Adiponectin, Adiponectin Receptors, and Epigenetic Regulation of Adipogenesis

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We studied the molecular mechanism of obesity-induced insulin resistance and adipogenesis. Plasma adiponectin and adiponectin receptor (AdipoR1) in muscle are down-regulated in obesity. Analysis of muscle-specific AdipoR1 knockout mice revealed the pivotal role of adiponectin/AdipoR1 in the regulation of mitochondrial biogenesis via AMPK- and SIRT1-mediated PGC-1 α activation as well as Ca²⁺-dependent up-regulation of PGC-1 α expression. Reduced adiponectin/AdipoR1 signals in muscle in obesity appear to cause PGC-1 α inactivation as well as down-regulation and consequently impaired mitochondrial biogenesis and insulin resistance. In the epigenetic analysis of adipogenesis, we demonstrated that adipocyte-specific formaldehyde-assisted isolation of regulatory elements (FAIRE) peaks are associated with genes up-regulated by adipogenesis, whereas preadipocyte-specific FAIRE peaks are associated with genes down-regulated by adipogenesis. Computational motif analyses of adipocyte-specific FAIRE peaks confirmed PPAR γ and CCAAT-enhancer binding proteins (C/EBPs) on the top list, consistent with their crucial roles in adipogenic transcription, and also revealed NFIA and NFIB to be important regulators of proper adipocyte differentiation.

Obesity is increasing worldwide and causes various diseases such as diabetes, cardiovascular disease, and some forms of cancer. To determine the molecular causes of these obesity-linked diseases, we have been working on adiponectin, an adipokine that is most abundantly expressed in white adipose tissue and becomes down-regulated in obesity to develop various obesity-related diseases. We have also been working on adipogenesis itself with the hope that the identification of the molecular mechanism of adipogenesis may shed light on the causes of obesity and obesity-related diseases.

ADIPONECTIN AND ADIPONECTIN RECEPTORS

Adiponectin (Scherer et al. 1995; Hu et al. 1996; Maeda et al. 1996; Nakano et al. 1996) is an adipokine that protects against diabetes and atherosclerosis. Plasma adiponectin levels are decreased in obesity, insulin resistance, and type 2 diabetes (Kadowaki et al. 2006). Administration of adiponectin has been shown to elicit glucose-lowering effects and ameliorate insulin resistance (Berg et al. 2001; Yamauchi et al. 2001). Adiponectin knockout mice exhibit insulin resistance and diabetes (Kubota et al. 2002). Adiponectin has also been shown to increase fatty acid oxidation by stimulation of AMP-activated protein kinase (AMPK) (Yamauchi et al. 2002) and also by peroxisome proliferator-activated receptor α (PPAR α) (Yamauchi et al. 2003a).

We previously reported the cloning of complementary DNAs encoding adiponectin receptors (AdipoR) 1 and 2

by expression cloning (Yamauchi et al. 2003b). Liver expresses both AdipoR1 and AdipoR2, whereas skeletal muscle expresses predominantly AdipoR1. Both receptors are predicted to contain seven transmembrane domains (Yamauchi et al. 2003b) but to be structurally and functionally distinct from G-protein-coupled receptors (GPCRs). Adiponectin receptors may thus comprise a new receptor family (Fig. 1).

Using AdipoR1 and/or AdipoR2 knockout mice, we previously showed that AdipoR1 and AdipoR2 act as the major receptors for adiponectin in vivo and have important roles in the regulation of glucose metabolism and insulin sensitivity in vivo (Yamauchi et al. 2007). Importantly, obesity decreases not only plasma adiponectin but also adiponectin receptors in liver and skeletal muscle to cause adiponectin resistance (Kadowaki et al. 2006). Moreover, in the liver, AdipoR1 activates the AMPK pathway to suppress gluconeogenesis and lipogenesis, and AdipoR2 activates the PPAR α pathway to stimulate fatty acid oxidation and suppress inflammation and oxidative stress (Yamauchi et al. 2007). In skeletal muscle, insulin resistance has been reported to be associated with mitochondrial dysfunction (Patti et al. 2003; Petersen et al. 2004). However, the exact cause of mitochondrial dysfunction has yet to be clarified. To clarify whether decreased adiponectin/AdipoR1 signaling could be associated with mitochondrial dysfunction, we analyzed muscle-specific AdipoR1-knockout (muscle-R1KO) mice (Iwabu et al. 2010) and attempted to determine the molecular mechanism whereby adiponectin/AdipoR1 would exert their biological effects.

