Biochimica et Biophysica Acta 1831 (2013) 915–923

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

Bone morphogenic proteins signaling in adipogenesis and energy homeostasis ☆

Salvatore Modica, Christian Wolfrum *

Institute of Food, Nutrition and Health, ETH Zurich, Switzerland

ARTICLE INFO

Article history:
Received 18 November 2012
Received in revised form 7 January 2013
Accepted 10 January 2013
Available online 23 January 2013

Keywords:
Bone morphogenic proteins
Mesenchymal stem cells
Adipogenesis
Brown adipocyte
White adipocyte
Energy homeostasis

ABSTRACT

A great deal is known about the molecular mechanisms regulating terminal differentiation of pre-adipocytes into mature adipocytes. In contrast, the knowledge about pathways that trigger commitment of mesenchymal stem cells into the adipocyte lineage is fragmented. In recent years, the role of members of the bone morphogenic protein family in regulating the early steps of adipogenesis has been the focus of research. Findings based on these studies have also highlighted an unexpected role for some bone morphogenic protein in energy homeostasis via regulation of adipocyte development and function. This review summarizes the knowledge about bone morphogenic proteins and their role in adipocyte commitment and regulation of whole body energy homeostasis. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

1. Introduction

During gastrulation several specialized cells such as adipocytes, myocytes and chondrocytes originate from the mesoderm layer [1]. Today it is commonly accepted that with regard to adipocyte development, two types of cells are generated: (i) one that functions as a precursor for white fat, a tissue devoted to store energy in the form of triglycerides for the time of starvation, and (ii) one precursor that gives rise to brown fat, a tissue that burns lipids to produce heat via non-shivering thermogenesis [2–4]. White adipose tissue (WAT) and brown adipose tissue (BAT) are organized to form the adipose tissue organ, a multi-depot organ distributed in our body with a discrete anatomy and a high physiological plasticity [5–7]. WAT can mainly be found in two locations: below the skin (subcutaneous) and in the abdomen (visceral). BAT, was believed to be absent in adult humans, however recently, using fluoro-deoxyglucose (FDG)-positron emission tomography (PET) combined with computer tomography (CT), it was clearly demonstrated that also adult humans possess BAT, which seems to be functional as well [8–11].

Morphologically, white and brown adipocytes differ by their lipid droplet size and abundance. White adipocytes store triglycerides in a single large lipid droplet and contain few mitochondria. On the contrary, brown adipocytes show higher numbers of mitochondria and accumulate triglycerides in several small lipid droplets [12]. The ability of brown adipocytes to dissipate energy is conferred by uncoupling protein 1 (UCP1). This protein is expressed uniquely in BAT, where it uncouples cellular respiration from ATP production, thus dissipating energy as heat [13]. This adaptive non-shivering thermogenesis is important not only for maintaining body temperature, but to some extent, also for the maintenance of body weight. Indeed, genetic ablation of BAT in mice resulted in susceptibility to develop obesity [14], whereas overexpression of UCP1 protected from diet induced obesity [15].

For long time white and brown adipocytes have been assumed to share a common developmental origin as they have a similar differentiation program and a similar expression patter of genes [16]. However, recent studies have clearly demonstrated that brown adipocytes share a common precursor with muscle cells [17]. This is not surprising as both cell types are rich in mitochondria and can perform oxidative phosphorylation as well as adaptive thermogenesis. Actually, the common origin of these two cells was already suggested in 1551 by the Swiss naturalist Konrad Gessner when he described brown adipocytes as neither fat, nor muscle [18]. Almost 450 years later Seale et

Abbreviations: WAT, white adipose tissue; BAT, brown adipose tissue; MSC, mesenchymal stem cell; UCP1, uncoupling protein 1; PRDM16, PR domain containing 16; PPARγ, peroxisome proliferator-activated receptor gamma; BMP, bone morphogenic protein; PGC1, peroxisome proliferator-activated receptor gamma coactivator 1; CtBP, C-terminal-binding protein; SMAD, small mother against decapentaplegic; TGF-β, tumor growth factor-β; ALK, activin receptor-like kinase; BMPR, bone morphogenic receptor; ActR, activin receptor; GDF, grown/differentiation factor; FGF, fibroblast growth factor; WNT, wingless; Rb, retinoblastoma; Pref-1, pre-adipocyte factor-1; C/EBP, CCAAT/enhancer binding protein; RIP140, receptor-interacting protein 140; PG, prostaglandin; HSL, hormone sensitive lipase; CNS, central nervous system; SNS, sympathetic nervous system; VMH, ventromedial hypothalamus; hPSCs, human pluripotent stem cells

☆ This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

* Corresponding author at: Institute for Food, Nutrition and Health, ETH Zurich, Schorenstrasse 16, 8603 Schwerzenbach, CH, Switzerland. Tel.: +41 44 6557451.
E-mail address: Christian-wolfrum@ethz.ch (C. Wolfrum).

1388-1981/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbalip.2013.01.010

al. [17] confirmed these suggestions by demonstrating that brown adipocytes share a common origin with skeletal muscle cells and have phenotypic features similar to both adipocytes and myocytes. Thus, brown adipocytes can be classified as "adipomyocytes", i.e. muscle cells that have developed the capacity to accumulate lipids.

Initial lineage tracing studies showed that cells expressing engrailed-1 (En1) located in the central dermomyotome can give origin to interscapular brown fat [19]. In addition, brown adipocyte cell precursors were shown to have a myogenic gene expression signature [20]. Finally, through in vivo fate mapping studies, the existence of a common Myf5$^+$ progenitor for both skeletal muscle and brown adipocytes was demonstrated [17]. Myf5 is a key early myogenic transcription factor whose expression was thought to be specific to committed skeletal muscle cells. Notably, the Myf5$^+$ precursor cell population does not give rise to white adipocytes although this concept has been recently challenged [21].

In line with this, the observation that brown adipocytes and muscle cells share a common precursor is the result from myogenin-deficient mice, which suggests that myocyte precursor cells that are not able to terminally differentiate into muscle cells can give rise to brown adipocyte accumulation in skeletal muscle fibers [22].

The factor responsible for the commitment of the Myf5$^+$ precursor cells into brown adipocytes or myocytes has been shown to be PR domain containing 16 (PRDM16) [17]. PRDM16 can control a bi-directional cell fate between muscle and BAT and by repressing the expression of myogenic factors PRDM16 is responsible for the determination of brown adipocytes from the Myf5$^+$ progenitors. Indeed, loss of PRDM16 from brown fat precursors results in an impaired brown fat formation, whereas the expression of myogenic markers is increased [17]. On the other hand, ectopic expression of PRDM16 in myoblast results in the appearance of a brown phenotype [17]. Notably, it was shown that peroxisome proliferator-activated receptor gamma (PPARγ) mediated the effects of PRDM16 and activation of PPARγ was able to convert myogenic cells to white-like adipocytes, whereas conversion into brown adipocytes was possible only by the coexpression of PRDM16. Thus, PRDM16 acts at an early stage to influence a brown lineage decision. Indeed, the expression of PRDM16 has been shown to be regulated by bone morphogenic protein 7 (BMP-7), a factor responsible for trigging commitment of mesenchymal stem cells (MSCs) specifically into the brown adipogenic lineage [23].

In order to repress the myogenic lineage in brown fat precursors, some repressing factors are likely to play an important role. In this respect, PRDM16 has been shown to have the ability to simultaneously activate and repress genes via a mutually exclusive interaction with peroxisome proliferator-activated receptor gamma coactivator 1 α/β (PGC1α/β) or C-terminal-binding protein (CtBP) corepressors [24]. Moreover, it has also been shown that PRDM16 can repress the myogenic signature via mir-193b and mir-365 [25]. By coactivating PPARα, PRDM16 promotes the expression of the cluster mir-193b-365, which blocks the entire program of myogenesis in C2C12 myoblast to promote, under adipogenic conditions, the formation of brown adipocytes. In contrary, blocking mir-193b and/or mir-365 in primary brown preadipocytes results in impaired brown adipogenesis by enhancing RUNX1T1 and increased expression of myogenic markers.

The adipose organ is constantly being remodeled in order to respond to environmental cues. Apart from the population of endogenous brown adipocytes, a second population of inducible brown adipocytes exists (referred to as "brite" or "beige") [26]. Thus, in rodents chronic cold exposure determines the appearance of brite adipocytes in white depots [27–29]. Brite adipocytes are not derived from Myf5$^+$ expressing progenitors [17,30], posing the question from which cellular origin these cells arise. One possibility that was suggested is the trans-differentiation of white to brite adipocytes [31]. However, also separate progenitors, which are negative for Myf5 may exist [21]. These findings are in line with the observation that BAT cells in the interscapular region are genetically different from those localized in WAT depots [32].

