review

Understanding adipose tissue development from transgenic animal models

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Abstract The World Health Organization has recognized obesity as a health problem of pandemic proportions. Recent work led to major breakthroughs in the understanding of the molecular basis of adipose tissue development with the cloning and characterization of numerous genes involved in fat cell differentiation and metabolism. Transgenesis has proved very useful in establishing the physiological roles of these genes. Here we review transgenic models made to study adipose tissue's metabolic and trophic responses. Genetic modifications unexpectedly associated to alterations of adipose tissue development are also examined because of their potential involvement in obesity and energy balance regulation. After a description of the methodologies commonly used, we review the data obtained on transcription factors, metabolism, signal transduction, secreted products, and models of lipodystrophy. An overview of such integrative studies leads to a better understanding of the physiology of adipose tissue development. Alterations in expression levels of proteins involved at different steps of a regulatory pathway highlight the complementary roles of genes in the regulation of adipose tissue development. However, lack of phenotypes also illustrates the capacity of animals to set up adaptive mechanisms.—Valet, P., G. Tavernier, I. Castan-Laurell, J-S. Saulnier-Blache, and D. Langin. **Understanding adipose tissue development from transgenic studies. *J. Lipid Res.* 2002.** 43: **835–860.**

Supplementary key words fat cell • obesity • gene invalidation • additive or random insertional transgenesis • targeted transgenesis

Transgenesis has contributed to a better understanding of adipose tissue (AT) homeostasis and development mechanisms. A very large number of transgenic animal models have been created to study the involvement of AT proteins in obesity. The aim of the present paper is to give an overview of the transgenic studies performed in this field. We have focused the review on genes that are expressed in AT (Tables 1 and 2). The impact of the central nervous system control of food intake on AT development has been dealt with elsewhere and will not be covered here (1, 2).

TRANSGENIC TECHNIQUES APPLIED TO ADIPOSE TISSUE STUDIES

Since the initial studies on AT (3, 4), the technology has consistently been under development. In the introductory section, we describe the principles and specific applications in the field.

Animal models

The mouse remains the species of choice to produce transgenic animals and to analyze the consequences of the modifications introduced in its genome. The laboratory mouse is the premier animal model for the study of human diseases. The major and sometimes obvious reasons are briefly that *i*) its small size means that it is easy to manipulate and, it lives in colony so keeping mouse lines requires less room than growing other transgenic mammals; *ii*) mating can occur at only 6 weeks and the generation time is around 12 weeks; *iii*) numerous (6–12) animals per litter are obtained; and *iv*) prices, depending on the strain used, are relatively low. The mouse model is made even more attractive because of the extensive and varied genetic tools available. These tools include a high-resolution genetic map, near complete sequencing of the

Abbreviations: ACC, acetylCoA carboxylase; ADD1, adipocyte determination and differentiation factor 1; AGT, angiotensinogen; aP2/ALBP, adipocyte lipid binding protein; AR, adrenergic receptor; ASP, acylation-stimulating protein; AT, adipose tissue; BAT, brown adipose tissue; 11β HSD-1, 11β hydroxysteroid deshydrogenase type 1; C/EBP, CCAAT-enhancer binding protein; CMV, cytomegalovirus; DGAT, diacylglycerotransferase; Cre, cyclization recombination; DT-A, diphtheria toxin A; EDG, endothelial differentiation gene; ES, embryonic stem; FAS, fatty acid synthase; FAT, fatty acid transporter; FOXC2, Forkhead box C2; GH, growth hormone; GLUT 4, glucose transporter 4; HMG, high mobility group protein; HSL, hormone sensitive lipase; IGF, insulin growth factor; IR, insulin receptor; IRES, internal ribosome entry site; IRS, insulin receptor substrate; iNOS, inducible NO synthase; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome-proliferator activated receptors; PTP1B, protein tyrosine phosphatase 1B; RXR, retinoid X receptor; SREBP, sterol responsive element binding protein; TNFα, tumor necrosis factor α; TZD, thiazolidinediones; UCP, uncoupling protein; WAT, white adipose tissue.

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TABLE 1. Models of additive transgenesis for the study of adipose tissue

