Journal of Diabetes & Metabolism
ISSN: 2155-6156
OPEN ACCESS Freely available online
Review Article

Recent Updates of Irisin's Anti-Obesity Research

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ABSTRACT

Obesity, one of the most common metabolic disorders, has become a worldwide disease that poses substantial health and economic burdens to individuals and society. Recently, "browning" of human white adipose tissue has become an attractive therapeutic strategy for the management of obesity and obesity-related metabolic diseases as the fat-burning brown-like beige adipose tissue enhances whole-body energy metabolism. Irisin, a hormone secreted by skeletal muscle and other organs after exercise and cold exposure, gives rise to keen interest because of its promising therapeutic potential for the browning of white adipocytes. This review will cover recent major progresses in the "anti-obesity" research of irisin with an emphasis on the browning effect of irisin in human. We further discuss emerging questions and new directions in the irisin research.

Keywords: Irisin; Adipocytes; Obesity

INTRODUCTION

Obesity and its related metabolic complications, including metabolic syndrome and type 2 diabetes, are increasingly spreading issues worldwide. Obesity results from excessive energy intake compared to energy expenditure, leading to increased adipose tissue mass and ectopic fat accumulation. Thus, increasing energy expenditure becomes a potential therapeutic strategy for obesity and its associated complications. Currently, lifestyle intervention is the preferred method of obesity treatment, however, it is suffered from low efficacy and high drop-out rate [1].

Other than maintaining the physical activity and postural retention, skeletal muscle also plays an important role in whole body energy metabolism [2]. In response to contraction, skeletal muscle can secrete lots of cytokines and proteins termed myokines, which exert beneficial effect on peripheral and remote organs and are identified as one of underlying mechanism for energy metabolism [3]. For example, interleukin 6, one of the well-studied myokine, plays important roles in glucose homeostasis and muscle atrophy [4]. Fibroblast growth factor 21, which is secreted by skeleton muscles, regulates not only muscle mass and function [5], but also diet-induced obesity and insulin resistance [6].

Irisin is a recently discovered, exercise-induced myokine in mice and humans [7]. It was shown to promote "browning" of subcutaneous white adipocytes by increasing the expression of mitochondrial UCP1. Furthermore, this hormone has demonstrated beneficial effects on different tissues and organs [8,9]. Importantly, as a myokine and adipokine, irisin is secreted after exercise and associated with thermogenesis, insulin resistance, glucose regulation and bone metabolism [10].

In this review, we discuss the current knowledge about irisin and its thermogenesis effects in both mice and human, and propose possible new directions for the future investigation of irisin.

THREE TYPES OF ADIPOCYTES

Animals and animal care

According to different developmental origins and functional features, adipocytes can be divided into white, beige (also known as "brite") and brown adipocytes. Brown adipocytes originate from a subpopulation of embryonic myogenic factor 5 (Myf5) positive precursors that can also give rise to skeletal muscle [11]. Brown adipocytes express a high basal level of thermogenic genes, including mitochondria uncoupling protein 1 (*UCP1*). The rediscovery of brown adipose tissue (BAT) in adults using positron emission tomography (PET) [12-14] evokes considerable interest in exploring the therapeutic potential of brown adipocytes or inducible brown-like adipocytes for weight loss and anti-obesity. Indeed, a number of prospective studies have reported an inverse correlation of BAT with body mass index (BMI) and adiposity [14,15].

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Received: March 06, 2020, **Accepted:** April 22, 2020, **Published:** April 29, 2020

Citation: Zhang Y, Yang L, Ding Y (2020) Recent Updates of Irisin's Anti-Obesity Research. J Diabetes Metab. 10:843. doi: 10.35248/2155-6156.20.11.843

Originating from Myf5 negative precursors, white adipocytes have broad distribution in the body. They are found in the entire subcutaneous region of organs and hollow viscera, therefore often being classified into two types: subcutaneous and visceral white adipocytes. In term of the role in thermogenesis, unlike brown adipocytes that dissipate chemical energy as heat, white adipocytes have low UCP1 expression and function as energy storage [16].