In addition to the appearance of brite adipocytes in WAT depots, brown adipocytes have been also identified in skeletal muscle, which might develop from Myf5$^+$ satellite cells to increase the oxidative capacity of the skeletal muscle [33]. Even though it is still unclear to which extend brite cells can participate to energy expenditure, the appearance of brown adipocytes in both WAT and muscle has been suggested to increase energy expenditure and resistance to obesity [34,35]. Thus, therapies aiming to promote these events to counteract obesity need the understanding of brown fat lineage commitment.

Although the discovery of the origin of brown adipocytes is an important finding, the localization and identification of the specific brown fat/myocyte progenitor cells during mouse embryogenesis remains to be established. Thus, it will be important to map the PRDM16 embryonic expression pattern to get insight into both localization and identity of the common precursors during early development. Further cues could be obtained from dissecting the signaling pathways responsible for the spatial and temporal expression of PRDM16. Of note in that respect are the BMP proteins, which are morphogens that play a key role in the development of many tissues, including adipose tissue. In fact, in mice lacking Schnurri-2, a down-stream mediator of the BMP-2 signaling pathways, the development of WAT is dramatically impaired [36]. In contrast the development of BAT is compromised in BMP-7 knock-out mice [23]. Among the BMP proteins, BMP-2 and BMP-4 have been proposed to promote the development of WAT, while BMP-7 has been implicated in the development of BAT [23,36]. BMP-7 has been shown to drive the complete brown fat differentiation program, including PRDM16 expression. Nevertheless, apart from small mother against decapentaplegic (SMAD) transcription factors, the knowledge of the down-stream mediators of the BMP signaling responsible for adipogenesis is limited.

2. Bone morphogenic proteins

Bone morphogenic proteins (BMPs) are pleiotropic members of the transforming growth factor β superfamily (TGF-β). As morphogens, BMPs are synthesized from localized sources from where they diffuse into the surrounding tissue to provide positional information in a dose-dependent manner [37]. Originally identified as factors that induce the formation of bone and cartilage, BMPs have been shown to play an important role in the development and function of many other tissues such as intestine, kidney, muscle, brain and hematopoietic as well as adipose tissue [38]. The activity of BMPs was initially recognized in the 1960s [39]. However, the factors responsible for bone formation stimulation remained elusive until the purification and sequencing of BMP-3 (osteogenin) and the cloning of human BMP-2 and BMP-4 in the late 1980s [40,41]. Since then, around 20 BMP family members have been identified nowadays in vertebrates and invertebrates [42] (Table 1). Of these, BMP-2, BMP-4, BMP-6, and BMP-7 are well-established mediators of both osteogenesis and adipogenesis from mesenchymal stem cells (MSCs) [43]. BMPs are secreted as precursor protein dimers that are cleaved by proteinases to yield the mature active form of the protein [44]. BMPs bind to serine–threonine kinase receptors that transduce their signal to the nucleus via SMAD proteins [45].

3. Bone morphogenic protein receptors

Two types of serine–threonine kinase receptors are required to transduce BMP signaling: type I and type II receptors. Both receptors have a short extracellular domain, a single membrane-spanning domain and an intracellular domain containing a serine–threonine kinase domain [46]. The specificity of BMP binding to type I receptors is affected by type II receptor. Moreover, BMP-receptor binding and signaling activity can be regulated by co-receptor factors [46].

With respect to BMP type I receptors, seven receptors have been identified, which are referred to as activin receptor-like kinase (ALK-1 to ALK-7). These seven receptors are further classified into

Table 1
Members of the mammalian BMP subfamily of the TGF-β superfamily and components of the BMP signal transduction pathways. Important corresponding references are noted in the table.

BMP ligand [46]	Receptor type I [46]	Receptor type II [46,47]	R-SMAD [48]	Co-SMAD [48]	I-SMAD [48]
GDF-1	ALK-1	BMPR-IIA	SMAD1 [36,46]	SMAD4 [45]	SMAD6 [45,51,52]
BMP-2 (BMP-2A) [36,41,43,46,63–65,73,74]	ALK-2	BMPR-IIB	SMAD2 [46]		SMAD7 [45,50]
BMP-3 (BMP-3A) [40]	ALK-3 (BMPR-IA)		SMAD3 [46]		
BMP-3B (GDF-10) [40]	ALK-4		SMAD5 [46]		
GDF-3	ALK-5		SMAD8 [46]		
BMP-4 (BMP-2B) [41,43]	ALK-6 (BMPR-IB)				
BMP-5	ALK-7				
BMP-6 [43,46]					
BMP-7 [23,43,46,73,74,86]					
BMP-8A					
BMP-8B [84]					
BMP-9 (GDF-2)					
BMP-10					
BMP-11 (GDF-11)					
BMP-12 (GDF-7)					
BMP-13 (GDF-6)					
BMP-14					
BMP-15 (GDF-9B)					

three groups depending on their similarity in structure and function: a) the BMPR-I group (ALK-3/BMPR-IA and ALK-6/BMPR-IB), b) the ALK-1 group (ALK-1 and ALK-2), c) the TβR-I group (ALK-4/ActR-IB, ALK-5/TβR-I and ALK-7). Signal transduction from members of the ALK-1 and BMPR-I groups results in activation of SMAD1/5/8, while the TβR-I group can activate SMAD2/3 [46].

With respect to type II receptors, three receptors have been identified: BMPR-II, ActR-II and ActR-IIB. Two isoforms of BMPR-II exist, one with a long C-terminal tail after the kinase domain, and one without the C-terminal tail. The former is expressed in most type of cells, while the latter only in a few types. While type II receptors are widely expressed in many tissues, the pattern of expression of type I receptors can be more restricted (e.g. BMPR-IB and ALK-1). The specificity of BMP binding to type I receptors is affected by type II receptors. Indeed, BMP-4 and BMP-2 preferentially bind to ALK-3 and ALK-6, BMP-6 and BMP-7 to ALK-2, BMP-9 and BMP-10 to ALK-1 and ALK-2, growth/differentiation factor 5 (GDF-5) to ALK-6 (Table 1).

4. Bone morphogenic protein signal transduction

Located at the N-terminus of the serine–threonine kinase domain, the glycine and serine (GS) rich-domain of type I receptor is the rate-limiting step in BMP signal transduction (Fig. 1). Although type II receptor is constitutively active and a small fraction of type I and type II receptors pre-exists as homo- and heterodimers, upon ligand binding the fraction of type I–II heterodimers is significantly increased [46]. When heterodimers are formed, type II receptor phosphorylates the GS domain of type I receptor, a key event that starts the intracellular signal transduction. Notably, a constitutively active form of type I receptor can transduce intracellular signals even in the absence of ligand and type II receptor. Thus, it is the type I receptor which determines the specificity of the intracellular signals by acting as a down-stream component of type II receptor [47].

SMAD proteins are the major signal transducers responsible for TGF-β signaling from the cytoplasm into the nucleus [48]. Eight different SMADs have been identified in mammals. Generally, SMAD1, SMAD5, and SMAD8 are receptor-regulated SMADs (R-SMADs) in BMP signaling pathways, while SMAD2 and SMAD3 are responsive to TGF-β signaling. Nevertheless, specificity of activation of R-SMADs by TGF-β family ligands is not as strict as previously thought. Thus, BMP receptors have been shown to phosphorylate SMAD2 in various cells [49], i.e. in endothelial cells SMAD1 and SMAD5 are activated by TGF-β via ALK-1 and ALK-2. Upon activation by type II receptor, type I receptor phosphorylates R-SMAD. Subsequently, R-SMADs form a complex with common partner SMAD (co-SMAD) and this complex translocates into the nucleus to bind to the promoter of target genes [45]. SMAD4 is the only co-SMAD in mammalians shared by both BMP and TGF-β signaling pathways. Inhibitory SMADs (I-SMADs), which are SMAD6 and SMAD7, are negative regulator of the action of R-SMADs and/or co-SMADs [45]. I-SMADs interact with activated type I receptors, but unlike R-SMADs they are not released and thus prevent the activation and R-SMADs [45]. For example, SMAD7 inhibits both BMP and TGF-β signaling, whereas SMAD6 preferentially represses BMP signaling. SMAD7 interacts with type I receptor of the ALK-1, BMPR-I and TβR-1 groups and in the nucleus can disrupt the formation of a functional SMAD–DNA complex [50]. SMAD6 inhibits signals from the BMPR-I group [51] and can compete with SMAD4 by forming a complex with SMAD1. Interestingly, R-SMADs, co-SMADs, I-SMADs, form a negative feed-back loop for the regulation of TGF-β family signaling as I-SMADs are strongly induced by TGF-β/activins and BMPs [52] (Table 1).