Promoter	Tissue Target	Reference	Gene	Chapter	Paragraph	AT Phenotype
aP2/ALBP	AT	79	FoxC2	Transcription factors	Forkhead box C2	Reduced abdominal WAT
		113	GLUT4	Lipid and glucose metabolism	Glucose metabolism	Increased AT
		124–126	UCP1		Thermogenesis	Reduced subcutaneous AT
		132	β_1-AR	Endocrine responses and signal transduction	Adrenergic receptors	Reduced AT
		136	β_3-AR			Not studied
		138	α_2-AR			Increased AT in mice lacking β_3-AR under high fat diet
		184	11β HSD-1		Glucocorticoids	Visceral obesity
		189	Leptin	Adipocyte secretions	Cytokines	Variations in AT weights depending on age
		31, 216	DT-A	Genetic ablation of AT – Models of lipodystrophies	Transgenesis strategies and depletion of WAT	Reduced WAT after 6 months of age
		210, 212, 214, 215, 217	A-ZIP/F			Loss of WAT. Reduced BAT
		211, 213	nSREBP1c			Reduced WAT. Increased BAT
UCP1	BAT	136	β_3-AR	Endocrine responses and signal transduction	Adrenergic receptors	Not studied
		30, 218, 219, 220	DT-A	Genetic ablation of AT – Models of lipodystrophies	Transgenesis strategies and depletion of BAT	Reduced BAT. Increased body lipid content
GLUT4	Muscle and AT	115–117	Glutamine: fructose-6-phosphate-amido transferase	Lipid and glucose metabolism	Glucose metabolism	No difference
PEPCK	Depending on the promoter fragment used (liver and adipose tissue)	17				
	Liver, skin	86	ApoC1	Lipid and glucose metabolism	Lipoprotein metabolism	Reduced visceral AT and loss of subcutaneous AT
		88	ApoAII			Increased AT
	Liver, kidney and several parts of the gut	176	IGFII	Endocrine responses and signal transduction	Insulin growth factors and associated proteins	No difference
Serum amyloid P	Liver	190, 215	Leptin	Adipocyte secretions	Cytokines	Reduced AT
Leptin (proximal 762 bp)	Ubiquitous	173	GH	Endocrine responses and signal transduction	Growth hormone	Reduced AT in female
Major histo-compatibility complex class I (H-2K)	Ubiquitous	84	Truncated HMGIc	Transcription factors	Other transcription factors	Increased WAT to lipomas
CMV	Ubiquitous	83	Truncated HMGIc	Transcription factors	Other transcription factors	Increased WAT to lipomas
		177, 178	IGFBP1	Endocrine responses and signal transduction	Insulin growth factors and associated proteins	No difference
		179	IGFBP2			No difference

genome, and technologies that allow direct and specific manipulation of its genome. From 1980 to 1985, the mouse represented the only species in which developmental physiologists and molecular biologists were able to introduce foreign DNA by microinjection into the fertilized egg or zygote. Furthermore, isolation of pluripotent embryo-derived stem cells, which are essential for homologous recombination, is only possible from the mouse.

Before starting a transgenesis protocol, it remains very important to spend time on the choice of the appropriate mouse strain. It must possess characteristics that allow easy preparation and injection of embryo, and the capacity to develop the expected phenotype. The inbred strains C57BL/6, BALB/cBy, 129/Sv, and the F1 hybrids C57BL/6 × CBA/Ca or C57BL/6 × DBA/2 have proved very useful. However, mice with various genetic backgrounds could behave differently and present various metabolic responses when submitted to the same pharmacological or physiological stimuli. As early as in the 1970s, Hummel and collaborators (5) studied the influence of the genetic background on the db/db mutation that is now known to be associated to a lack of the long-form of the leptin receptor. The db/db C57BL/6 mice develop obesity whereas the db/db C57BL/Ks mice are obese and diabetic. Chronic intraperitoneal infusion of leptin significantly reduced body fat in lean db/+ C57BL/6 but not in C57BL/Ks mice (6). Comparison of the effects of varying dietary macronutrient content on the body composition in AKR or SWR mice showed that the AKR strain had a greater percentage of carcass fat and was more responsive to the effects of dietary fat composition compared with the SWR strain (7). Differences in cold-sensitivity between uncoupling protein (UCP) 1-deficient congenic and F1 hybrid mice have also been reported (see below) (8).

The rat is the other rodent used as transgenic animal, e.g., as cardiovascular model of hypertension (9). It also represents a tool in the pharmacological studies related to diabetes (10), to lipoprotein metabolism (11) or to obesity (see below). However, practical and technical disadvantages of the rat model include difficulties in seeing the vaginal plug of the female and the low percentage of transgenic founders varying from 5% to 10% according to the strain (vs. 25% in the mouse). Moreover, although embryonic stem (ES) cells were isolated in vitro, cultured, and used to produce chimeras by injection into rat blastocysts, these chimeras have never demonstrated germ line transmission (12).

Genetic mutations of genes responsible for human disease do not always result in appropriate pathological models in rodents. Moreover, a number of invasive techniques, e.g., to study cardiovascular function, are available, but are limited to single experiments because the mouse does not survive the analysis. For these reasons, some investigators have chosen as transgenic models larger mammals like the rabbit. This animal, more relevant in cardiovascular research than the rodents as a model for human hypertrophic cardiomyopathy (13) and in lipoprotein metabolism and/or atherosclerosis (14), also presents some drawbacks however. Sexual maturity occurs between 20 and 24 weeks (6 to 8 in the mouse), the cost of generating and maintaining a rabbit colony is high, only 50% of the zygotes recovered are fertilized (vs. 80% to 90% in mouse), mosaicism is frequently seen in the founder, and the success rate for generating transgenic animals is very low (1%). Nevertheless, the rabbit represents the smallest domestic animal that can be used to produce recombinant protein with therapeutic and commercial interest in its blood or milk (15).