Figure 1. Cloning of and functional analysis of adiponectin receptors. Adiponectin receptors AdipoR1 and AdipoR2 are predicted to be integral membrane proteins with seven transmembrane domains, whose amino terminus is internal and carboxyl terminus is external opposite to G-protein-coupled receptors (GPCRs) (Yamauchi et al. 2003b, 2007).

Decreased PGC-1 α and Mitochondria in Muscle-R1KO

Muscle-R1KO mice showed decreased phosphorylation of AMPK. Moreover, the administration of adiponectin increased the phosphorylation of AMPK in the muscle of control littermates but not in that of muscle-R1KO mice, whereas adiponectin increased the phosphorylation of AMPK in the liver of both genotypes. Muscle-R1KO mice also exhibited decreased molecules involved in mitochondrial biogenesis, such as PGC-1 α , at both messenger RNA and protein levels. Adiponectin increased the expression levels of PGC-1 α in muscle of control littermates but not in that of muscle-R1KO mice. Muscle-R1KO mice showed decreases in molecules involved in mitochondrial biogenesis such as oestrogen-related receptor α (Esrr α), molecules involved in transcription such as nuclear respiratory factor 1 (Nrf1), and molecules involved in mitochondrial DNA replication/translation such as mitochondrial transcription factor A (Tfam) (Iwabu et al. 2010). Moreover, the expression levels of several oxidative phosphorylation and other mitochondrial genes as well as mitochondrial content were significantly reduced in muscle-R1KO mice. Mitochondrial function was also decreased in muscle-R1KO mice (Iwabu et al. 2010). In soleus muscle of muscle-R1KO mice, type I fibers were reduced by 20%. To study the effect of AdipoR1 ablation on skeletal muscle function, we challenged control and muscle-R1KO mice with involuntary physical exercise and assessed muscle endurance during treadmill running. Muscle endurance was significantly lower for muscle-R1KO mice than control mice (Iwabu et al. 2010). We next examined the expression of metabolic genes and found that molecules involved in fatty acid oxidation were significantly decreased in muscle-R1KO mice, which was associated with decreased β oxidation and increased triglyceride content in skeletal muscle. Muscle-R1KO mice exhibited decreased expression levels of mitochondrial and cytoplasmic oxidative-stress-detoxifying genes such as manganese superoxide dismutase (*Sod2*) and catalase (*Cat*), respectively, and increased oxidative stress such

as thiobarbituric acid reactive substance (TBARS) in muscle (Iwabu et al. 2010).

Insulin Resistance in Muscle-R1KO Mice

Upon glucose administration, plasma glucose and insulin levels were significantly higher in muscle-R1KO mice than in control mice, suggesting that muscle-R1KO mice have insulin resistance (Fig. 2). We performed a hyperinsulinemic–euglycemic clamp experiment. Disruption of AdipoR1 in muscle did not significantly alter endogenous glucose production, whereas it significantly decreased the glucose disposal rate and the glucose infusion rate, indicating decreased insulin sensitivity in muscle (Iwabu et al. 2010).