In the past decade, beige or brite adipocytes was discovered as the another type of adipocytes. They sporadically reside within white adipose tissue and share many biochemical characteristics with brown adipocytes including multilocular lipid droplets and UCP1 expression [11,17,18]. UCP1 positive beige adipocytes were reported to derive from Myf5-negative, platelet-derived growth factor receptor α (PDGFRα) positive mesenchymal precursors [11,19]. However, their cellular origin remains incompletely understood. On the other hand, the amount of beige adipocytes in the body increases dramatically, along with the robust expression of thermogenic genes under certain external cues, such as chronic cold exposure and β3-adrenergic agonists [20]. This phenomenon of differentiation from precursors or trans-differentiation from white adipocytes in the postnatal stages is called "browning" [21-23]. Increased thermogenesis by "browning" otherwise-energy-storing white adipocytes has a promising potential to combat obesity and obesity-related diseases.

IRISIN, A PROMISING MYOKINE AND ADIPOKINE

In 2012, Boström P *et al* observed a greater expression of the fibronectin type III domain containing 5 (FNDC5) gene and protein in the muscles of transgenic PPAR-γ co-activator-1α (PGC1α) mice, compared to the wild-type mice [7]. FNDC5 is a transmembrane protein comprising of one signal peptide, one fibronectin III domain and one hydrophobic membrane-anchored domain [24,25]. In response to certain stimuli, FNDC5 can be C-terminally cleaved and the released N-terminus, named "irisin", is a 112 amino acid protein confirmed by mass spectrometry analyses [7]. Irisin shares the identical amino acid sequence among most mammalian species, indicating the conservation and essentiality of its potential functions [7].

Over the past eight years, the biochemical and structural features of irisin have been available in multiple reports. Irisin is likely a glycosylated protein as culture medium in FNDC5-overexpressing cells showed multiple bands in western blot analysis [26]. Furthermore, our research groups generated recombinant human irisin protein (hr-irisin) using the yeast expression system. The purified hr-irisin showed 3 bands with a molecular weight of 25, 22, and 15 kDa in SDS-PAGE and western blot analysis. We predicted that the glycosylation on hr-irisin can occur on asparagine 7 and asparagine 52. Subsequent site-directed mutagenesis on these two sites supported the bioinformatics prediction, and the resultant glycosylated mutant in the yeast expression system showed the same molecular weight as the PNGase-F-treated wild type protein [26]. On the other hand, glycosylated irisin was biologically functional. The structural basis of irisin's biological functions has also been uncovered in the *E. coli* expression system [27]. Schumacher *et al*, demonstrated that irisin contains an N-terminal fibronectin type III (FNIII)-like domain and forms a continuous eight-stranded β-sheet dimer [27]. The structural details of irisin recently assisted the identification of its receptor as the αV integrin receptor from MLO-Y4 osteocytes by the Spiegelman group [28].

Irisin is highly expressed in skeletal muscle and increases with endurance exercise and cold exposure [29]. The plasma concentration of irisin in mice was dramatically elevated after three weeks of free wheel running [16]. Similarly, healthy human adults with 10-weeks endurance exercise training showed a 2-fold increase in circulating irisin levels, comparing with controls without endurance training [7]. Mechanistically, exercise can induce skeletal muscle mitochondrial biogenesis by increasing the expression and activity of PGC1α, which results in elevated FNDC5 expression, followed by increased irisin secretion [7]. Not only secreted by skeletal muscle, irisin is also secreted by adipose tissue. The pattern of adipose-FNDC5/irisin expression and secretion is varying among fat deposits at different locations and also affected by exercise and nutritional status [30]. Subcutaneous adipose tissue shows the highest secretion level of FNDC5/irisin, while fasting reduces adipose-FNDC5/irisin secretion. In addition, adipose-FNDC5/irisin has a feedback secretion pattern, similar to other well-known adipokines (e.g., leptin) [30]. Irisin supports a possible new interaction between skeletal muscle and adipocytes, which is called the muscle-adipose tissue axis.