Additive or random insertional transgenesis

The main application of additive transgenesis has been to express genes specifically in AT. This became possible because of the in vivo characterization of promoter regions conferring AT-specific expression (**Table 1**). The most widely used promoters are the adipocyte lipid binding protein (aP2/ALBP) and phosphoenolpyruvate carboxykinase (PEPCK) promoters (16, 17) that target both white adipose tissue (WAT) and brown adipose tissue (BAT), and the UCP1 promoter that targets only BAT (18, 19). Brinster and Palmiter established the characteristics of a transgene, which are essential to achieve proper expression (20). Linearized constructs integrate more efficiently than circular DNA (21), plasmid sequences are undesirable (22), and introns increase the expression of the transgene and the frequency of transgenic mice (23). A poly(A) tail is also an essential structure in the design of the transgene, because the activation of mRNA degradation is tightly coupled to deadenylation (24). Transcription terminators localized in the 3′ untranslated region, and particularly the stem-loop structures also belong to the elements involved in the enhancement of the transcription. Upstream of the open reading frame, a minimum length of the 5′ untranslated region (20 to 100 nucleotides) is required to obtain efficient translation. Generally, the mRNAs translated at a high rate possess a short 5′ untranslated region with a Kozak consensus sequence around the AUG (25). During the initiation of translation, the 40S ribosomal subunit binds to the cap structure (m^7GpppN) and scans the 5′ untranslated region in order to reach a functional initiation codon. Some mRNAs without a cap are, however, efficiently translated. In that case, the ribosomes bind to an internal ribosome entry site (IRES). The IRES can therefore be used to favor translation of a second cistron in a bicistronic mRNA. Several gene products could then be expressed under the control of the same promoter (26). It is often of great interest to target the nuclear compartment (e.g., for expression of transcription factors); this is done by adding to the transgene a short sequence named nuclear localization signals (27). Other elements controlling gene expression are located either upstream of the transcription initiation site and/or downstream of the poly(A) addition site. They include matrix and scaffold attachment regions, AT-rich sequences joining the nuclear matrix in the vicinity of genes, and locus control regions (28). Matrix and scaffold attachment regions generally enhance gene expression in cooperation with resident regulatory elements, thereby blocking chromosomal position effects. Intact locus con-

trol regions are required for complete insulation, i.e., to establish position-independent, copy number-dependent expression of transgenes with homologous or heterologous promoters. To avoid the engineering of complicated DNA constructs and to ensure a pattern of expression identical to that of the endogenous mouse gene, a large genomic fragment containing the most important regulatory sequences can be microinjected (29).

Besides targeted expression, other applications can be achieved using additive transgenesis. Tissue-specific ablation is possible through linkage of the sequence coding for the poisonous element of a toxin [such as diphtheria toxin subunit A (DT-A)] to WAT or BAT promoters (30, 31). The DT-A protein inactivates elongation factor-2 resulting in inhibition of protein synthesis and cell death. In this approach, cell killing is irreversible provided that threshold levels of intracellular toxin are attained. A conditional or inducible system exists that is based on the expression of the thymidine kinase gene of the herpes simplex virus (32). In the presence of nucleoside analogs, only the cells expressing thymidine kinase are killed. Suppression of gene expression in vivo can be achieved through targeted ablation (see below) but also through the expression of antisense RNA (33, 34). This method for gene disruption is technically easier to undertake than homologous recombination. Furthermore, it may represent a means to overcome in utero lethality by using promoter regions only active at birth. However, the decrease in the targeted protein expression is variable. Targeted oncogenesis has been used to induce hibernomas using the large tumor antigen of the simian virus 40 and derive immortalized brown fat cell lines (35, 36).

The DNA solution is generally injected into the pronucleus of a one-cell fertilized embryo which is reimplanted in the uterus of a pseudopregnant foster mother at this stage of development or after the first cellular division. This is the method of choice in the mouse. Pronuclear injection of DNA often results in multiple copies of the transgene arranged as head to tail concatamers inserted randomly into the host genome generally at a single integration site. Sometimes many integration sites co-exist that could represent an advantage in generating two or more lines from the same founder. Thus, screening of the F1 generation must rely on Southern blot analysis and not only on PCR amplification of the transgene. Upon breeding, transgenic DNA is inherited as a simple Mendelian trait. If this is not observed, it is assumed that integration of the transgene occurred after the first round of replication in the single fertilized egg cell and that the resulting transgenic mouse is mosaic for the transgene.

Targeted transgenesis

Targeted transgenesis using homologous recombination is the technique of choice to invalidate (knockout) or to introduce a subtle and precise mutation into a gene (**Table 2**). Homologous recombination relies on the naturally occurring but rare event of recombination between the targeting vector and its endogenous counterpart. The prerequisite is to know the exon-intron structure and the sequence of at least part of the targeted gene. The gene targeting construct is made up with genomic parts of sufficient lengths that surround a positive selectable gene encoding an enzyme conferring antibiotic resistance (neomycin, puromycin, or hygromycin). This vector is then electroporated into ES cells. Evans and Kaufman have shown that it is possible to establish culture cells derived from the inner cell mass of the early embryo at the blastocyst stage (37). Mouse ES cells remain diploid even after being cultured for several weeks and conserve the ability to proliferate vigorously in an undifferentiated state.