Adiponectin Induces Ca²⁺ Influx by AdipoR1

To determine molecular mechanisms of muscle phenotypes of AdipoR1 knockout mice, we used C₂C₁₂ myocytes. Interestingly, treatment of C₂C₁₂ myocytes with adiponectin resulted in an increase in the intracellular Ca²⁺ concentration measured by fura-2-based fluorescent imaging (Iwabu et al. 2010). Removal of extracellular free Ca²⁺ by EGTA almost completely abolished the adiponectin-induced Ca²⁺ response. Suppression of AdipoR1 expression using a specific siRNA largely reduced the calcium response of C₂C₁₂ myocytes to adiponectin. These results indicate that the influx of extracellular Ca²⁺ after adiponectin treatment of C₂C₁₂ myocytes is mediated by AdipoR1 (Iwabu et al. 2010). Incubation of C₂C₁₂ myocytes with adiponectin increased the phosphorylation of the AMPK α subunit at Thr 172, and EGTA partially suppressed adiponectin-induced increased AMPK phosphorylation, suggesting that adiponectin stimulates AMPK phosphorylation in part via a Ca²⁺-dependent fashion. We also examined whether adiponectin-induced Ca²⁺ influxes into skeletal muscle might be impaired in muscle-R1KO mice through in vivo imaging. We found that, whereas treatment of soleus muscle with adiponectin resulted in an increase in the intracellular Ca²⁺

Figure 2. Muscle-specific AdipoR1 knockout mice showed insulin resistance and hyperglycemia. (Modified from Iwabu et al. 2010; ©Macmillan.)

concentration as measured by fura-2-based fluorescent imaging in control mice, muscle-R1KO mice almost completely abolished the calcium response of soleus muscle to adiponectin, consistent with the observation that adiponectin significantly increased the phosphorylation of CaMKI, an intracellular substrate of CaMKK β , by AdipoR1 in skeletal muscle in vivo. We also attempted to examine whether simultaneous activation of Ca²⁺ signaling and AMPK/SIRT1 pathways by exercise, independent of AdipoR1, could rescue phenotype in muscle-R1KO mice. Two weeks of exercise significantly ameliorated insulin resistance and increased mitochondrial content and function such as citrate synthase activities in muscles of muscle-R1KO mice (Iwabu et al. 2010).

Here, we provide evidence that muscle-R1KO mice exhibit decreased mitochondrial content and enzymes. PGC-1 α is a key regulator of mitochondrial content and function (Wu et al. 1999; Mootha et al. 2003, 2004; Handschin and Spiegelman 2008). We have demonstrated that muscle-R1KO mice exhibit decreased PGC-1 α expression as well as decreased deacetylation

and activation of PGC-1 α (data not shown) (Iwabu et al. 2010). Thus, adiponectin induced Ca²⁺ influx via AdipoR1, thereby activating CaMKK β , which led to increased PGC-1 α expression. Moreover, adiponectin/AdipoR1 activated AMPK and SIRT1, thereby inducing PGC-1 α deacetylation and activation (Fig. 3). These data indicate that adiponectin and AdipoR1 stimulate increases in both the expression and activation of PGC-1 α , in a similar fashion to exercise. Although the degree of decreased PGC-1 α expression was approximately 40% in muscle-R1KO mice, the extent of decreased mitochondrial biogenesis and decreased exercise endurance was comparable to that observed for muscle-specific PGC-1 α -knockout mice, which may be explained by the finding that adiponectin and AdipoR1 increase not only PGC-1 α expression but also PGC-1 α activity. Importantly, decreases in the expression of PGC-1 α and downstream mitochondrial genes were also observed in type 2 diabetic patients and individuals at increased risk of developing diabetes owing to their family history (Mootha et al. 2003, 2004; Patti et al. 2003) as well as in obese

Figure 3. Adiponectin via AdipoR1 activates AMPK–SIRT1–PGC-1 α in muscle. (Modified from Iwabu et al. 2010; ©Macmillan; see also Cantó et al. 2009, 2010.)

diabetic *db/db* mice (Iwabu et al. 2010). These data indicate that decreased adiponectin/AdipoR1 signals in muscle in pathophysiological conditions such as obesity may have causal roles in the development of PGC-1 α dysregulation and mitochondrial dysfunction.