Besides exercise, many factors can affect the expression and secretion of irisin. For example, α-lipoic acid upregulated *FNDC5* mRNA expression and irisin secretion in cultured adipocytes [31], while increased irisin secretion was observed in rat muscle cells when treated with saturated free fatty acids [32]. In addition to small molecule metabolites, hormones can impose different effects on the irisin section. Leptin is a hormone from produced in adipose tissue that can control food intake and energy homeostasis. Interestingly, leptin upregulated the *FNDC5* expression in murine C2C12 myocytes, while downregulated its expression in murine differentiated subcutaneous adipocytes [33]. On the other hand, myostatin, a hormone released by myocytes, decreased *PGC1*α expression and irisin production in C2C12 cells [34], and some inflammatory cytokines (e.g., IL1β and TNFα) reduced the *FNDC5* mRNA level and irisin secretion in skeletal muscle [35]. These results state the complexity of irisin regulation network and suggest its important physiological functions.

IRISIN COMBATS OBESITY IN MICE

As we discussed previously, the formation of beige adipocytes could increase energy expenditure and thereby favor the combat against obesity [12-14]. It was reported that FNDC5 treatment of primary subcutaneous white adipocytes during differentiation dramatically increases the levels of brown adipocytes markers *UCP1*, *PRDM16*, and *CIDEA* and enhance energy expenditure and oxygen consumption [7]. Wu *et al.* further demonstrated that irisin enhances the browning of white adipocytes in mice, especially with a high expression level of the specific beige adipocytes marker *CD137* in white adipocytes [21]. Our research groups have also made important contributions to irisin's anti-obesity research. We observed the browning of rat primary adipocytes and 3T3L1-derived mature adipocytes when treated with r-irisin for 4 days [26]. Importantly, we further uncovered the activation of the p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal–related kinase (ERK) pathways in irisin-induced thermogenic gene

expression [26]. In the Bostrom's study, over-expression of *FNDC5* gene in mice resulted in a moderately increased level of circulating blood irisin and energy expenditure, along with decreased body weight [7]. To confirm the effects of irisin at the protein level, we administered hr-irisin into high fat induced obese mice daily for two weeks and observed weight loss and improvement of insulin resistance [26]. The following two mechanisms may contribute the anti-obesity effect of irisin: 1) the browning of subcutaneous white adipose tissue [26], leading to enhanced energy expenditure, and 2) enhancing lipolysis by activating hormone-sensitive lipase (HSL) via cAMP-PKA-HSL/perilipin pathway, which in turn ameliorates lipid metabolic derangements and insulin resistance in obese mice [36,37].

IRISIN COMBATS OBESITY IN HUMAN

The physiological concentration of circulating irisin in human body has so far been controversy in literatures [38,39]. The majority of publications described the positive correlation between the plasma irisin level and adiposity, while some reports indicated the negative relation between irisin and the amount of fat mass. Furthermore, the circulating irisin level was positively correlated with BMI and glucose and negatively associated with insulin and cholesterol levels in many studies [40,41]. However, Moreno-Navarrete JM *et al.* reported that the circulating irisin level in adipose tissue is negatively associated with obesity but positively associated with the expression levels of brown adipose tissue markers [42]. This contradiction may be originated from the use of different detection methods or ELISA kits that rely on the sensitivity and detection limitations of different anti-irisin antibodies [10,43].

Although animal studies suggest the therapeutic potential of irisin in fighting obesity and diabetes [7,21,26], the therapeutic use of irisin to combat obesity in human remains poorly evaluated [29,44,45]. Up to date, only a small numbers of studies focused on the investigation of irisin's effects on human adipocytes. In one study by Raschke *et al.*, after incubating with primary human subcutaneous pre-adipocytes during adipocytes differentiation period of 14 days, irisin showed no effect on the beige differentiation of human preadipocytes, suggesting no beneficial anti-obesity effects of irisin in humans [44]. However, another work reported a strong induction of brown and beige genes in primary human adipocytes isolated from neck biopsies after FNDC5/irisin treatment for 6 days [29]. Lee *et al.* also found that FNDC5/irisin enhanced thermogenic program in subcutaneous but not omental adipocytes, although the extent of thermogenic activation was less than that in neck adipocytes [29]. Similarly, Huh *et.al* demonstrated that irisin significantly upregulated the expression of browning genes, including *UCP1*, *PRDM16*, and *CIDEA*, when subcutaneous human mature adipocytes were treated for 8 days [45]. They also observed the activation of the glucose and lipid metabolism pathways after irisin treatment. Consistent with these reports on the beneficial effects of irisin, our groups treated human primary mature adipocytes derived from subcutaneous WAT for 4 days and found the significantly enhanced expression of general brown genes and increased cellular mitochondrial respiration and glycolysis [46].