In order to distinguish random insertion, also named illegitimate recombination, from homologous recombination, the thymidine kinase cassette is generally attached to the 3′ end of the targeting vector (38). Hence, the number of ES clones to analyze is considerably diminished. Cells from a positive clone are introduced into recipient blastocysts and then these embryos are reintroduced into a pseudopregnant female. To determine the positive animals, the easiest technique is coat color selection. Indeed, ES cells usually come from 129/Sv mice with agouti color and the blastocysts are from C57BL/6 black mice. The more the coat of the offspring is agouti, the more the ES cells have been involved in the development of all cell types of the embryo. The chimeric animals are mated with C57BL/6 mice in order to obtain heterozygous animals for the mutation. It is usually necessary to get the mutation on the two alleles to obtain a phenotype, thus hemizygous offspring must be intercrossed in order to obtain homozygous mice.

Systems for conditional expression of transgenes

The importance of being able to control the expression and disruption of a transgene became clear when it was impossible to establish transgenic lines because the transgene product, or lack of it, was deleterious for the animal's development or impaired the fertility of the adult. Moreover, an alteration of gene expression at early embryonic stages is more likely to activate compensatory pathways that mask phenotypes. New approaches have been developed that place the genetic manipulations under stringent temporal and spatial control. Classical systems for inducible mammalian gene expression have typically encountered limitations such as low level of expression, basal leakiness, and toxic or nonspecific effects of inducing agents (39). Inducible systems that combine functional domains from prokaryotic, eukaryotic, and viral proteins to create chimeric transactivators capable of modulating gene expression in a drug-dependent manner were then developed. The other technology was based on the use of an enzymatic system where the enzyme is able to recombine DNA fragments at small specific sites.

The first system developed by Gossen and Bujard is an allosteric off switch where the transcription of the gene of interest is suppressed by the addition of tetracycline or its structural analog doxycycline (40, 41). In this system, the repressor of *Escherichia coli* Tn10 tetracycline resistance operon (tetR) is fused to the activation domain of the herpes simplex virus VP16 to produce the transactivator tTA,

TABLE 2. Models of knockout mice for the study of adipose tissue

Chapter	Paragraph	Gene	AT Phenotype	Reference	Note
Transcription factors	CCAAT-enhancer binding proteins	C/EBPβ and/or C/EBPδ	Reduced WAT	59	85% death at birth
		C/EBPα	Reduced AT	60	Perinatal death
	Peroxisome-proliferator activated receptors and retinoid X receptors	PPARγ	Reduced BAT*	64–66	Death in utero Rescue with tetraploid chimeras * Only one null mouse
		PPARβ	Reduced WAT	69, 70	
		RXRα		71, 72	Death in utero
		RXRα ablation in AT	Lack of obesity with high fat diet	53	Tissue-specific gene knockout
	Sterol responsive element binding proteins	SREBP1	No difference	76	85% death in utero
	Other transcription factors	HMGIc	Reduced WAT in Lep^{ob}/Lep^{ob} mice	85	
Lipid and glucose metabolism	Lipoprotein metabolism	VLDL receptor	Reduced visceral WAT, lack of subcutaneous WAT	87	
		LPL		90	Perinatal death
		LPL ablation in AT	Reduced weight gain in Lep^{ob}/ Lep^{ob} mice	89	Rescue in skeletal muscle
	Fatty acid transport and metabolism	ASP	Moderate decrease in AT	91, 92	
		CD36/FAT	Defective fatty acid uptake	93, 94	
		DGAT	Moderate decrease in AT	95	
		ACC2	Reduced AT	97	
	Adipose tissue lipolysis	HSL	Moderate decrease in gonadal AT. Increase in BAT	100, 101	
		aP2/ALBP	Increased WAT on Lep^{ob}/Lep^{ob} mice	104–108	
		Perilipin	Reduced AT	109, 110	
	Glucose metabolism	GLUT4	Reduced AT	114	
		GLUT4 ablation in AT	Insulin resistance, no difference in fat mass	51	Tissue-specific gene knockout
	Thermogenesis	UCP1	No difference for BAT	119	
		UCP2	No difference for AT	120, 123	
		UCP3	No difference for AT	121, 122	
		EiF4ebp1	Reduced AT	130	
Endocrine responses and signal transduction	Adrenergic receptors	β_3-AR	Small increase in WAT	134, 135	Disruption via oocyte microinjection
	GTP binding proteins	Gαs	Influence of imprinting on fat mass	139, 140	Death in utero of homozygous mice
	Protein kinase A	RIIβ	Reduced WAT	148, 149	
	Insulin and Insulin pathway	IR	Reduced dermal WAT	152	Perinatal death
		IR ablation in BAT	BAT atrophy	158	Tissue-specific gene knockout
		IRS1/IRS2	Marked reduction of WAT in newborn mice	161	
		p85α subunit of PI3K	Increased insulin sensitivity	163	
		PTP1B	Resistant to diet-induced obesity	164	
	Insulin growth factors and associated proteins	IGF1	Decreased epididymal WAT under sucrose fed diet	177, 178	Growth deficiency
		IGF2	Increased AT	174	Growth deficiency
		IGF1 receptor		174	Death at birth
		IGF2 receptor		174	Death in utero
	Estrogens	Aromatase	Increased abdominal WAT	180	
		Estrogen receptor α	Increased WAT	181	
		Prolactin receptor	Reduced abdominal WAT	187	