In skeletal muscle, AdipoR1 regulates insulin sensitivity by several mechanisms. First is the activation of S6K1, which has been reported to be able to cause insulin resistance by increased phosphorylation of Ser 636/639 in IRS-1. S6K1 is significantly inhibited by AMPK. In skeletal muscle of muscle-R1KO mice, AMPK activation was reduced and activation of S6K1 and phosphorylation of Ser 636/639 in IRS-1 was indeed increased (Iwabu et al. 2010). Second is increased oxidative stress, which has been causally linked to insulin resistance by increased phosphorylation of Ser 302 in IRS-1 through JNK activation. Several oxidative stress detoxification genes are regulated by AMPK and PGC-1 α , and the expression levels of these genes, such as *Sod2* and *Cat* were reduced, which was associated with increased thiobarbituric acid reactive substances (TBARS) in skeletal muscle of muscle-R1KO mice (Iwabu et al. 2010). Third is increased triglyceride content, which has been associated with insulin resistance by increased phosphorylation of Ser 302 in IRS-1 through JNK activation. Molecules involved in fatty acid oxidation are regulated by AMPK and PGC-1 α , and the expression levels of these genes, such as *Mcad*, were reduced, which was associated with increased triglyceride content in skeletal muscle of muscle-R1KO mice (Iwabu et al. 2010). Consistent with increased TBARS and triglyceride content, JNK activation and phosphorylation of Ser 302 in IRS-1 were indeed increased (Iwabu et al. 2010).

Exercise has been reported to have beneficial effects on longevity and lifestyle-related diseases and at the same time to activate Ca²⁺, AMPK, SIRT1, and PGC-1 α pathways. In this study, we clearly demonstrated that adiponectin and AdipoR1 regulate PGC-1 α and mitochondria via Ca²⁺ and AMPK/SIRT1 (Fig. 3). Therefore, agonism of AdipoR1 as well as strategies to increase AdipoR1 in muscle could be exercise mimetics.

In conclusion, adiponectin-AdipoR1 has crucial physiological and pathophysiological roles in muscle and is involved in the regulation of Ca²⁺ signaling, PGC-1 α expression and activation, mitochondrial function and oxidative stress, glucose and lipid metabolism, exercise endurance, and insulin resistance (Iwabu et al. 2010). This study suggests that agonism of AdipoR1, as well as strategies to increase AdipoR1 in muscle, may be logical approaches to providing a new treatment modality for mitochondrial dysfunction, insulin resistance, and type 2 diabetes linked to obesity.

EPIGENETIC REGULATION OF ADIPOGENESIS

Coordinated actions of transcription factors and cofactors on regulatory DNA sequences produce transcriptional activation of the eukaryotic gene. Therefore, identification and mapping of the genome's regulatory

elements are critical for understanding how cell-type-selective regulation of genes in the genome is achieved.

Traditionally, regulatory elements have been identified by DNase I hypersensitivity assay combined with Southern blot analysis (Heintzman et al. 2009; Siersbæk et al. 2011). That assay coupled with microarray or high-throughput sequencing (DNase-ChIP or DNase-seq) was effectively applied in genome-wide identification of open chromatin regions.

To understand the molecular mechanisms of adipogenesis, which is regulated by a cascade of various transcription factors such as PPAR γ and C/EBP α , it is important to identify some upstream epigenetic regulatory mechanisms. Lieb and colleagues recently developed formaldehyde-assisted isolation of regulatory elements (FAIRE) as a simple procedure to isolate nucleosome-depleted DNA from chromatin (Fig. 4) (Giresi et al. 2007). FAIRE detects open chromatin structure in much the same way as the DNase I hypersensitivity assay, but with advantages, such as obviating the need for clean nuclei preparation and laborious enzyme titrations. Coupled with high-throughput sequencing (FAIRE-seq), FAIRE allows unbiased identification of potential regulatory elements without prior knowledge of binding factors (Waki et al. 2011).