In the nucleus, SMADs regulate the expression of target genes by directly binding to DNA sequences and recruiting coactivators and/or corepressor proteins (Fig. 1). Among the DNA binding proteins that interact with BMP-specific SMADs are RUNX2 and Schnurri-2. BMP-specific R-SMADs and RUNX2 cooperatively promote the transcription of genes responsible for osteoblast differentiation [53]. Upon BMP-2 stimulation, Schnurri-2 translocates into the nucleus where it interacts with the complex SMAD1/4 and C/EBPα to induce PPARγ2, a key transcription factor for adipogenesis [36].

5. Bone morphogenic proteins and adipogenesis: form mesenchymal stem cells to mature adipocytes

Adipogenesis is a multistep process that can be described in the context of two main phases: commitment and terminal differentiation. Cell fate determination is affected by signaling molecules that are involved in the evolution of mesodermal tissues. These include fibroblast growth factors (FGFs), wingless (WNT), and members of the TGF-β family such as the aforementioned BMPs. These molecules can act in an endocrine, autocrine and a paracrine fashion. In the latter case they are produced from the microenvironment i.e. niche and provide instructions for commitment of precursor cells into pre-adipocytes [54]. Upon commitment, in order to become mature adipocytes, pre-adipocytes need to be released from suppressive signaling molecules including members of the WNT family [55], protein of the retinoblastoma (Rb) family [56,57] pre-adipocyte factor 1 (Pref-1), a member of the Notch/Delta/Serrate family of epidermal growth factor-like repeat-containing proteins [58], and Necdin, a member of the melanoma-associated antigen family of protein [59].

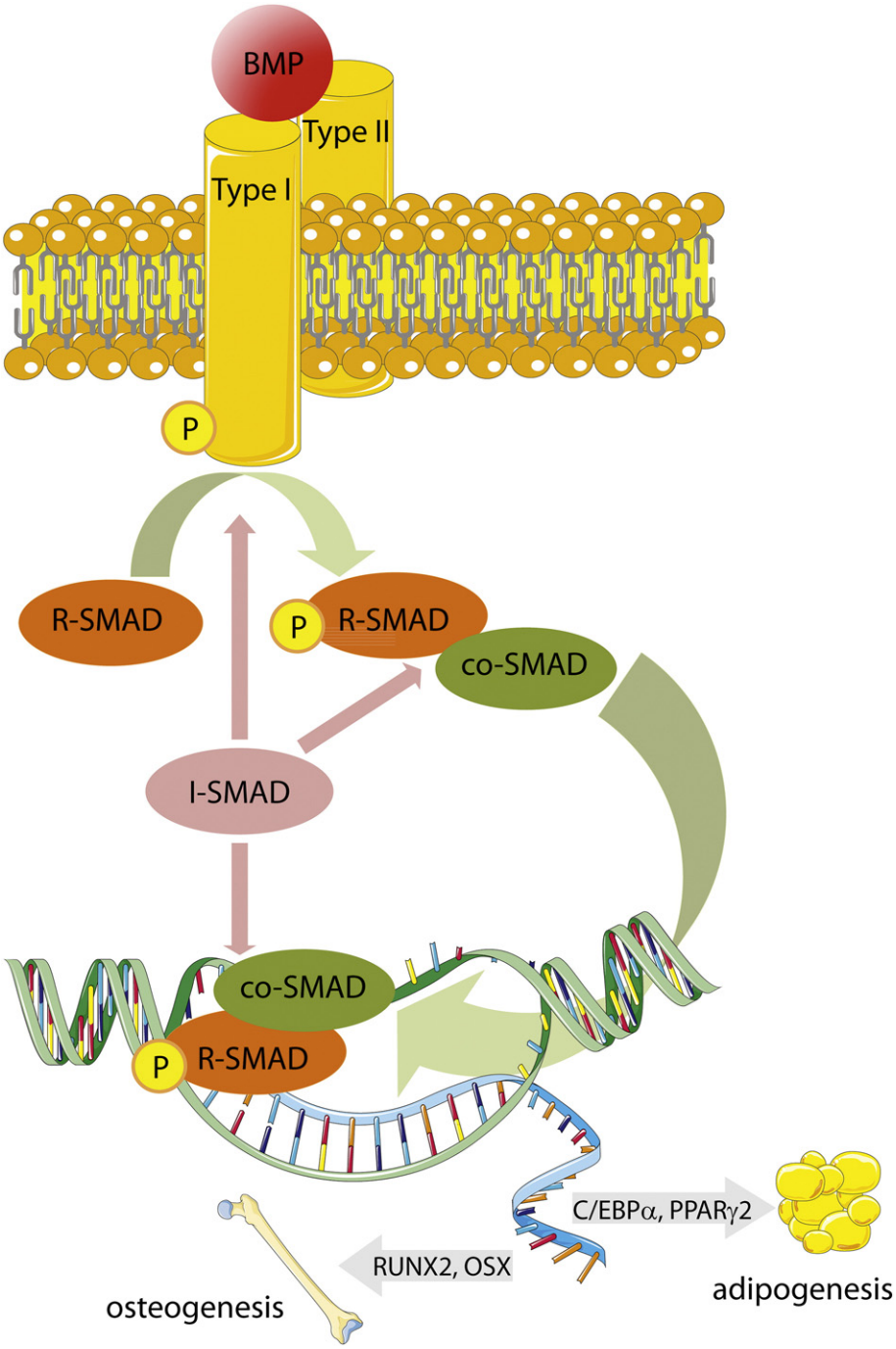

Fig. 1. The BMP signaling pathway. Two types of serine–threonine kinase receptors transduce BMP signals named type I and type II receptors. Initially, BMP ligands promote the formation of type I–II heterodimers. Consequently, type II receptor phosphorylates type I receptor, which in turn activates R-SMADs (SMAD1–2–3–5–8) by phosphorylation. Upon activation, R-SMAD forms a complex with co-SMAD (SMAD-4), which translocates into the nucleus to regulate the expression of target genes such as RUNX2 and OSX (osteogenesis) or C/EBPα and PPARy2 (adipogenesis). I-SMADs (SMAD6–7) interfere with phosphorylation of R-SMADs, formation of R-SMAD/co-SMAD complex and formation of a functional SMAD/DNA complex.

Notably, Necdin and the Rb family members also selectively suppress brown adipogenesis at an early stage [56,59].

Although the knowledge about the commitment state of adipogenesis is unclear, a great deal of information is available about the terminal differentiation process of adipocytes. After being released from suppression, pre-adipocytes progress towards a mature adipocyte state through a transcriptional cascade of events that includes members of the transcription factors CCAAT/enhancer binding protein (C/EBPs) and the master regulator of adipogenesis PPARγ. Notably, some factors have been identified that are responsible for a white or brown adipocyte differentiation. In this regard, members of the Rb protein family, and the nuclear corepressor receptor-interacting protein 140 (RIP140) promote a white phenotype [60–62], while PRDM16, as alluded to above, is required to determine a brown phenotype [35]. BMPs have been also suggested to play an important role in the control of white versus brown adipose fate determination by acting at the commitment stage of adipogenesis. In particular, it has been shown that BMP-2 and BMP-4 can promote commitment of MSCs into white pre-adipocytes [63–65], whereas BMP7 drives brown pre-adipocyte commitment [23].

BMPs are niche factors that provide instructive cues to MSCs. As morphogens, BMPs are either secreted from the niche in proximity of MCSs to act as paracrine effectors or blood-derived factors secreted

from other organs to act as endocrine factors. The effects of BMPs on fat development seem to be evolutionary conserved as Drosophila larvae missing the BMP-7 homologue glass bottom boat (gbb-60A) display impaired fat body development [66]. In line with this observation, different BMP gradients might instruct the formation of the different fat depots distributed in our body. Since there is a relationship between the way fat is distributed in our body and our metabolic phenotype [67], how the embryonic BMP gradients shape our fat distribution might influence the susceptibility of developing obesity and other metabolic diseases.