TABLE 2. (*Continued*)

Chapter	Paragraph	Gene	AT phenotype	Reference	Note
Adipocyte secretions	Cytokines	TNFα	Small decrease in AT under high-fat diet	193	
		TNF receptor 1	Increased insulin sensitivity in Lep^{ob}/Lep^{ob} mice	196	
		iNOS	Protection against high fat diet-induced insulin resistance	198	
		eNOS	Improved insulin sensitivity	199	
	Adhesion molecules	Interleukin 6	Mature onset obesity	201	
		Mac-1	Increased AT	202	
		ICAM-1	Increased AT	202	
		ADAMTS-1	Reduced AT	203	
	Angiotensinogen	Angiotensinogen	Reduced AT	205	
	Detoxification molecules	MTI and MTII	Increased AT	207	
	Lipids	Edg2	Reduced AT	209	50% perinatal death
Genetic ablation of adipose tissue. Models of lipodystrophies	Other models	lysosomal lipase	Decreased until loss of AT	221	

which binds to tet operator (tetO) DNA sequences upstream of target genes and activates transcription. In the allosteric off switch, the permanent addition of tetracycline or its analog to the drinking water to avoid transcriptional activity may lead to deleterious effects. Therefore, an alternative system, the "allosteric on switch" system, has been developed. Using chemical mutagenesis and genetic selection, a mutant TetR was identified that only binds tetO in the presence of the antibiotic. Fusing this to VP16 produces a new transactivator named rtTA (42, 43). In mice, different lines with transgenic transactivator, usually under the control of the cytomegalovirus (CMV) promoter and responder with TetO minimal promoter driving the gene of interest, have to be generated and crossed to obtain the double-transgenic offspring. Both transgenes segregate independently, making further breeding experiments tedious. Moreover, the copy number of the individual components in the genome of double-transgenic offspring may vary in different combinations and could be unbalanced. Steroid hormones and their receptors constitute the molecular basis of alternative allosteric on switch systems (44).

Using the recombinase activity of the cyclization recombination (Cre) gene from the P1 bacteriophage, conditional transgenesis and knockout became available. Cre recombinase catalyzes the recombination of two 34 bp-long consensus sites, the loxP (locus of X-over of P1) sites, without additional cofactors. With the Cre recombinase system, it is possible to excise loxP-flanked DNA segments (45). The structure of the conditional transgene is simple. The promoter and the gene of interest are separated by a loxP-flanked Stop region, which does not allow transcription initiated from the promoter to go through. When the Stop region is removed by Cre-mediated excision, the gene is expressed. Many variations have been added to this system. The conditional transgene can be expressed under the control of lineage/cell-specific promoter and the Cre recombinase under the control of either lineage/cell type-specific or ubiquitous promoters. The conditional transgene may also be driven by a ubiquitous promoter while the Cre expression is lineage/cell type-specific. This approach has been used to study the relationship between brown and white adipocyte lineages (46). It is possible to control Cre recombinase expression in a temporal manner using fusion protein between Cre recombinase and a mutated form of the ligand-binding domain of steroid nuclear receptor. These chimeric proteins do not bind the corresponding endogenous hormone, progesterone or estradiol, but keep the capacity to bind antagonists like RU486 or Tamoxifen (47, 48). Finally, some attempts were made to combine both the tetracycline Tet-off system and Cre technology in transgenic mice (49).

Another major application of the Cre/LoxP system is spatial and/or temporal gene invalidation (50–53). Through homologous recombination, the wild-type allele is replaced by a functional allele containing LoxP sites encompassing coding exons. Mice with the floxed allele are crossed with transgenic mice expressing the Cre recombinase to produce tissue-specific gene knockout (54). The system is also

used to remove the selectable marker from the targeted allele. Indeed there is convincing evidence that the presence of a selectable marker expression cassette can influence the expression of the neighboring genes (55). Such interference can create a phenotype that does not reflect the role of the targeted gene. Furthermore, a neomycin gene resistance construct has a cryptic acceptor site between the phosphoglycerokinase promoter and the neo^r coding region. This creates abnormal splicing events when the phosphoglycerokinase promoter-neo^r cassette is sitting in an intron with the same transcriptional orientation as the gene (56, 57).

TRANSCRIPTION FACTORS AND REGULATION OF ADIPOGENESIS

A tremendous amount of information has been collected on the molecular regulation of adipocyte differentiation (58). The most comprehensive set of data comes from studies on preadipocyte cell lines. When treated with appropriate media, the fibroblast-like preadipocytes undergo differentiation and acquire the morphology and characteristics of lipid-laden adipocytes. Despite the wealth of information obtained on these models, it is important to keep in mind the inherent differences with in vivo AT development. The immortalized preadipocyte cells are aneuploid. This property may induce differences in gene expression compared with adipocytes. Moreover, the cells are cultured out of the normal environment for AT, e.g., normal extracellular matrix in the presence of several cell types that can interact with each other. In that respect, the recent production of animal models with altered expression of key transcription factors for fat cell development has provided essential support for the current model of adipocyte differentiation (**Fig. 1**). Several classes of transcription factors and nuclear factors have been implicated in the control of adipocyte differentiation (58). Two groups of factors appear to be essential, CCAAT-enhancer binding proteins (C/EBPs) and peroxisome-proliferator activated receptors (PPARs). In vivo experiments, however, suggest the involvement of other proteins, but their exact contribution to the transcriptional network of adipogenesis awaits further studies.