Genome-Wide Profiling of Open Chromatin Regions in 3T3-L1 Adipocytes by FAIRE-seq

Our study took an unbiased approach to mapping adipocyte-specific regulatory elements in the genome by using FAIRE in 3T3-L1 adipocytes (on days 0 and 8 of differentiation) and NIH-3T3 fibroblasts (Fig. 4) (Waki et al. 2011). This approach identified in the genome 37,781 FAIRE peaks in 3T3-L1 on day 0 and 26,611 on day 8, plus 36,111 in NIH-3T3 cells—all with a false discovery rate of 10⁻⁴. Using ChIP-seq analyses, we also generated genome-wide maps of binding sites for PPAR γ , the master regulator of adipocyte differentiation, for RXR α , its heterodimer partner, for histone H3 lysine 4 trimethylation (H3K4me3), and for CCCTC-binding factor (CTCF). Figure 5 shows a representative map of results generated near *Pparg*, a transcription factor up-regulated by differentiation and important in adipocyte differentiation (Waki et al. 2011). Consistent with previous observations, 28% of the FAIRE peaks were detected near the transcription start sites (TSSs; 6500 base pairs) and are referred to as promoter FAIRE peaks, whereas 72% were located outside known TSSs and are referred to as nonpromoter FAIRE peaks. Average profiling revealed that a FAIRE signal, H3K4me3, and histone H3 lysine 27 acetylation (H3K27ac) were observed at TSSs of actively transcribed genes (Waki et al. 2011). On the other hand, nonpromoter FAIRE peaks were accompanied by mono-modal enrichment of H3K4me1 and were devoid of H3K4me3 enrichment, a condition described as the signature of enhancers (Waki et al. 2011).

We next compared the FAIRE peaks in 3T3-L1 cells on days 0 and 8 and in NIH-3T3 cells. The promoter FAIRE peaks were relatively constant among the three groups.

Figure 4. High-throughput sequencing formaldehyde-assisted isolation of regulatory elements (FAIRE-seq) and ChIP-seq in adipocyte differentiation (modified from Giresi et al. 2007).

More than 70% of those peaks in day 0 and day 8 3T3-L1 cells and in NIH-3T3 cells were shared by all three groups. In contrast, nonpromoter FAIRE peaks showed dynamic change. The three groups shared only 25%, 45%, and 26% of nonpromoter FAIRE peaks, respectively, in day 0 and day 8 3T3-L1 cells and NIH-3T3 cells. Moreover, a significant proportion of the nonpromoter FAIRE peaks were cell-type specific, implying the role of nonpromoter regulatory elements in cell-type-specific transcriptional regulation (Waki et al. 2011).

To determine whether nonpromoter FAIRE peaks were functionally associated with cell-type-specific gene expression, we analyzed the relationship between the presence of the adipocyte- or preadipocyte-specific nonpromoter FAIRE peaks and the change in gene expression during adipocyte differentiation (Waki et al. 2011). Those

FAIRE peaks were enriched in the vicinity of genes, expression levels of which were highly induced or suppressed during adipocyte differentiation. Importantly, as the number of the adipocyte-specific FAIRE peaks associated with a gene increased, the fraction of up- or down-regulated genes increased or decreased, respectively, whereas as the number of associated preadipocyte-specific FAIRE peaks increased, the fraction of up- or down-regulated genes decreased or increased, respectively (Waki et al. 2011).

Analysis of Binding Sites for PPAR γ and RXR α in 3T3-L1 Adipocytes

PPAR γ is key regulator of adipocyte development (Nielsen et al. 2008; Tontonoz and Spiegelman 2008).

Figure 5. *Pparg* gene locus. (Modified from Waki et al. 2011.)

To determine the contribution of PPAR γ to adipocyte-specific transcriptional regulation, we conducted ChIP-seq analyses using antibodies specific for either PPAR γ or RXR α in 3T3-L1 adipocytes at 36 h and 8 days after induction of differentiation (Fig. 6). The number of PPAR γ binding sites increased during differentiation, whereas the number of RXR α binding sites remained virtually constant. Significant overlap between the PPAR γ and RXR α binding sites was consistent with the heterodimer formation of PPAR γ and RXR α (Kliwer et al. 1992; Wakabayashi et al. 2009). To determine the contribution of PPAR γ to the adipocyte-specific open chromatin regions, we calculated percentage fractions of the FAIRE peaks that were stratified by FAIRE signal in 3T3-L1 on days 0 and 8, and that overlapped either the PPAR γ binding sites or C/EBP α binding sites in 3T3-L1 reported by Schmidt et al. (2011). Both PPAR γ and C/EBP α binding sites were enriched in the fractions of adipocyte-specific FAIRE peaks, and they respectively accounted for 45.3% and 11.7% of the adipocyte-specific FAIRE peaks (Fig. 6). These data support the role of PPAR γ and C/EBP α as primary transcription factors that drive adipocyte-specific gene expression.