Several reasons may account for these reported controversies regarding the anti-obesity effects of irisin in human. First, the concentrations of irisin used in these studies varies to a large extent [29,44-46]. Raschke and coworkers treated human pre-mature subcutaneous adipocytes with 200 ng/mL recombinant FNDC5 or 60 ng/mL irisin [44], while Lee and coworkers treated human neck adipocytes with 100 nM recombinant FNDC5 [29]. Furthermore, 10 nM or 50 nM r-irisin were used to treat human subcutaneous adipocytes in the Huh's study [45]. In our studies, we found that as low as 5 nM, hr-irisin is able to evaluate mitochondrial respiration and UCP1 expression in human adipocytes and fresh adipose tissue [46]. On the other hand, the level of circulating irisin in human plasma was detected by quantitative mass spectrometry by the AQUA method to be 3.6-4.3 ng/ml [47], and it was 0.3 ng/ml of irisin in mice plasma [28]. Therefore, the concentrations of irisin used in the mice and human experiment are commonly tens to hundreds times higher than the detected circulating level.

Second, the adipocytes in different locations have different features that may contribute to varying results of irisin studies. Furthermore, human adipose tissue consists of adipocytes, stem cells, fibro-vascular components and other cell types, which interact closely [48,49]. The generation of human primary mature adipocytes for irisin studies involves dedifferentiation and redifferentiation *in vitro* and the transcription profiles, epigenetic characters and potential functions can vary in different studies [50,51]. To address this limitation, we recently developed a human adipose tissue culture system that can more accurately evaluate the *in vivo* therapeutic roles of irisin [46]. We observed that 4-day irisin treatments significantly induced BAT related genes, including *UCP1* and *PRDM16*, in freshly isolated human subcutaneous WAT fragments, but no significant increase of these genes' expression in perirenal BAT or visceral WAT at the tissue culture level. Furthermore, the browning action of hr-irisin on subcutaneous WAT was supported by the significant increase expression of UCP1 via activating P38 MAPK and ERK signaling pathways, which were not activated by r-irisin in perirenal BAT or visceral WAT [46,52]. De Oliveira M et.al treated human subcutaneous white adipocytes with 20 nM irisin for 24 h and found that irisin improved UCP1 production, lipid profile, oxidative stress, and DNA damage, without altering adipokine, PPARG, and FNDC5 levels [53]. In addition, our group recently found that irisin increases mitochondrial respiration of mature adipocytes derived from human visceral WATs and but does not upregulate their UCP1 expression [52]. Collectively, the above studies support the beneficial effects of irisin on human subcutaneous WATs and demonstrate the varied responses of different types of adipose tissues to irisin treatment.

Another potential reason of current controversies of the irisin's anti-obesity effects can be highly diverse genetic backgrounds of human subjects. In our previous study, we observed that subcutaneous WAT fragments from 8 donors exhibited obviously different responses to irisin-induced UCP1 overexpression, ranging from 0.5 to 60 times in comparison to saline control [46]. To elucidate the underlying molecular mechanism of individual different responses, we quantitated the basal levels of beige biomarker genes in different donors, and found that breast fats of different donors contained different abundance of brown/brite adipocytes. Importantly, the relative expression changes of *UCP1* in irisin-treated breast fat positively correlated with basal levels of *UCP1, TMEM26, PRDM16, CD137,* and *FNDC5* [46]. In addition to different fat compositions of individuals, genetic mutations/variations that affect beige/brown fat development and energy metabolism can lead to varied

responses to irisin, including polymorphisms in *UCP1* (–3826A/G) and β3-adrendergic receptor (*ADRB3*) (64 Trp/Arg) [54,55], *EHMT1* [56] and *LGR4* expression [57].

Furthermore, irisin can have different effects on adipocytes on different development stages. Our group also found that irisin affects preadipocytes differentiation [46]. After treating human primary preadipocytes with hr-irisin in adipogenic differentiation medium for 14 days, we found that irisin inhibits the lipid accumulation, reduces the expression levels of *ADIPOQ* and *CEBPB*, and suppresses the expression of UCP1 in the mRNA and protein levels [46]. These results that irisin inhibits adipogenic differentiation of preadipocytes, supported by a previous report [45].