The role of BMPs on adipogenesis is related to the dosage of these morphogens and to the stage of cell development. For instance, in bone marrow stromal cells, BMPs mainly promote osteogenesis while inhibiting adipogenesis [68–70]. On the contrary, BMP-4 has been shown to promote adipogenesis in embryonic stem cell-derived embryonic bodies [71]. In C3H10T1/2 cells, which are mouse embryonic fibroblasts established from 14-to-17-day-old embryos of the C3H mouse strain that show characteristics of MSCs [72], high concentration of BMP-2 and BMP-7 promotes osteogenesis, whereas low concentrations promote adipogenesis [73,74].

A key finding suggests that BMP-4 is involved in the earliest stages of adipogenic commitment. It is in fact possible to commit the MSC line C3H10T1/2 into a pre-adipocyte cell lineage by means of 5-azacytidine [63]. This treatment results in de-methylation of BMP4 promoter with consequent high expression levels of BMP-4. This event is critical as the BMP4 antagonist Noggin blocks the development of adipocyte characteristics [63].

Although early studies have established a link between BMPs and adipogenesis, only recently a more specific role for some BMP in directing white versus brown adipogenesis has emerged. While BMP-2 and BMP-4 have been shown to trigger commitment of C3H10T1/2 cells into white pre-adipocytes [63–65], BMP-7 can direct MSCs towards a brown fat lineage [23]. Notably, both BMP-4 and BMP-7 can promote lipid accumulation in NIH-3T3 cells, a cell line with no adipogenic features, but only BMP7 can induce the expression of brown fat selective genes such as UCP1 and PRDM16. BMP-7 does not only commit MSCs to a brown adipogenic lineage by itself, but it also can act in concert with other differentiating agents such as rosiglitazone to promote a brown phenotype in primary cultures derived from interscapular brown fat and in different embryonic fibroblast cell lines [23]. Finally, the more convincing evidence that BMP7 and BMP-2 are specifically required for BAT and WAT development, respectively, comes from knock-out studies. BMP-7 knock-out mice have a marked reduction in BAT, but not WAT, size [23], while Schnurri-2 knock-out mice display a white, but not brown, fat mass reduction [36]. This can be explained by the fact that Schnurri-2 is responsive to BMP-2 signaling by entering the nucleus to interact with SMAD1/4 and C/EBPα to promote PPARγ expression [36].

In addition to trigger commitment of MSCs into the adipogenic lineage, BMPs can also promote differentiation of already committed pre-adipocytes. In this regard, BMP-2 can promote maturation of 3T3-L1 and 3T3-F44A pre-adipocytes [74]. Notably, the BMP-2 induced terminal differentiation can be enhanced by rosiglitazone, suggesting a cross talk between BMP signaling and PPARγ [96]. Also BMP-7 can promote terminal differentiation of brown pre-adipocytes, even in the absence of an induction cocktail, but not that of white pre-adipocytes [23].

In conclusion, different members of the BMP family can determine a brown versus white adipogenesis (Fig. 2). Other members of this family will need to be investigated to address their role in white versus brown adipogenesis.

6. The dual role of bone morphogenic proteins in adipogenesis and osteogenesis

Bone marrow MSCs can either differentiate into adipocyte or osteoblast, an event that mutually excludes the other. In the bone, under normal conditions, the commitment into these two lineages is tightly regulated in favor of bone formation. Thus, the physiological milieu of the bone marrow must contain appropriate levels of certain hormones, nutrients and growth factors that promote osteoblastogenesis while suppressing adipogenesis. Changes in this milieu that can occur with aging, or through certain diseases and/or pharmacological treatments are responsible for loss of bone mass and an increase in bone morrow adiposity. For example, chronic activation of PPARγ by rosiglitazone or by the naturally occurring prostaglandin J2 (PGJ2) results in promotion of adipogenesis and inhibition of osteogenesis [75,76]. Indeed, obese subjects treated with PPARγ ligands to promote insulin sensitivity are more susceptible to bone fracture [77]. The inhibition of bone formation by PPARγ agonists occurs in part by suppression of master regulators of osteogenesis such as RUNX2, OSX and DLX5. On the other hand, PPARγ insufficiency was shown to enhance osteogenesis through osteoblast formation from bone marrow progenitors [78]. In addition to pharmacological treatments, aging is also responsible for a decrease in bone mineral density and a simultaneous increase in bone morrow adiposity. This event observed in humans and in aged-mice as well as in a mouse model of premature aging (SAMP6) is characterized at the molecular levels by an increased expression of PPARγ and a parallel decreased expression of RUNX2 [79].

Osteoblasts secrete several members of the BMP family, thus regulating in an autocrine and paracrine way bone mass by inducing bone marrow MSC differentiation [80,81]. Moreover, BMPs can enhance the metabolic function of mature osteoblasts [80,81]. SMADs and p38-MAPK are key mediators of BMP-driven osteogenesis by activating both RUNX2- dependent and -independent pathways that converge on OSX.

The specificity of the intracellular signal that determines, whether the MSC is committed to the osteogenic or adipogenic lineage is in part related to the type of BMP receptor involved. In this context, BMPR-IA has been linked to adipogenesis, while BMPR-IB has been linked to osteogenesis [82]. In line with the above mentioned findings, it was shown that committed pre-adipocytes display high levels of BMPR-IA compared to the parental C3H10T1/2 line, while the more osteogenic receptor BMPR-IB is expressed at low levels [63]. In addition to the type of receptor, also the concentration of BMPs has an effect on directing MSC into fat or bone cells. Thus, low dose of BMP-2 promotes adipogenesis, whereas high dose promotes osteogenesis [74].

Just like for osteogenesis, also the BMP-driven adipogenic commitment is mediated by simultaneous activation of SMAD transcription factors and p38-MAPK. SMAD signaling promotes PPARγ transactivation via cooperation with C/EBPα and the zinc finger transcription factor Schnurri-2. Indeed, after BMP-2 stimulation, Schnurri-2 shuttles into the nucleus where it interacts with SMAD1/4 complex and C/EBPα to promote PPARγ expression [36]. In line with this, overexpression of SMAD6, an antagonist of SMAD1, blocks the expression of PPARγ in C3H10T1/2 MSCs and consequently differentiation into the adipogenic lineage by BMP-2 [83].

7. Bone morphogenic proteins and energy homeostasis

BMPs have initially been thought to have a biological relevance only in the determination of the MSC fate. However, recent findings highlight a new role for BMPs in energy balance (Fig. 2). In this regard, BMP-8B, a molecule that is expressed in the central nervous system (CNS) and in mature brown adipocytes, has been recently shown to act as player of the thermogenic machinery in BAT rather than a factor trigging commitment/differentiation of MSCs into the adipogenic lineage [84]. Indeed, BMP-8B is not required for normal BAT development, as shown in BMP-8B knock-out mice, but it functions to promote a greater thermogenic response in times of increased demand of heat production such as cold conditions and overfeeding. Mechanistically, at the peripheral level, BMP-8B is produced from mature brown adipocytes where it acts in an autocrine way to increase P38-MAPK signaling, which is important for an adequate UCP1 induction, and to promote activation of the hormone sensitive lipase (HSL), which would be