Clustering of Multiple Adipocyte-Specific Nonpromoter FAIRE Peaks and PPAR γ Binding Sites

Genes that were highly induced by adipocyte differentiation often harbored multiple adipocyte-specific FAIRE peaks and/or PPAR γ binding sites in their vicinity, as suggested by the linear correlation between the number of the associated adipocyte-specific FAIRE peaks and the robustness of the induction of the gene by adipocyte differentiation. To determine whether these multiple regions have functional regulatory elements, we selected AdipoR2 (Yamauchi et al. 2003b). Although AdipoR2 was regulated by PPAR γ and PPAR α , conventional 22-kb promoter studies failed to identify the response element. Our ChIP-seq analysis revealed a cluster of multiple PPAR γ /RXR α binding sites in the intron 1, downstream from the TSS of AdipoR2 (Waki et al. 2011). We iden-

tified putative DR-1 motifs in these binding sites and tested them by gel-shift assay and luciferase assay. These motifs were indeed bound by the PPAR γ /RXR α heterodimer and were functional in the luciferase assay, suggesting the functionality of these elements and the advantage of a genome-wide approach over the conventional “promoter-bashing” approach to identifying such response elements (Waki et al. 2011).

Sequence Motif Analyses of DNA Sequences of the Adipocyte-Specific Nonpromoter FAIRE Peaks: Identification of NFI Family Transcription Factors Involved in Adipogenesis

Next, we performed enrichment analyses of known motifs using AME in the MEME suite and the TRANSFAC (Wingender et al. 2000) and JASPER (Bryne et al. 2008) motif databases to identify motifs enriched in either adipocyte- or preadipocyte-specific FAIRE peaks compared with the background (Fig. 7). Figure 8 shows the top of the list of TRANSFAC motifs enriched in the adipocyte- and preadipocyte-specific FAIRE peaks (Waki et al. 2011). The motifs for PPAR γ (and other DR1 motifs) and C/EBPs are on the list, consistent with their critical roles in adipogenic transcription. Motif analyses using the JASPER motif database showed enrichment of the motifs for PPAR γ , C/EBPs, and Zfp423 (Gupta et al. 2010), a recently identified adipogenic regulator.

There were several other motifs for transcription factors, their functions not previously linked to adipocyte differentiation. We focused on a motif for the NFI family of transcription factors (Waki et al. 2011). The murine NFI family consists of NFIA, NFIB, NFIC, and NFIX and was identified as a site-specific DNA binding protein that bound to the adenovirus origin of replication. It forms a dimer to bind to the symmetric consensus sequence TTGGC(N5)GCCAA. We first examined the expression change of these factors in *in vitro* adipocyte differentiation and found that the expressions of NFIA and NFIB were significantly induced during differentiation of 3T3-L1 and another adipogenic cell line, 3T3-F442A. Consistent with this pattern, both NFIA and NFIB were highly

Figure 6. Comparisons between PPAR γ binding sites (ChIP-seq) and adipocyte-enriched FAIRE peaks. RXR α binding was constant, whereas PPAR γ binding increased during differentiation. Consistent with heterodimer formation of PPAR γ and RXR α , 51% of adipocyte-enriched FAIRE peaks overlapped binding sites for PPAR γ , supporting the primary roles of PPAR γ as a major adipogenic factor. (Modified from Waki et al. 2011.)