Chronic low-grade inflammatory condition is a well characterized feature in obesity, with pro-inflammatory factors persistently expressed in adipocytes [58,59]. It was reported that irisin decreased the expression of inflammatory makers and alleviate inflammation by inhibiting TLR4/MyD88 and nuclear factor kappa B (NFκB) signaling pathway [60,61]. Irisin also induced the phenotypic switching of adipose tissue macrophages from an M1-like (pro-inflammatory) to an M2-like (anti-inflammatory) phenotype [34,61]. Recently, our group found that the expression of pro-inflammatory genes (*TNF-α*, *IL-6*, *MCP-1*, and *MIP-1α*) significantly decreased, while the expression of anti-inflammatory gene *IL10* increased, after human visceral WAT and subcutaneous WAT treated with irisin for 4 days [52].

Taken together, the responses or anti-obesity actions of irisin are dependent on different local concentrations of irisin and other inflammatory cytokines, different distribution (subcutaneous versus visceral) of the adipose tissue, and various stages of adipocyte differentiation (Figure 1).

OTHER EFFECTS OF IRISIN

In addition to its effects on adipocytes, irisin has demonstrated potential roles in many other organs and tissues. From the therapeutic perspective, irisin is considered to be beneficial in type 2 diabetes [7,26], cardiovascular diseases [62-64], some neurodegenerative diseases (e.g., Alzheimer and Parkinson) [65-68], osteoporosis [69,70] [71], intestinal injury [72], sarcopenia [73,74] and different kinds of cancer [75,76]. Furthermore, the circulating irisin level is negatively correlated with chronic kidney disease [9,77] and non-alcoholic fatty liver disease [78,79]. In this regard, the use of irisin and its derivatives for the management of obesity should consider and balance their potential actions on other important organs and tissues.

RECEPTOR OF IRISIN

Since the discovery of irisin in 2012, scientists have continuously searched for its receptor for obtaining an advanced understanding and control of its functions. In 2014, our group used a live cell binding method to explore the binding property of irisin and found 3T3-L1 derived adipocytes have a yet-identified irisin receptor on the cell membrane [26]. Later, our group also demonstrated the binding of irisin to the cell membrane of H9C2 cells, suggesting the presence of irisin receptor in cardiomyoblasts [62].

Recently, Kim *et al.* revealed that irisin-induced thermogenic gene program was mediated through the αV/β5 integrin receptor [28]. To identify irisin receptor, they first found that irisin stimulates a strong integrin-like signaling in murine osteocytes [28]. Next, they identify the integrin αV/β5 as irisin receptor using a mass spectrometry-based quantitative proteomics method and further confirmed that the integrin αV/β5 has a high affinity with irisin and is required for the cellular response to irisin. By using differential hydrogen-deuterium exchange linked to mass spectrometry, Kim *et al* demonstrated that irisin directly binds to the integrin αV/β5, and the three-dimensional structure of the proximal motif of irisin resembles the well-known "RGD" motif of fibronectin [28]. Furthermore, they injected irisin along with the control RGD peptide or cycloRGDyK, a specific inhibitor for the integrin αV, and found that cycloRGDyK blocks the activation of irisin-induced signal pathways and decreases the expression