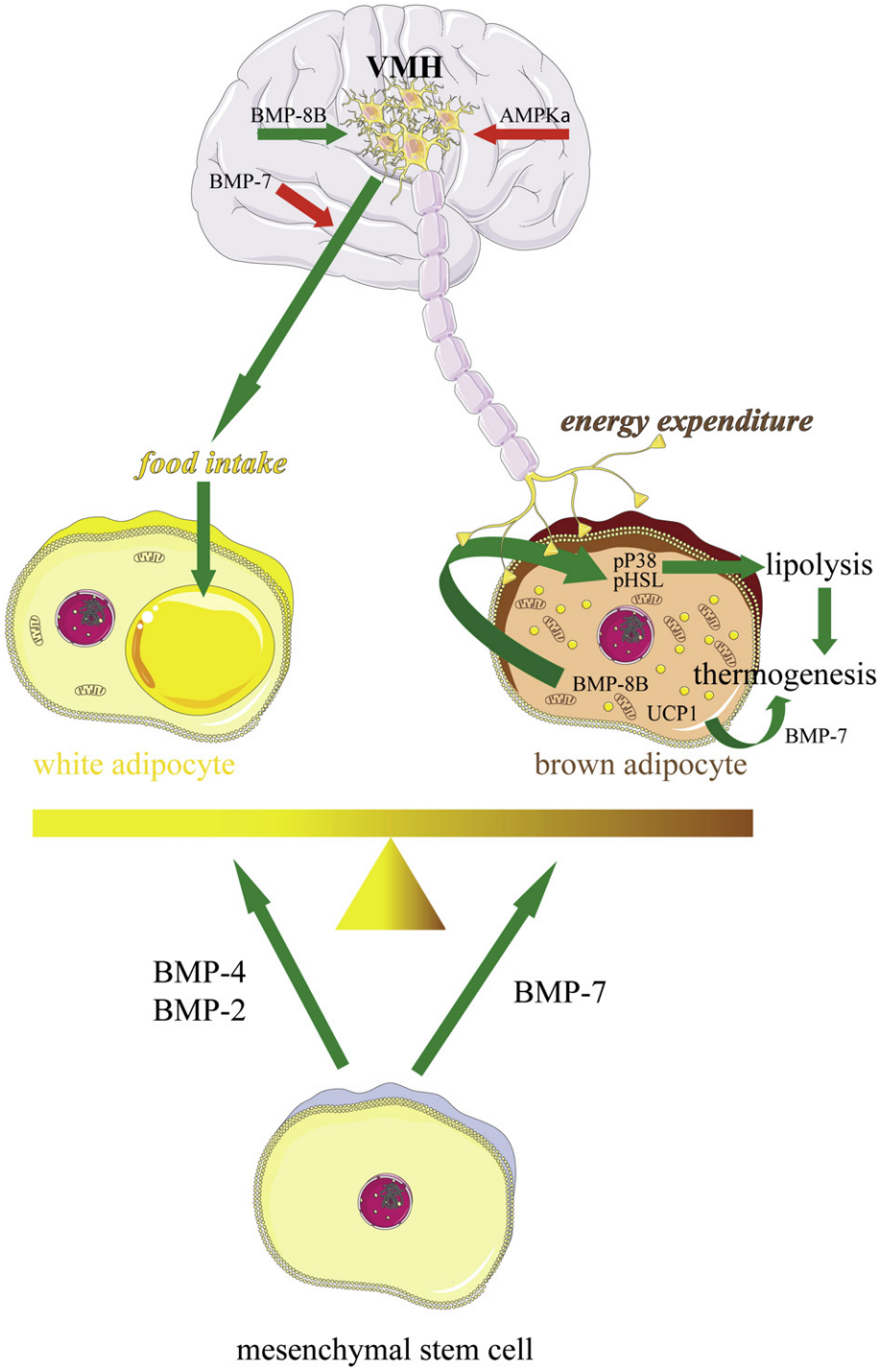

Fig. 2. Role of bone morphogenic proteins in adipogenesis and energy homeostasis. BMP-2 and BMP-4 trigger commitment of MSCs into white adipocytes, whereas BMP-7 promotes the brown adipocyte lineage. In addition, BMP-7 can also affect energy homeostasis. At the peripheral level, BMP-7 acts on mature brown adipocytes by promoting thermogenesis via induction of UCP1. At the central level, BMP-7 acts on the VMH to block food intake. BMP-8B is another factor regulating energy homeostasis, however without affecting adipogenesis. BMP-8B is produced from mature brown adipocytes where it acts in an autocrine manner to synergize with the SNS to regulate lipolysis, and thermogenesis, by inducing the phosphorylation and consequent activation of P38-MAPK (pP38-MAPK) and HSL (pHSL). At the central level, BMP-8B also acts on the VHM to increase sympathetic tone of BAT and consequent thermogenesis. Notably, AMPKα antagonizes the central effects of BMP-8B. In summary, due to the opposing role of white and brown adipocytes in energy intake and energy expenditure, their metabolic equilibrium is fundamental for the maintenance of body weight. Green arrows indicate positive effects, red arrows indicate inhibitory effects.

responsible for a greater lipolytic activity and likely thermogenic activity in response to a β-adrenergic stimulation [84].

Besides its peripheral function in BAT, BMP-8B also has a central thermogenic role being expressed in key hypothalamic nuclei controlling energy balance in the ventral medial hypothalamus (VMH) area. Indeed, central treatment with BMP-8B enhances thermogenesis with an increase in the sympathetic tone of BAT. The importance of the coordination between the central and peripheral activities of BMP-8B is demonstrated by BMP-8B knock-out mice that are susceptible to weight gain due to impaired thermogenesis. This phenotype becomes even more evident when mice are challenged by a high fat diet. Interestingly, overexpression of AMPKα in the CNS completely inhibits the activation of BAT by BMP-8B, suggesting an opposing role of AMPKα versus BMP-8B for the maintenance of the energy balance via BAT.

Besides BMP-8B, BMP-7 has been shown to positively impact on energy homeostasis by promoting brown adipogenesis and BAT-related energy expenditure. Unlike BMP-8B, BMP7 it is not expressed in mature brown adipocytes, suggesting that it does not work as an autocrine factor, but rather as an endocrine factor. Moreover, BMP-7 is required for BAT development as BMP-7 knock-out mice show smaller BAT which could be due to an in-appropriate thermogenic response. Several regions of the brain, including the VMH, express BMP-7 suggesting that similar to BMP-8B also BMP-7 might regulate BAT function via a central mechanism [85]. Indeed, besides promoting energy expenditure via its direct effect on BAT development, recent findings also highlight a central role for BMP-7 in appetite regulation [86]. Systemic treatment of diet-induced obese mice with BMP-7 not only resulted in increased energy expenditure, but also in a decreased food intake. Weight loss and reduced appetite were also observed in *ob*/*ob* mice treated with BMP-7, suggesting that BMP-7 functions through a leptin independent mechanism [86].

Although some BMPs have been positively correlated with energy expenditure, other family members have a negative impact on the maintenance of body weight. Recently an increased expression of BMPR-IA, which binds with high affinity to BMP-2 and BMP-4, but not to BMP-7 [87], has been reported for both visceral and subcutaneous white fat depots of obese subjects [88]. Furthermore, corroborating the link between BMP-2/BMP-4 and obesity [89], BMPR-IA knock-out mice are protected from high fat diet induced obesity [90].

Taken together, these last findings indicate a critical and complex role for BMPs in systemic energy homeostasis via regulation of adipocyte development and also mature function.

8. Therapeutic opportunities based on bone morphogenic proteins

The continuously expanding epidemic of obesity clearly demonstrates that currently available medical treatments are not effective. A successful treatment must impact the whole-body energy balance by increasing energy expenditure and/or reducing body fat accumulation. To this end, a better understanding of the molecular mechanisms regulating adipogenesis of both brown and white adipose tissue is required. Although we have accumulated a great deal of knowledge about the terminal differentiation process of adipogenesis and this knowledge continues to be refined, still little is known about the commitment of stem cells into the adipocyte lineage.

Theoretically, by increasing the size of BAT it should be possible to raise energy expenditure. It has been calculated that in humans as little as 50 g BAT, which is less than 0.1% of body weight, could use up to 20% of basal calorific needs when maximally stimulated [91]. Indeed, dissipation of as little as 17 kcal/day, which is about 0.6% of daily total energy expenditure, could lead to a weight loss of 1 kg/year [92,93]. Potential therapeutics to elicit such an effect could for example mimic the action of BMP-7. Such kind of therapeutics might also function beyond their role in promoting BAT recruitment by PRDM16, as it has been shown that BMP-7 can reverse obesity and regulate appetite through mTOR in the brain [86].

BMP-7 clearly is an important factor for BAT formation as evidenced by the finding that BMP-7 promotes commitment of MSCs into the brown adipogenic lineage. In adult humans such a process could occur after chronic cold exposure, which induces hyperplasia and hypertrophy of BAT [94]. So far it is not known whether chronic cold exposure promotes BMP-7 expression either in BAT, or in WAT.

Novel opportunities for the design of anti-obesity drugs could also come from the recently uncovered role of BMP-8B in the regulation of energy balance both at the peripheral and the central level. AMPK is already considered as a drug target for the treatment of insulin resistance [95]. Thus, the newly uncovered relationship with BMP-8B and AMPK should influence the development of specific therapeutic interventions without the deleterious cardiovascular side effects associated with previously reported sympathetic nervous system (SNS) drugs.

