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Visions & Reflections

Metabolic stress regulates basic transcription through acetyl-coenzyme A

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Abstract. The acetylation and auto-acetylation of general transcription factors has recently been demonstrated, but the functional significance of these modifications is unclear. The presence of acetyl-coenzyme A activates basal transcription, and acetylation of transcription factor IIB (TFIIB) has been shown to activate transcription in several contexts. If auto-acetylation is an important pathway in eukaryotes, the regulatory pathways for acetyl-coenzyme A should be important in transcription regulation. Fasting represents an acute metabolic stress which should elevate levels of acetyl-coenzyme A, while mitochondrial aging represents a cumulative stress. We show that tissue-specific levels of acetylated TFIIB change dramatically in response to fasting in mice, suggesting a role for metabolism in the direct regulation of transcription. We also observed a large increase in acetyl-TFIIB in tissues from aged mice relative to younger mice. Sir2 family deacetylases, which regulate acetyl-coenzyme A synthesis, have recently been shown to impart longevity in a variety of organisms through a pathway related to calorie restriction. We hypothesize that protein acetylation and Sir2-related deacetylation may be tied to the metabolic regulation of transcription through the availability and action of acetyl-coenzyme A on key transcription factors and transcriptional regulators.

Key words. Auto-acetylation; acetyl-coenzyme A; transcriptional regulation; transcription factor IIB; metabolic stress; fasting; aging.

Recently, we demonstrated that the transcription factor IIB (TFIIB) auto-acetylates, a novel post-translational modification to a ubiquitous and highly conserved general transcription factor (GTF) [1]. We isolated acetylated TFIIB from actively proliferating HeLa cells. Thus, auto-acetylation may play a physiologically relevant role, although its effect on specific eukaryotic promoters remains to be determined [2]. In addition, we found that the small subunit of the transcription factor IIF (TFIIF) also becomes auto-acetylated in vitro [2], suggesting that auto-acetylation may be a more general phenomenon. Certainly the acetylation of transcription factors by factor acetyltransferases (FATs) is nothing new [3], but the auto-catalytic activity of these GTFs is novel. The addition of acetyl-coenzyme A activates transcription in the absence of histones by increasing the affinity of transcription factor IID (TFIID) for promoter DNA [4], thus demonstrating that acetylation may play an important role in regulating transcription in addition to its influence on chromatin remodeling. The intrinsic enzyme-like activity of these key elements in transcription initiation may point to an important role for acetylation in the regulation of gene expression. Yet, precisely how acetylation modulates transcriptional function has not been studied in detail.

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Differential acetylation of TFIIB in response to metabolic stress in mice

Perhaps the first question is whether auto-acetylation is a viable pathway *in vivo*, or is simply a test-tube phenomenon. If the auto-acetylation pathway is indeed active in eukaryotes, we would expect that conditions which drastically change the availability of acetyl-coenzyme A would also change the level of acetylation of TFIIB.

Acetyl-coenzyme A is a key metabolite which is compartmentalized in cells. Its cytoplasmic and mitochondrial levels are regulated primarily by acetyl-coenzyme A synthetase (Acs or AceCS), acetyl-coenzyme A carboxylase (ACC) and the pyruvate dehydrogenase complex (PDH) in prokaryotes, archaea and eukaryotes [5–7]. Fatty acid synthesis and oxidation also impact the availability of acetyl-coenzyme A. The two localized isoforms of Acs produce acetyl-coenzyme A from acetate, coenzyme A and ATP. Previous work on the cytosolic Acs1 isoform suggested that the highest levels of expression in mice are in kidneys, ovaries, testes and liver, while the mitochondrial isoform, Acs2, is found mainly in heart and kidney, while being absent from the liver [8, 9]. Levels of acetyl-coenzyme A may increase in kidneys during fasting due to increased Acs1 activity. During starvation, increased synthesis of acetyl-coenzyme A by Acs1 in the kidneys allosterically activates pyruvate carboxylase, which stimulates gluconeogenesis. It is also likely that fasting causes an increase in acetyl-coenzyme A in the liver. It has been shown that low glucose availability lowers ACC-β expression [10, 11], decreasing levels of malonyl-coenzyme A and thus allowing fatty acids to enter the mitochondria for oxidation into ketone bodies. With increased fatty acid oxidation, the availability of acetyl-coenzyme A is also increased, and may account for the increased TFIIB acetylation observed in liver. The high mitochondrial count of the liver also likely contributes to this acute response to hunger.