Figure 7. Motif analysis: Identification of novel factors. Known motif mapping compares frequency of binding motifs of known transcription factors between adipocyte- or preadipocyte-enriched FAIRE peaks.

expressed in a variety of adipose tissue depots in addition to the brain (Waki et al. 2011). We next examined the effect of siRNA knockdown of NFIA and NFIB on adipogenic gene regulation and adipocyte differentiation. Interestingly, induction of the expression of the adipogenic transcription factors PPAR γ and C/EBP α and of downstream genes was significantly suppressed by siRNA knockdown of either NFIA or NFIB. Consistent with the gene expression change, we observed significant reduction of lipid accumulation as judged by oil red O staining, suggesting physiological roles for NFIA and NFIB in adipocyte differentiation. We confirmed the effect of NFIA and NFIB knockdown on adipogenesis using independent pooled siRNA.

We demonstrated that genome-wide mapping of open chromatin regions by FAIRE-seq is a simple, accurate

method that allows a snapshot view of regulatory elements in the genome. Although open chromatin regions detected by FAIRE-seq include promoters of transcribed genes, enhancers, and insulators, open chromatin regions that vary in two different conditions probably contain regulatory elements that play roles in the specific biological process (Waki et al. 2011). By comparing open chromatin regions in preadipocytes and adipocytes, we identified the adipocyte- and preadipocyte-specific FAIRE peaks in the genome (Waki et al. 2011). Functionally, we demonstrated that the adipocyte-specific FAIRE peaks were associated with genes up-regulated by adipogenesis, whereas the preadipocyte-specific FAIRE peaks were associated with genes down-regulated by adipogenesis. Adipocyte gene expression appears to be mediated through multiple regulatory elements distal

Figure 8. Known motif analysis (AME, FIMO)—JASPAR CORE database. (Modified from Waki et al. 2011.)

to TSSs: Greater induction of gene expression by differentiation means greater likelihood that more adipocyte-specific FAIRE peaks are associated with the gene. This implies that optimal gene transcriptional regulation may require coordinated actions of multiple regulatory elements. Therefore, although valuable and informative, the proximal promoter assay may not always be sufficient (Waki et al. 2011).

Computational motif analysis is used to discover new transcription-factor binding motifs in sequences inferred from genome-wide studies such as ChIP-seq. In genome-wide ChIP analysis of transcription factors, motif analysis is used to obtain their accurate binding motifs and discover unknown DNA binding factors that colocalize with the transcription factors of interest. The analyses, however, rely on prior knowledge about transcription factors and the regions to be analyzed are limited to their binding sites. In contrast, the combination of motif analyses and mapping of regulatory elements by FAIRE-seq does not require such prior knowledge and hence offers a distinct advantage in unbiased screening for novel transcription factors that are important in given biological processes. In our study, we retrieved the motifs for PPAR γ and C/EBPs and for known regulators that top the list of the motifs identified in the adipocyte- or preadipocyte-specific FAIRE peaks (Waki et al. 2011). Furthermore, we demonstrated that NFIA and NFIB were functionally required for proper adipocyte differentiation (Waki et al. 2011). These results demonstrated that motif analyses of cell-type-specific FAIRE peaks are useful in identifying regulators of a biological process in an unbiased manner.

Siersbæk et al. (2011) recently used DNase-seq to investigate genome-wide change in open chromatin structure at various time points during 3T3-L1 differentiation. They reported a dramatic increase in the number of open chromatin sites in the first hours of differentiation. Such regions included what they termed “hot spots” that were bound by multiple adipogenic regulators, facilitating binding of PPAR γ and C/EBP α during the late stage of differentiation. We found that the DNase I hypersensitive sites in 3T3-L1 cells on day 0 or 6 in the Siersbæk study significantly overlapped the FAIRE peaks on day 0 or 8 in our study (78.8% and 80.9%, respectively), suggesting that both methods detect similar open chromatin regions.

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Cold Spring Harb Symp Quant Biol 2011 76: 257-265 originally published online April 4, 2012
Access the most recent version at doi:[10.1101/sqb.2012.76.010587](https://doi.org/10.1101/sqb.2012.76.010587)

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