Besides the connection between muscle and BAT, a new link between bone morrow and BAT has now emerged based on the findings that a particular hematopoietic cocktail containing cytokines known to drive hematopoiesis such as BMP-7 can generate brown adipocytes from human pluripotent stem cells (hPSCs) [96]. This is consistent with observations from Charles Huggins, who showed that rodents exposed to cold conditions increased not only BAT size, but also the marrow adiposity [97,98]. Moreover, it has been shown that murine bone morrow-derived adipocytes show a temperature-inducible expression of PRDM16 and UCP1 [99]. Taken together, a novel opportunity to treat obesity could be based on the use of a particular hematopoietic cocktail to transform autologous hPSCs into brown adipocytes for consequent sub-cutaneous implantation in obese subjects.

In conclusion, it has to be stated that research on adipose tissue formation and metabolic control in relation to BMP signaling is still in its early stages. As adipose tissue plays an important role in energy homeostasis, uncovering the detailed molecular mechanisms whereby BMPs regulate commitment of MSCs into the adipogenic lineage and balance energy intake with energy expenditure could provide new avenues for the design of an original class of drugs to counteract the epidemic of obesity.

Acknowledgements

Our work is supported by grant from EU FP7 project DIABAT.

References

[1] A.I. Caplan, S.P. Bruder, Mesenchymal stem cells: building blocks for molecular medicine in the 21st century, Trends Mol. Med. 7 (2001) 259–264.
[2] N. Billon, C. Dani, Developmental origins of the adipocyte lineage: new insights from genetics and genomics studies, Stem Cell Rev. 8 (2012) 55–66.
[3] S. Enerback, The origins of brown adipose tissue, N. Engl. J. Med. 360 (2009) 2021–2023.
[4] S. Gesta, Y.H. Tseng, C.R. Kahn, Developmental origin of fat: tracking obesity to its source, Cell 131 (2007) 242–256.
[5] S. Cinti, The adipose organ: morphological perspectives of adipose tissues, Proc. Nutr. Soc. 60 (2001) 319–328.
[6] S. Cinti, The adipose organ, Prostaglandins Leukot. Essent. Fatty Acids 73 (2005) 9–15.
[7] S. Cinti, Between brown and white: novel aspects of adipocyte differentiation, Ann. Med. 43 (2011) 104–115.
[8] A.M. Cypess, S. Lehman, G. Williams, I. Tal, D. Rodman, A.B. Goldfine, F.C. Kuo, E.L. Palmer, Y.H. Tseng, A. Doria, G.M. Kolodny, C.R. Kahn, Identification and importance of brown adipose tissue in adult humans, N. Engl. J. Med. 360 (2009) 1509–1517.
[9] M. Saito, Y. Okamatsu-Ogura, M. Matsushita, K. Watanabe, T. Yoneshiro, J. Nio-Kobayashi, T. Iwanaga, M. Miyagawa, T. Kameya, K. Nakada, Y. Kawai, M. Tsujisaki, High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity, Diabetes 58 (2009) 1526–1531.
[10] W.D. van Marken Lichtenbelt, J.W. Vanhommerig, N.M. Smulders, J.M. Drossaerts, G.J. Kemerink, N.D. Bouvy, P. Schrauwen, G.J. Teule, Cold-activated brown adipose tissue in healthy men, N. Engl. J. Med. 360 (2009) 1500–1508.
[11] K.A. Virtanen, M.E. Lidell, J. Orava, M. Heglind, R. Westergren, T. Niemi, M. Taittonen, J. Laine, N.J. Savisto, S. Enerback, P. Nuutila, Functional brown adipose tissue in healthy adults, N. Engl. J. Med. 360 (2009) 1518–1525.
[12] A. Koppen, E. Kalkhoven, Brown vs white adipocytes: the PPARgamma coregulator story, FEBS Lett. 584 (2010) 3250–3259.
[13] H. Aquila, T.A. Link, M. Klingenberg, The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane, EMBO J. 4 (1985) 2369–2376.
[14] B.B. Lowell, S.S. V, A. Hamann, J.A. Lawitts, J. Himms-Hagen, B.B. Boyer, L.P. Kozak, J.S. Flier, Development of obesity in transgenic mice after genetic ablation of brown adipose tissue, Nature 366 (1993) 740–742.
[15] J. Kopecky, G. Clarke, S. Enerback, B. Spiegelman, L.P. Kozak, Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity, J. Clin. Invest. 96 (1995) 2914–2923.
[16] K.W. Park, D.S. Halperin, P. Tontonoz, Before they were fat: adipocyte progenitors, Cell Metab. 8 (2008) 454–457.
[17] P. Seale, B. Bjork, W. Yang, S. Kajimura, S. Chin, S. Kuang, A. Scime, S. Devarakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnicki, D.R. Beier, B.M. Spiegelman, PRDM16 controls a brown fat/skeletal muscle switch, Nature 454 (2008) 961–967.
[18] K. Gessner, Tigurine Historiae Animalium Lib. I de Quadripedibus uiuiparis, 1551.
[19] R. Atit, S.K. Sgaier, O.A. Mohamed, M.M. Taketo, D. Dufort, A.L. Joyner, L. Niswander, R.A. Conlon, Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse, Dev. Biol. 296 (2006) 164–176.
[20] J.A. Timmons, K. Wennmalm, O. Larsson, T.B. Walden, T. Lassmann, N. Petrovic, D.L. Hamilton, R.E. Gimeno, C. Wahlestedt, K. Baar, J. Nedergaard, B. Cannon,

Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 4401–4406.
[21] J. Sanchez-Gurmaches, C.M. Hung, C.A. Sparks, Y. Tang, H. Li, D.A. Guertin, PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors, Cell Metab. 16 (2012) 348–362.
[22] P. Hasty, A. Bradley, J.H. Morris, D.G. Edmondson, J.M. Venuti, E.N. Olson, W.H. Klein, Muscle deficiency and neonatal death in mice with a targeted mutation in the myogenin gene, Nature 364 (1993) 501–506.
[23] Y.H. Tseng, E. Kokkotou, T.J. Schulz, T.L. Huang, J.N. Winnay, C.M. Taniguchi, T.T. Tran, R. Suzuki, D.O. Espinoza, Y. Yamamoto, M.J. Ahrens, A.T. Dudley, A.W. Norris, R.N. Kulkarni, C.R. Kahn, New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure, Nature 454 (2008) 1000–1004.
[24] S. Kajimura, P. Seale, T. Tomaru, H. Erdjument-Bromage, M.P. Cooper, J.L. Ruas, S. Chin, P. Tempst, M.A. Lazar, B.M. Spiegelman, Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex, Genes Dev. 22 (2008) 1397–1409.
[25] L. Sun, H. Xie, M.A. Mori, R. Alexander, B. Yuan, S.M. Hattangadi, Q. Liu, C.R. Kahn, H.F. Lodish, Mir193b-365 is essential for brown fat differentiation, Nat. Cell Biol. 13 (2011) 958–965.
[26] J. Wu, P. Bostrom, L.M. Sparks, L. Ye, J.H. Choi, A.H. Giang, M. Khandekar, K.A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W.D. van Marken Lichtenbelt, J. Hoeks, S. Enerback, P. Schrauwen, B.M. Spiegelman, Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human, Cell 150 (2012) 366–376.
[27] B. Cousin, S. Cinti, M. Morroni, S. Raimbault, D. Ricquier, L. Penicaud, L. Casteilla, Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization, J. Cell Sci. 103 (Pt 4) (1992) 931–942.
[28] A. Vegiopoulos, K. Muller-Decker, D. Strzoda, I. Schmitt, E. Chichelnitskiy, A. Ostertag, M. Berriel Diaz, J. Rozman, M. Hrabe de Angelis, R.M. Nusing, C.W. Meyer, W. Wahli, M. Klingenspor, S. Herzig, Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes, Science (New York, N.Y.) 328 (2010) 1158–1161.
[29] P. Young, J.R. Arch, M. Ashwell, Brown adipose tissue in the parametrial fat pad of the mouse, FEBS Lett. 167 (1984) 10–14.
[30] N. Petrovic, T.B. Walden, I.G. Shabalina, J.A. Timmons, B. Cannon, J. Nedergaard, Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes, J. Biol. Chem. 285 (2010) 7153–7164.
[31] A. Vitali, I. Murano, M.C. Zingaretti, A. Frontini, D. Ricquier, S. Cinti, The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes, J. Lipid Res. 53 (2012) 619–629.
[32] B. Xue, J.S. Rim, J.C. Hogan, A.A. Coulter, R.A. Koza, L.P. Kozak, Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat, J. Lipid Res. 48 (2007) 41–51.
[33] T.J. Schulz, T.L. Huang, T.T. Tran, H. Zhang, K.L. Townsend, J.L. Shadrach, M. Cerletti, L.E. McDougall, N. Giorgadze, T. Tchkonia, D. Schrier, D. Falb, J.L. Kirkland, A.J. Wagers, Y.H. Tseng, Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 143–148.
[34] K. Almind, M. Manieri, W.I. Sivitz, S. Cinti, C.R. Kahn, Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 2366–2371.
[35] P. Seale, S. Kajimura, W. Yang, S. Chin, L.M. Rohas, M. Uldry, G. Tavernier, D. Langin, B.M. Spiegelman, Transcriptional control of brown fat determination by PRDM16, Cell Metab. 6 (2007) 38–54.
[36] W. Jin, T. Takagi, S.N. Kanesashi, T. Kurahashi, T. Nomura, J. Harada, S. Ishii, Schnurri-2 controls BMP-dependent adipogenesis via interaction with Smad proteins, Dev. Cell 10 (2006) 461–471.
[37] M.C. Ramel, C.S. Hill, Spatial regulation of BMP activity, FEBS Lett. 586 (2012) 1929–1941.
[38] B.L. Hogan, Bone morphogenetic proteins: multifunctional regulators of vertebrate development, Genes Dev. 10 (1996) 1580–1594.
[39] M.R. Urist, Bone: formation by autoinduction, Science 150 (1965) 893–899.
[40] F.P. Luyten, N.S. Cunningham, S. Ma, N. Muthukumaran, R.G. Hammonds, W.B. Nevins, W.I. Woods, A.H. Reddi, Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation, J. Biol. Chem. 264 (1989) 13377–13380.
[41] J.M. Wozney, The bone morphogenetic protein family and osteogenesis, Mol. Reprod. Dev. 32 (1992) 160–167.
[42] D. Chen, M. Zhao, G.R. Mundy, Bone morphogenetic proteins, Growth Factors 22 (2004) 233–241.
[43] Q. Kang, W.X. Song, Q. Luo, N. Tang, J. Luo, X. Luo, J. Chen, Y. Bi, B.C. He, J.K. Park, W. Jiang, Y. Tang, J. Huang, Y. Su, G.H. Zhu, Y. He, H. Yin, Z. Hu, Y. Wang, L. Chen, G.W. Zuo, X. Pan, J. Shen, T. Vokes, R.R. Reid, R.C. Haydon, H.H. Luu, T.C. He, A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor cells, Stem Cells Dev. 18 (2009) 545–559.
[44] D.B. Constam, E.J. Robertson, Regulation of bone morphogenetic protein activity by pro domains and proprotein convertases, J. Cell Biol. 144 (1999) 139–149.
[45] R. Nishimura, K. Hata, F. Ikeda, T. Matsubara, K. Yamashita, F. Ichida, T. Yoneda, The role of Smads in BMP signaling, Front. Biosci. 8 (2003) s275–s284.
[46] K. Miyazono, Y. Kamiya, M. Morikawa, Bone morphogenetic protein receptors and signal transduction, J. Biochem. 147 (2010) 35–51.
[47] P.B. Yu, H. Beppu, N. Kawai, E. Li, K.D. Bloch, Bone morphogenetic protein (BMP) type II receptor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells, J. Biol. Chem. 280 (2005) 24443–24450.
[48] C.H. Heldin, K. Miyazono, P. ten Dijke, TGF-beta signalling from cell membrane to nucleus through SMAD proteins, Nature 390 (1997) 465–471.
[49] M. Murakami, H. Kawachi, K. Ogawa, Y. Nishino, M. Funaba, Receptor expression modulates the specificity of transforming growth factor-beta signaling pathways, Genes Cells 14 (2009) 469–482.
[50] S. Zhang, T. Fei, L. Zhang, R. Zhang, F. Chen, Y. Ning, Y. Han, X.H. Feng, A. Meng, Y.G. Chen, Smad7 antagonizes transforming growth factor beta signaling in the nucleus by interfering with functional Smad–DNA complex formation, Mol. Cell. Biol. 27 (2007) 4488–4499.
[51] K. Goto, Y. Kamiya, T. Imamura, K. Miyazono, K. Miyazawa, Selective inhibitory effects of Smad6 on bone morphogenetic protein type I receptors, J. Biol. Chem. 282 (2007) 20603–20611.
[52] W. Ishida, T. Hamamoto, K. Kusanagi, K. Yagi, M. Kawabata, K. Takehara, T.K. Sampath, M. Kato, K. Miyazono, Smad6 is a Smad1/5-induced smad inhibitor. Characterization of bone morphogenetic protein-responsive element in the mouse Smad6 promoter, J. Biol. Chem. 275 (2000) 6075–6079.
[53] Y.W. Zhang, N. Yasui, K. Ito, G. Huang, M. Fujii, J. Hanai, H. Nogami, T. Ochi, K. Miyazono, Y. Ito, A RUNX2/PEBP2alpha A/CBFA1 mutation displaying impaired transactivation and Smad interaction in cleidocranial dysplasia, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 10549–10554.
[54] D.L. Jones, A.J. Wagers, No place like home: anatomy and function of the stem cell niche, Nat. Rev. Mol. Cell Biol. 9 (2008) 11–21.
[55] S.E. Ross, N. Hemati, K.A. Longo, C.N. Bennett, P.C. Lucas, R.L. Erickson, O.A. MacDougald, Inhibition of adipogenesis by Wnt signaling, Science 289 (2000) 950–953.
[56] J.B. Hansen, C. Jorgensen, R.K. Petersen, P. Hallenborg, R. De Matteis, H.A. Boye, N. Petrovic, S. Enerback, J. Nedergaard, S. Cinti, H. te Riele, K. Kristiansen, Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4112–4117.
[57] A. Scime, G. Grenier, M.S. Huh, M.A. Gillespie, L. Bevilacqua, M.E. Harper, M.A. Rudnicki, Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha, Cell Metab. 2 (2005) 283–295.
[58] C.M. Smas, H.S. Sul, Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation, Cell 73 (1993) 725–734.
[59] Y.H. Tseng, A.J. Butte, E. Kokkotou, V.K. Yechoor, C.M. Taniguchi, K.M. Kriauciunas, A.M. Cypess, M. Niinobe, K. Yoshikawa, M.E. Patti, C.R. Kahn, Prediction of preadipocyte differentiation by gene expression reveals role of insulin receptor substrates and necdin, Nat. Cell Biol. 7 (2005) 601–611.
[60] F. Picard, M. Gehin, J. Annicotte, S. Rocchi, M.F. Champy, B.W. O'Malley, P. Chambon, J. Auwerx, SRC-1 and TIF2 control energy balance between white and brown adipose tissues, Cell 111 (2002) 931–941.
[61] J.H. Steel, R. White, M.G. Parker, Role of the RIP140 corepressor in ovulation and adipose biology, J. Endocrinol. 185 (2005) 1–9.
[62] Z. Wang, C. Qi, A. Krones, P. Woodring, X. Zhu, J.K. Reddy, R.M. Evans, M.G. Rosenfeld, T. Hunter, Critical roles of the p160 transcriptional coactivators p/CIP and SRC-1 in energy balance, Cell Metab. 3 (2006) 111–122.
[63] R.R. Bowers, J.W. Kim, T.C. Otto, M.D. Lane, Stable stem cell commitment to the adipocyte lineage by inhibition of DNA methylation: role of the BMP-4 gene, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13022–13027.
[64] H. Huang, T.J. Song, X. Li, L. Hu, Q. He, M. Liu, M.D. Lane, Q.Q. Tang, BMP signaling pathway is required for commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 12670–12675.
[65] Q.Q. Tang, T.C. Otto, M.D. Lane, Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 9607–9611.
[66] O. Khalsa, J.W. Yoon, S. Torres-Schumann, K.A. Wharton, TGF-beta/BMP superfamily members, Gbb-60A and Dpp, cooperate to provide pattern information and establish cell identity in the Drosophila wing, Development 125 (1998) 2723–2734.
[67] S. Gesta, M. Bluher, Y. Yamamoto, A.W. Norris, J. Berndt, S. Kralisch, J. Boucher, C. Lewis, C.R. Kahn, Evidence for a role of developmental genes in the origin of obesity and body fat distribution, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 6676–6681.
[68] T.L. Chen, W.J. Shen, F.B. Kraemer, Human BMP-7/OP-1 induces the growth and differentiation of adipocytes and osteoblasts in bone marrow stromal cell cultures, J. Cell. Biochem. 82 (2001) 187–199.
[69] J.M. Gimble, C. Morgan, K. Kelly, X. Wu, V. Dandapani, C.S. Wang, V. Rosen, Bone morphogenetic proteins inhibit adipocyte differentiation by bone marrow stromal cells, J. Cell. Biochem. 58 (1995) 393–402.
[70] R.C. Pereira, A.M. Delany, E. Canalis, Effects of cortisol and bone morphogenetic protein-2 on stromal cell differentiation: correlation with CCAAT-enhancer binding protein expression, Bone 30 (2002) 685–691.
[71] M.F. Taha, M.R. Valojerdi, S.J. Mowla, Effect of bone morphogenetic protein-4 (BMP-4) on adipocyte differentiation from mouse embryonic stem cells, Anat. Histol. Embryol. 35 (2006) 271–278.
[72] C.A. Reznikoff, D.W. Brankow, C. Heidelberger, Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division, Cancer Res. 33 (1973) 3231–3238.
[73] I. Asahina, T.K. Sampath, P.V. Hauschka, Human osteogenic protein-1 induces chondroblastic, osteoblastic, and/or adipocytic differentiation of clonal murine target cells, Exp. Cell Res. 222 (1996) 38–47.
[74] E.A. Wang, D.I. Israel, S. Kelly, D.P. Luxenberg, Bone morphogenetic protein-2 causes commitment and differentiation in C3H10T1/2 and 3T3 cells, Growth Factors 9 (1993) 57–71.
[75] O.P. Lazarenko, S.O. Rzonca, L.J. Suva, B. Lecka-Czernik, Netoglitazone is a PPAR-gamma ligand with selective effects on bone and fat, Bone 38 (2006) 74–84.
[76] B. Lecka-Czernik, E.J. Moerman, D.F. Grant, J.M. Lehmann, S.C. Manolagas, R.L. Jilka, Divergent effects of selective peroxisome proliferator-activated receptor-gamma 2