C/EBPs

The C/EBPs belong to the basic-leucine zipper class of transcription factors. Six isoforms that play a role in the differentiation of several cell types, including hepatocytes and adipocytes, have been characterized. In culture systems of adipocyte differentiation, C/EBPβ and δ are expressed early but transiently. The factors have been shown to transactivate the C/EBPα and PPARγ genes. C/EBPα is induced later than C/EBPβ and δ. Its expression precedes the induction of many genes characteristic of the adipocytes. A severe phenotype is observed in mice lacking C/EBPβ and δ (59). Eighty five percent of the pups die within 24 h of birth. The survivors show markedly decreased accumulation of lipid and low expression of UCP1 in BAT. Epididymal WAT is reduced in adults but, unexpectedly, there is no alteration of C/EBPα and PPARγ gene expression or fat cell size. The decreased fat pad weight may therefore result from a lower number of adipocytes in knockout animals. However, embryonic fibroblasts derived from C/EBPβ and δ-null mice cannot differentiate into adipocytes and do not express C/EBPα and PPARγ. These findings suggest that, in vivo, some alternative pathways compensate for the lack of C/EBPβ and δ. The phenotype of C/EBPα null mice is also severe (60). The pups die within 8 h postpartum. They are lethargic and do not suck the mothers. Decreased expression of glucose 6-phosphatase and PEPCK in liver may explain the hypoglycemia observed at birth. Repeated injections of glucose allow the rescue of pups up to 40 h. Unlike wild-type mice, the C/EBPα null pups do not accumulate lipid in BAT and WAT. Fibroblasts from C/EBPα$^{-/-}$ mice have been used to investigate in vitro the role of the transcription factor in AT differentiation (61). Through expression and activation of PPARγ, the cells undergo differentiation but they accumulate less lipid than wild-type cells due to a defective induction of lipogenic genes. No induction of endogenous PPARγ gene expression is observed, indicating the occurrence of cross-regulation between C/EBPα and PPARγ. Another clear defect is the absence of insulin-stimulated glucose transport, which is partly explained by a decreased expression of the insulin receptor (IR) and insulin receptor substrate 1 (IRS-1). In vivo, the early death of the C/EBPα$^{-/-}$ mice precluded investigation of AT development. To improve the survival of the animals, transgenic mice that express C/EBPα in liver under the control of the albumin enhancer/promoter were crossed with C/EBPα$^{-/-}$ mice (62). C/EBPα expression in liver restored the mRNA levels of known hepatic gene targets of C/EBPα. The presence of the transgene improved the survival of C/EBPα$^{-/-}$ mice, which were investigated at 7 days of age. The knockout animals showed a complete lack of subcutaneous and visceral WAT. Interscapular BAT was present and contained more lipid than wild-type BAT. Surprisingly, mammary gland WAT developed normally. The data demonstrate that C/EBPα is required for the differentiation of preadipocytes to white fat cells in most WAT depots. However, the transcription factor is dispensable for the development of BAT and mammary gland WAT. The nature of the compensatory mechanisms is presently unknown. Another important role of C/EBPα is the control of cellular proliferation. The factor controls growth inhibition through repression of the transcription factor E2F. To demonstrate that the pathway was critical in vivo, the wild-type C/EBPα gene was replaced using homologous recombination by E2F repression-deficient alleles (63). In contrast to C/EBPα$^{-/-}$ mice, homozygotes for the knockin alleles reached adulthood. However, the animals showed severe hypotrophy of gonadal fat pads.

PPAR and retinoid X receptors

PPARs are members of the nuclear receptor superfamily. PPARs heterodimerize with retinoid X receptors (RXR) to bind DNA and activate transcription. PPARγ has