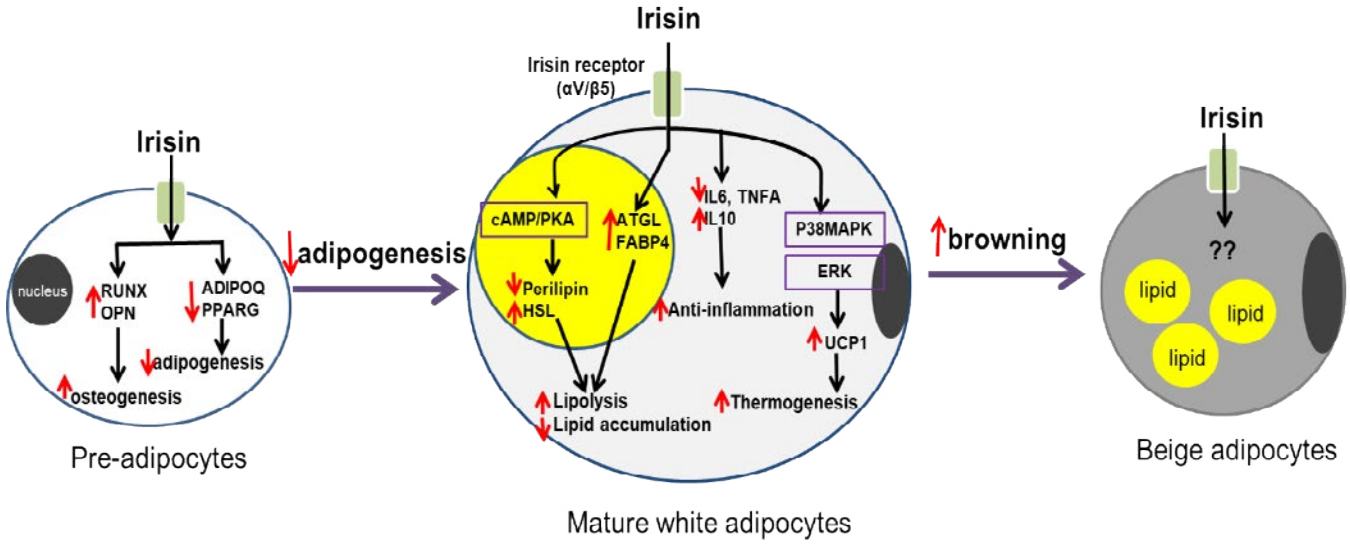

Figure 1: Irisin inhibits the adipogenesis of pre-adipocytes via decreasing expression of ADIPOQ and PPARG, while promotes osteogenesis via increasing RUNX and OPN expression. Irisin stimulates lipolysis via cAMP/PKA pathway, followed by decreased expression of perilipin and increased expression of HSL. Irisin also upregulates the expression of ATGL and FABP4, resulting the decreased lipid accumulation. Irisin plays a role in anti-inflammation by downregulating IL6, TNFA and upregulating IL10 in human white adipocytes. Irisin promotes the browning of white adipocytes by inducing the expression of UCP1 via the p38MAPK and ERK pathway. The effect of irisin on beige adipocytes needs to be further explored

of sclerostin. Moreover, cycloRGDyK blocked the irisin-induced overexpression of *UCP1* and *DIO2* in adipocytes, inferring the same integrin family proteins also serving as receptor for irisin in adipocytes [28].

CONCLUDING REMARKS AND FUTURE PROSPECTS

In recent years, there were many significant progresses in the irisin research. One major breakthrough is the identification of the αV family integrin complex as the irisin receptor in osteocytes and likely in adipocytes. On the other hand, irisin has demonstrated multifaceted effects on multiple human organs and tissues. It is unclear if other types of irisin receptors exist in other types of cells. A more complete investigation of irisin receptor in different organs and tissues is important to fully understand the biology and roles of irisin and leverage its therapeutic values.

Another promising direction in the irisin research is to elucidate the mechanisms and signal pathways that underlie the effects of irisin in human. Currently the irisin signaling has been studied well and includes that exercise induces the PGC1α expression, which upregulates the expression of FNDC5/irisin, leading to the increased thermogenic/brown fat program though, at least partly, the activation of PPARα. However, the signaling and mechanism of irisin in different types of adipose tissues and other organs/tissues remain less explored. A better understanding of signal pathways of irisin in adipocytes will provide new insights into the future study that aims to control and manipulate the irisin signaling for benefits human health, particularly obesity management.

In conclusion, despite conflicts and controversies concerning irisin's biological effects and physiological levels, the current findings and upcoming prospects in the irisin research are highly promising. Future in-depth studies, e.g., preclinical and clinical evaluation and medicinal chemistry efforts to develop irisin derivatives, are necessary to realize its full potential as a meaningful therapeutic strategy for fighting human obesity and obesity-related diseases.

DISCLOSURE OF CONFLICT OF INTEREST

None.

ACKNOWLEDGEMENT

This work was supported in part by Grant 00117544 from Congressionally Directed Medical Research Programs/Department of Defense (Y. Ding and L. J. Yang). We thank current and past members of the Yang and Ding lab for constructive and inspiring discussions on the irisin research topic over the past 6 years.

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