ligands on adipocyte versus osteoblast differentiation, Endocrinology 143 (2002) 2376–2384.
[77] A. Grey, Skeletal consequences of thiazolidinedione therapy, Osteoporos. Int. 19 (2008) 129–137.
[78] T. Akune, S. Ohba, S. Kamekura, M. Yamaguchi, U.I. Chung, N. Kubota, Y. Terauchi, Y. Harada, Y. Azuma, K. Nakamura, T. Kadowaki, H. Kawaguchi, PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors, J. Clin. Invest. 113 (2004) 846–855.
[79] E.J. Moerman, K. Teng, D.A. Lipschitz, B. Lecka-Czernik, Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways, Aging Cell 3 (2004) 379–389.
[80] H. Cheng, W. Jiang, F.M. Phillips, R.C. Haydon, Y. Peng, L. Zhou, H.H. Luu, N. An, B. Breyer, P. Vanichakarn, J.P. Szatkowski, J.Y. Park, T.C. He, Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs), J. Bone Joint Surg. Am. 85-A (2003) 1544–1552.
[81] M. Suzawa, Y. Takeuchi, S. Fukumoto, S. Kato, N. Ueno, K. Miyazono, T. Matsumoto, T. Fujita, Extracellular matrix-associated bone morphogenetic proteins are essential for differentiation of murine osteoblastic cells in vitro, Endocrinology 140 (1999) 2125–2133.
[82] D. Chen, X. Ji, M.A. Harris, J.Q. Feng, G. Karsenty, A.J. Celeste, V. Rosen, G.R. Mundy, S.E. Harris, Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages, J. Cell Biol. 142 (1998) 295–305.
[83] K. Hata, R. Nishimura, F. Ikeda, K. Yamashita, T. Matsubara, T. Nokubi, T. Yoneda, Differential roles of Smad1 and p38 kinase in regulation of peroxisome proliferator-activating receptor gamma during bone morphogenetic protein 2-induced adipogenesis, Mol. Biol. Cell 14 (2003) 545–555.
[84] A.J. Whittle, S. Carobbio, L. Martins, M. Slawik, E. Hondares, M.J. Vazquez, D. Morgan, R.I. Csikasz, R. Gallego, S. Rodriguez-Cuenca, M. Dale, S. Virtue, F. Villarroya, B. Cannon, K. Rahmouni, M. Lopez, A. Vidal-Puig, BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions, Cell 149 (2012) 871–885.
[85] K. Ohyama, R. Das, M. Placzek, Temporal progression of hypothalamic patterning by a dual action of BMP, Development 135 (2008) 3325–3331.
[86] K.L. Townsend, R. Suzuki, T.L. Huang, E. Jing, T.J. Schulz, K. Lee, C.M. Taniguchi, D.O. Espinoza, L.E. McDougall, H. Zhang, T.C. He, E. Kokkotou, Y.H. Tseng, Bone morphogenetic protein 7 (BMP7) reverses obesity and regulates appetite through a central mTOR pathway, FASEB J. 26 (2012) 2187–2196.
[87] W. Sebald, J. Nickel, J.L. Zhang, T.D. Mueller, Molecular recognition in bone morphogenetic protein (BMP)/receptor interaction, Biol. Chem. 385 (2004) 697–710.
[88] Y. Bottcher, H. Unbehauen, N. Kloting, K. Ruschke, A. Korner, D. Schleinitz, A. Tonjes, B. Enigk, S. Wolf, K. Dietrich, M. Koriath, G.H. Scholz, Y.H. Tseng, A. Dietrich, M.R. Schon, W. Kiess, M. Stumvoll, M. Bluher, P. Kovacs, Adipose tissue expression and genetic variants of the bone morphogenetic protein receptor 1A gene (BMPR1A) are associated with human obesity, Diabetes 58 (2009) 2119–2128.
[89] J.W. Son, M.K. Kim, Y.M. Park, K.H. Baek, S.J. Yoo, K.H. Song, H.S. Son, K.H. Yoon, W.C. Lee, B.Y. Cha, H.Y. Son, H.S. Kwon, Association of serum bone morphogenetic protein 4 levels with obesity and metabolic syndrome in non-diabetic individuals, Endocr. J. 58 (2011) 39–46.
[90] T. Schulz, T. Huang, Y. Mishina, Y. Tseng, Adipocyte-specific inactivation of type 1A bone morphogenetic protein receptor impacts systemic energy metabolism and fat physiology, Diabetes (2009), [Supplement 1].
[91] N.J. Rothwell, M.J. Stock, Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour, Clin. Sci. (Lond.) (1983) 19–23.
[92] E. Christiansen, L. Garby, T.I. Sorensen, Quantitative analysis of the energy requirements for development of obesity, J. Theor. Biol. 234 (2005) 99–106.
[93] J.C. Clapham, J.R. Arch, Thermogenic and metabolic antiobesity drugs: rationale and opportunities, Diabetes Obes. Metab. 9 (2007) 259–275.
[94] L. Bukowiecki, A.J. Collet, N. Follea, G. Guay, L. Jahjah, Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia, Am. J. Physiol. 242 (1982) E353–E359.
[95] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, J. Clin. Invest. 108 (2001) 1167–1174.
[96] M. Nishio, T. Yoneshiro, M. Nakahara, S. Suzuki, K. Saeki, M. Hasegawa, Y. Kawai, H. Akutsu, A. Umezawa, K. Yasuda, K. Tobe, A. Yuo, K. Kubota, M. Saito, Production of functional classical brown adipocytes from human pluripotent stem cells using specific hemopoietin cocktail without gene transfer, Cell Metab. 16 (2012) 394–406.
[97] C. Huggins, B.H. Blocksom, Changes in outlying bone marrow accompanying a local increase of temperature within physiological limits, J. Exp. Med. 64 (1936) 253–274.
[98] C. Huggins, S. Wiseman, A.H. Reddi, Transformation of fibroblasts by allogeneic and xenogeneic transplants of demineralized tooth and bone, J. Exp. Med. 132 (1970) 1250–1258.
[99] A. Krings, S. Rahman, S. Huang, Y. Lu, P.J. Czernik, B. Lecka-Czernik, Bone marrow fat has brown adipose tissue characteristics, which are attenuated with aging and diabetes, Bone 50 (2012) 546–552.