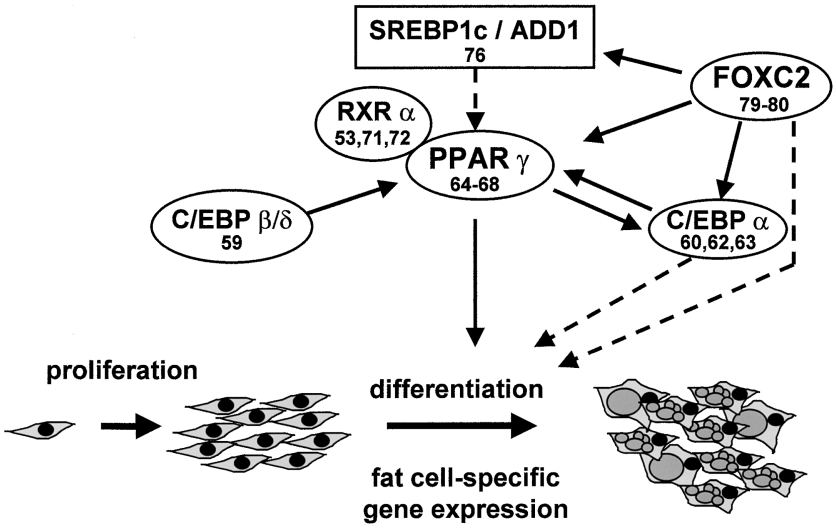

Fig. 1. The transcriptional control of adipogenesis. After proliferation of preadipocytes, the differentiation is promoted by several families of transcription factors. CCAAT-enhancer binding proteins (C/EBP)β and C/EBPδ are first expressed, followed by peroxisome-proliferator activated receptors γ (PPARγ), which in turn activate C/EBPα. C/EBPα exerts positive feedback on PPARγ to maintain differentiation. Sterol responsive element binding protein 1c (also named adipocyte determination and differentiation factor 1; ADD1/SREBP1) can increase the transcriptional activity of PPARγ. These factors induce the expression of genes that characterize the differentiated adipocyte phenotype. Forkhead box C2 (FOXC2) also activates genes that stimulate adipocyte differentiation (C/EBPα, PPARγ, and ADD1/SREBP1). References in parentheses correspond to transgenic overexpression or gene knockout of the proteins.

been shown to play a critical role in adipocyte differentiation. Two protein isoforms that differ in their amino terminus region have been characterized. PPARγ1 is expressed in several cell types including fat. PPARγ2 is almost exclusively expressed in AT. Targeted disruption of the PPARγ gene provokes cardiac malformation around embryonic day 10 due to a placental defect and in utero lethality (64–66). To bypass this developmental stage that precedes the appearance of AT, three different approaches have been used which showed that PPARγ was essential for fat development. First, to rescue the placental defect, chimeric embryos were produced with diploid PPARγ$^{-/-}$ cells and wild-type tetraploid cells that develop into extraembryonic lineages, such as placenta, but cannot contribute to the embryo formation (64). One homozygous animal developed to term. Although it had several defects and died shortly after birth, the pup lacked BAT. Second, a study of PPARγ$^{-/-}$ chimeric mice showed that adipocytes in WAT came exclusively from wild-type cells whereas other organs contained a mix of wild-type and negative cells (66). Third, ES cells or embryonic fibroblasts from PPARγ null mice did not differentiate into adipocytes (66). Cells from heterozygous animals had impaired lipid accumulation with decreased expression of C/EBPα, indicating that the cross talk between the two factors works in both directions (65). Furthermore, cell lines null for PPARγ were generated from mouse embryonic fibroblasts containing a floxed allele and a null allele (67). After immortalization, cells were infected with adenovirus expressing Cre recombinase to inactivate the floxed allele. In these cells, PPARγ but not C/EBPα restored adipogenesis. The data strongly suggest that PPARγ is the direct modulator of adipogenesis while the primary role of C/EBPα is maintenance of PPARγ level. The phenotype of heterozygous mice proved to be very informative (65, 68). Fed a standard diet, the animals had similar weight gain and fat mass as wild-type mice. However, they were resistant to high fat diet-induced obesity. Besides a direct role of PPARγ on the development of adipocyte hypertrophy, the phenotype may result from an increased expression of leptin accompanied by a decrease of food intake and an increase in energy expenditure. This is somewhat paradoxical because leptin production is usually proportional to adipocyte size. However, it has been shown that PPARγ agonists repress the leptin promoter activity. The ubiquitously expressed PPARβ (also named δ) has also been proposed to play a role in adipocyte differentiation. PPARβ null mice develop normally except that they are smaller than wild-type littermates (69, 70). Gonadal fat stores are reduced because of a decrease in cell number rather than cell size. To determine whether the reduction in fat pad mass was due to a loss of PPARβ function in adipocytes, mice with a selective depletion of PPARβ in AT were produced (70). No difference was observed between wild-type and transgenic animals, indicating that the decrease of fat mass in PPARβ$^{-/-}$ mice was a consequence of its expression in other tissues than AT.

As stated above, RXRs are the indispensable partners of PPARs. WAT expresses high levels of RXRα; however, its role cannot be investigated in RXRα$^{-/-}$ mice because the fetuses die in utero (71, 72). To alleviate this problem, specific ablation of RXRα was performed in AT using the Cre-Lox system (53). The authors produced a transgenic mouse line [aP2-Cre-ER$^{T2(tg/0)}$] that expresses a tamoxifen-inducible fusion protein between the Cre recombinase and a mutated ligand-binding domain of the human

estrogen receptor under the control of the aP2/ALBP promoter. Several rounds of crosses generated aP2-CreER$^{T2(tg/0)}$/RXRα$^{L2/-}$ mice in which the RXRα DNA binding domain is floxed on one allele and the RXRα gene is disrupted on the other. The technological tour de force yielded mice with spatiotemporal control of RXRα expression. Treatment of 4-week-old mice with Tamoxifen induced Cre-mediated excision of the floxed allele selectively in adipocytes. Mice with adipocyte ablation of RXRα did not develop obesity under a high fat diet or administration of monosodium glutamate, which provokes lesions in the hypothalamus. Adipocyte RXRα null mice had an impaired increase in plasma free fatty acid levels during fasting. The phenotype of the mice fed high fat diet is reminiscent of that of PPARγ$^{+/-}$ mice suggesting that PPARγ/RXRα heterodimers are indeed essential for the formation of hypertrophic adipocytes. Data in fasted mice reveal that RXRα is not only important for fat accretion but also for fat mobilization.

