin is not fully understood. We have recently demonstrated that feeding/insulin activates USF through DNA-PK, a kinase involved in DNA damage repair, and subsequently activates FAS transcription¹⁰. This insulin signaling pathway involving DNA-PK and USF is first initiated by PP1. Although the molecular mechanism is not well understood, the stimulation of PP1 by insulin has been well documented. For example, insulin inhibits breakdown and promotes synthesis of glycogen primarily by activating PP1. PP1 is known to be compartmentalized in cells by discrete targeting subunits¹⁵. The role of PP1 in transcriptional activation of FAS is to dephosphorylate/activate DNA-PK upon feeding or insulin treatment. USF-1 is then phosphorylated by DNA-PK, allowing recruitment of and acetylation by P/CAF, leading to promoter activation. We also demonstrated a requisite role of DNA-PK by employing DNA-PK deficient SCID mice; USF-1 phosphorylation and acetylation is attenuated, blunting transcriptional activation of FAS and de novo lipogenesis in fasting/feeding¹⁰. Thus we showed DNA-PK is a player in USF regulated transcriptional activation of the FAS gene.

USF regulated genes coding for other lipogenic and glycolytic enzymes, such as mitochondrial glycerol-3-phosphate acyltransferase, acetyl-CoA carboxylase and glucokinase, might be possible targets of DNA-PK mediated insulin signaling. Furthermore, in addition to USF, various transcription factors have been reported to regulate a battery of metabolic enzymes (those involved in glycolysis, gluconeogenesis and glycogen and triacylglycerol metabolism) that is regulated during fasting/feeding. What transcription factors aside from USF, if any, are phosphorylated by DNA-PK in response to feeding/insulin? In addition to phosphorylating transcription factor(s), DNA-PK might also play a role in regulating enzymes that are under control of feeding/insulin. In this regard, as an insulin signaling molecule, could DNA-PK potentially phosphorylate proteins including kinases that are activated by insulin? Last but not least, with DNA-PK's role as an insulin signaling molecule in activating lipogenesis, can DNA-PK serve as a pharmacological target for obesity and diabetes treatment? Identification of DNA-PK as a signaling molecule in activating lipogenic genes by insulin has brought us a step closer to understanding how cells respond to insulin.

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