Since acetyl-coenzyme A is a key product of fatty acid metabolism, we examined the effect of fasting on TFIIB acetylation in female mice (fig. 1). Our experiments show similar baseline levels of acetylated TFIIB in kidney, liver and ovary tissue extract, based on total protein loaded. We observed a tissue-specific increase of acetylated TFIIB with fasting in kidney and liver tissue, but we did not see any significant change in TFIIB acetylation in ovarian tissue. The increased acetyl-TFIIB observed in kidney and liver tissue correlate with the expected increases in acetyl-coenzyme A levels discussed above. The lack of change with regard to ovarian tissue suggests the relative independence of the reproductive system from short-term metabolic changes. These findings are significant because they illustrate that levels of acetyl-TFIIB change significantly in response to the metabolic stresses associated with fasting, including reduced glucose and insulin levels.

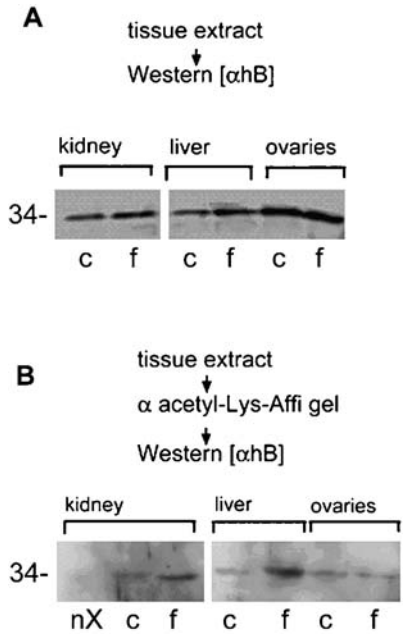

Figure 1. TFIIB acetylation modulated by fasting. C57BL/6J female mice were fasted for 24 h to observe differences in TFIIB acetylation. Mice were sacrificed, and tissue was removed and ground up. Proteins were extracted in buffer and separated using SDS gel electrophoresis. All experiments were done in triplicate and gave reproducible results. (*A*) Western blot with anti-TFIIB antibody demonstrating equal loading of protein samples in control (c) and fasted (f) mice. The protein size is shown to the left of the panel in kDa. (*B*) Tissue extract first selected for acetylated lysine residues, then blotted with anti-TFIIB antibody to show differential acetylation with fasting. Lanes represent no extract (nX), control (c) and fasted (f) mice.

We have previously shown that acetylation of TFIIB can dramatically affect transcription initiation on viral promoters [1]. The changes in acetylation status of TFIIB and other transcriptional factors and activators in response to metabolic stresses may activate differential transcriptional profiles. Not only so, but the higher general availability of acetyl-coenzyme A may result in the acetylation of many other proteins as well. Thus direct acetylation or auto-acetylation of transcription factors or transcriptional regulators in response to metabolic stresses may represent novel pathways for transcriptional regulation.

Differential acetylation of TFIIB with aging in mice

The metabolism of eukaryotes is known to change with aging, and even the number of mitochondria in cardiac myocytes increases with age [12]. The stresses of aging on metabolism are known to include glucose intolerance and hyperinsulinemia [13]. Aging has been linked to the generation of reactive oxygen species in the mitochondria and mutations of mitochondrial DNA [12, 14, 15]. These

changes result in mitochondrial loss of function and the subsequent accumulation of acetyl-coenzyme A, which could impact acetylation-mediated transcriptional regulation.

Acetylation has been recently shown to be closely tied to lifespan in a wide variety of organisms through the action of the Sir2 family of deacetylases [16]. This longevity effect has generally been ascribed to gene silencing through the deacetylation of histones and the preservation of chromosome telomeres. However, yeast from which all five Sir2 homologs have been deleted still maintain relatively normal bulk histone acetylation levels, perhaps indicating that there are also other mechanisms at work [17]. The mechanism of Sir2 in lifespan extension has been shown to be related to increased cellular respiration [18] and calorie restriction [19, 20], the very processes which are activated by and activate acetyl-coenzyme A generation. Indeed, Sir2 has been shown to activate Acs in bacteria and yeast by deacetylating a specific lysine residue, resulting in increased synthesis of acetyl-coenzyme A [21, 22]. This regulatory modification is highly conserved in Acs and its homologs. It is possible that increased production of acetyl-coenzyme A might activate cellular respiration and regulate the transcription of genes which may play a significant role in Sir2 lifespan extension.

