Promoting Adipose Specificity: The Adiponectin Promoter

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In the modern obesogenic environment, obesity, insulin resistance and type 2 diabetes mellitus are becoming a global epidemic. At the organismal level, obesity is causally linked to the metabolic dysfunction of energy balance and homeostasis in the adipose tissue (reviewed in Refs. 1–3). Thus, it is becoming more and more important to have an improved and comprehensive biological knowledge of the fat cell in an attempt to understand the associated pathologies. In mammals, adipose tissue depots have been classified into two distinct types: white adipose tissue (WAT), the primary site of energy storage, and brown adipose tissue, specialized for energy expenditure (4). Over the last 2 decades, with the discovery of WAT's capacity to secrete an array of hormones, a significant importance has been attributed to its endocrinal role. These hormones, known as adipokines, have revolutionized the conception of WAT biological function, consolidating the idea that it is not just a supplier and storer of energy but a dynamic organ that is central to metabolic regulation (reviewed in Refs. 5 and 6). The numbers of adipokines are expanding rapidly and include leptin, adiponectin, resistin, visfatin, serpin, lipocalin-2, omentin, vaspin, plasminogen activator inhibitor-1, retinol binding protein 4, *etc.* that exert systemic effects. WAT also secretes TNFα, IL-6, and macrophage chemoattractant protein 4 that exert inflammatory responses or cell migration.

One important experimental tool in conducting research on adipose tissue *in vivo* has been the adipose-specific transgenic or knockout mouse models. The major technical challenge to develop such genetic mouse model is to select the tissue-specific promoter that would drive the expression of transgene or cyclization recombination (Cre) recombinase exclusively in the adipose tissue. Wang *et al.* (7) took advantage of promoter segments from the adiponectin gene, the expression of which is selectively localized to the adipocytes. Adiponectin (also described as Adipo Q, Acrp30, apM1), a 30-kDa adipokine, was discovered in both human and rodent adipose tissue as well as in the cultured adipocytes by three different groups (8–10). Screening by Northern blotting for adiponectin mRNA expression in different mammalian tissues in all three groups had independently shown that adiponectin was almost exclusively expressed in adipocytes. Adiponectin is now considered to play an important role in enhancing insulin sensitivity, decreasing influx of nonesterified fatty acids (FAs), increasing FA oxidation in liver and muscle and decreasing expression of adhesion molecules within the vascular wall, resulting in the decrease in atherogenic risk (5). The plasma levels of adiponectin were found to positively correlate with the improved metabolic function (11, 12). The production of adiponectin in the adipocytes is under considerable transcriptional control mechanisms. For example, transcription of adiponectin has been shown to be up-regulated by peroxisome proliferator-activated receptor-γ, CCAAT/enhancer-binding protein-α, sterol-responsive-element-binding protein-1c, forkhead box 1, and specificity protein 1 and down-regulated by reactive oxygen species, TNFα, and IL-6 (reviewed in Ref. 13).

To achieve adipose-specific expression of a target gene, Wang *et al.* (7) used a 4.9-kb adiponectin promoter cassette containing the upstream promoter region, exon 1, and two ends of intron 1 of the adiponectin gene. Adipocyte-specific expression of the transgene was more efficient compared with the promoter cassette carrying only the upstream promoter region. Furthermore, using the adiponectin promoter cassette, the authors have shown that expression of functional Cre recombinase specifically

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2010-0316 Received March 17, 2010. Accepted March 22, 2010.

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Abbreviations: aP2, Adipocyte P2; Cre, cyclization recombination; FA, fatty acid; WAT, white adipose tissue.

in the adipose tissue. The Cre recombinase induced the β-galactosidase reporter expression, which was prevented by stop cassette flanked by two loxP sites. Although the use of adiponectin promoter cassette to drive the transgene expression in the adipose tissue is a new approach, a FA binding protein adipocyte P2 (aP2) promoter is being used by the research community for the past decades. aP2 is primarily detected in adipose tissue and its expression is highly regulated during adipocyte differentiation (14). Reports on the aP2-deficient mice suggest important biological functions of aP2 in the systemic glucose and lipid metabolism (15). However, it has been demonstrated that functional aP2 protein is also expressed in cardiomyocytes, dendritic cells, and macrophages (16). In particular, the deficiency of aP2 in macrophage has been shown to have a protective role in the development of atherosclerosis (17, 18). Similarly, the aP2 promoter fragment conventionally used for adipose-specific transgene expression also drives the expression of the target gene in macrophages and cardiomyocytes. Thus, there have been efforts to develop a different promoter cassette, which would enable us to have more stringent adipose-specific gene expression. Adiponectin promoter cassette developed by Wang *et al.* (7) is likely to achieve this goal.

The mouse model developed by Wang *et al.* (7) set the stage for future investigations in understanding the gene function *in vivo* in the adipose tissue. This model is different from the commonly used aP2 promoter driven models at least in three different ways. First, expression of the transgene appeared to be restricted only to the adipocytes. For example, the authors could not detect the Cre transgene in either the stromal vascular fraction of adipose tissue or the peritoneal macrophages. It is well known that in obesity, the macrophage plays an important role in modulating the inflammatory response in the adipose tissue. The absence of transgene expression in macrophage is therefore particularly interesting because this can bypass one compounding factor in interpreting the experimental data. Thus, these mice will probably be a better animal model for the study of inflammation in the context of obesity and diabetes. Second, although the levels of gene expression driven by aP2 promoter cassette in different fat depots vary considerably, in the adiponectin promoter-driven mouse model, expression of the transgene was relatively even across the different WAT and brown adipose tissue depots. Third, average expression levels of the transgene were quantitatively lower than in the mice carrying the aP2 promoter cassette. Thus, this model could be an important tool for the moderate expression of the target genes, particularly in cases of transcription factors or molecular switches. With the further characterization of this mouse model, we expect that more interesting data will be revealed. Because the authors indicated that for an effective knockout of a target gene using adipose-specific Cre expression, it is better to use a homozygous rather than conventional hemizygous state. Furthermore, because the adiponectin promoter activity is under the control of multiple transcription factors, it will be interesting to see whether the adiponectin promoter cassette is also regulatable *in vivo*.

Acknowledgments

I thank Konstantin V. Kandror (Department of Biochemistry, Boston University School of Medicine) for his critical reading and suggestions during the preparation of the manuscript.

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Disclosure Summary: The author has nothing to disclose.

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