Deacetylation of transcriptional proteins may also play a role in the metabolic regulation of transcription. Sir2 proteins have been shown to deacetylate histones, but that may not be their only or even primary function. Mammalian SIRT1, a Sir2 family member found in the nucleus, has been shown to regulate the deacetylation of p53 and FOXO transcription factors [23–25]. As with many histone acetyltransferases, it is quite possible that Sir2 deacetylases might act on other acetylated proteins, and not just histones.

If aging results in increased acetyl-coenzyme A levels in cells, TFIIB acetylation could be significantly elevated, along with other transcription factors. Altered transcriptional profiles may contribute to some of the effects of old age, such as reduced ability to regenerate tissue, impaired immunological response and even reproductive failure. With this in mind, we examined the acetylation of TFIIB as a function of aging in female mice (fig. 2).

We observed a striking increase in levels of acetylated TFIIB in ovaries and kidneys from older mice. It is notable that acetyl-TFIIB is especially abundant in the aged ovary in 8-month-old mice, which can no longer reliably reproduce. This acetylated protein may be due to either auto-acetylation or FAT activity. TFIIB does not share any homology with known acetyltransferases, and it can not be acetylated by classical histone acetyltransferases (HATs) such as CBP/p300 *in vitro* [26]. This suggests that acetyl-TFIIB is formed by auto-acetylation, or else by an unknown acetyltransferase. It is highly unlikely

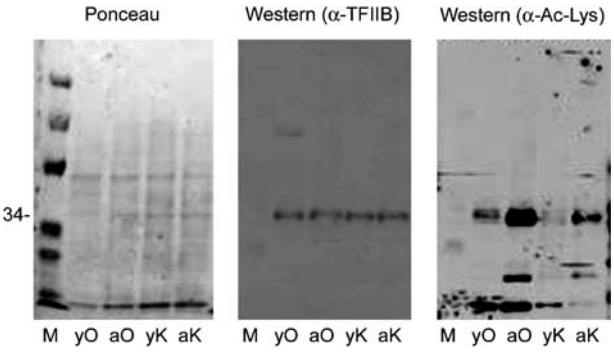

Figure 2. TFIIB acetylation changes with aging. 5 weeks young (y) and 8 months aged (a) C57BL/6J female mice were sacrificed, and ovarian (O) or kidney (K) tissue removed and processed as described previously. SDS gel electrophoresis was used to separate the proteins, which were subsequently transferred to a nitrocellulose membrane. Shown are a Ponceau stain (left), a Western blot using anti-TFIIB antibody (center) and a Western blot using anti-acetyllysine antibody (right). The position of the TFIIB protein is indicated to the left of the panel in kilodaltons. All experiments were done in triplicate and gave reproducible results.

that acetyl-coenzyme A levels increased enough to cause this change by auto-acetylation, because of the magnitude of the observed difference. We have shown previously that the auto-acetylation of TFIIB is reversible, but only in a heavy excess of coenzyme A, and even then it is a kinetically slow process [1]. Perhaps acetyl-TFIIB is accumulated over the lifespan of the mice. We speculate that this may indicate the lack of a specific deacetylase which might act upon acetylated TFIIB *in vivo*. This would result in a slow accumulation of acetyl-TFIIB, and could account for the larger percentage of acetylated TFIIB in older mice.

Perhaps there is further regulation of transcription factor acetylation that has not yet been characterized which may tie in to the fasting/calorie restriction paradigm discussed here. Taken together, these developments in acetylation may provide a glimpse of how metabolism may regulate transcription. We propose that the acetylation of TFIIB and other transcriptional proteins is capable of regulating transcription in response to fasting and aging. This would establish a novel role for acetyl-coenzyme A as a metabolic regulator of transcription.

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