PPARγ has also recently been proposed as the target for agouti in adipocytes and thus involved in insulin sensitivity (73). Dominant mutations of the agouti gene cause the development of obesity and insulin resistance. In terms of tissue distribution, species differences have been described since, unlike mouse agouti, human agouti is expressed in AT. Transgenic mice expressing agouti in adipocyte do not become obese or diabetic but exhibit high plasma levels of glucose and leptin. Signal transducers and activators of transcription such as STAT-1 and STAT-3 and PPARγ protein levels were elevated in transgenic mice. Moreover, these mice increased weight gain when a daily injection of insulin was performed. The authors suggest that insulin triggers the onset of obesity and that adipocyte agouti potentiates this effect through a direct action on PPARγ expression (74, 75).

Sterol responsive element binding proteins

Sterol responsive element binding protein 1c (also named adipocyte determination and differentiation factor 1; SREBP1c/ADD1) is a member of the basic helix-loop-helix leucine zipper family of transcription factors expressed in BAT, WAT, liver, and kidney. Studies in cellular models have shown that SREBP1c/ADD1 positively influences adipogenesis and stimulates the expression of genes involved in lipogenesis. However, in vivo, the role of SREBP1c/ADD1 in adipogenesis is more elusive. Eighty five percent of SREBP1 null mice die in utero at embryonic day 11 (76). The surviving animals appear normal with no effect on fat mass. The effect on AT gene expression was moderate with no changes in lipogenic genes such as lipoprotein lipase (LPL), acetylCoA carboxylase (ACC), and fatty acid synthase (FAS). Overexpression of a constitutively active form of SREBP1c/ADD1 in AT unexpectedly results in lipodystrophy (see below). SREBP2 is a member of the SREBP family expressed in AT in lower amounts than SREBP1c. Expression of a dominant positive form of SREBP2 was followed in liver and AT using the PEPCK promoter (77). No change in lipogenic enzyme gene expression was observed. However, transgene expression resulted in increased expression of LDL receptor and cholesterol biosynthetic enzyme mRNAs. This suggests that SREBP2 is a relatively specific activator of cholesterol synthesis in WAT.

Forkhead box C2

Study of the LPL promoter showed that members of the winged helix/forkhead gene family were expressed in differentiated adipocytes (78). Forkhead box C2 (FOXC2) is exclusively expressed in WAT and BAT of adult mice and humans. However, mice lacking FOXC2 die embryonically or perinatally with aortic arch, craniofacial, and skeletal defects (79, 80). To investigate its role in AT, overexpression was achieved with the aP2/ALBP promoter fused to the FOXC2 cDNA. Transgenic mice were leaner with a reduced amount of intraabdominal WAT (81). White fat cells from these depots looked like brown fat cells with a reduced size, multilocular lipid droplets, and numerous mitochondria. The increased thermogenic capacity of WAT might contribute to the lean phenotype. Profound changes in gene expression underlie the new function of WAT. There was an increase in genes important for energy dissipation, such as PPARγ coactivator 1, cytochrome oxidase II, and UCP1. Moreover, the sensitivity of the β-adrenergic signal transduction pathway was enhanced. The increased mRNA levels for several components of the insulin-signaling cascade may explain the increased insulin sensitivity of the transgenic animals fed a high-fat diet. Concomitantly, expression of genes involved in adipogenesis such as C/EBPα, PPARγ, and SREBP1c/ADD1 was also increased. Altogether, FOXC2 appears as a master gene. Its induction protects against obesity and diet-induced insulin resistance but also participates in the maintenance of the white fat cell phenotype.

Other transcription factors

Other factors expressed in WAT and BAT have been shown to influence adipogenesis. The AP-1 family consists of dimeric complexes of Fos- and Jun-related proteins. Overexpression of ΔfosB, a natural and functional truncated form of FosB, led to increased bone formation and reduced WAT (82). Bone marrow stromal cell culture from ΔfosB mice treated with either osteogenic or adipogenic agents showed a significant decrease in adipose cell number and maturation and increased expression of genes associated with the osteoblast lineage. Adipocytes and osteoblasts are cells of mesenchymal origin that are believed to originate from a common pluripotent precursor. It is therefore conceivable that ΔfosB positively regulates osteoblastogenesis at the expense of adipogenesis.

A role for high mobility group protein Ic (HMGIc) in AT development was recently found in vivo. HMG proteins bind to an adenine/thymine-rich region on the minor groove of DNA and have been shown to play a role in chromatin structure as well as gene-specific transcriptional activation. HMGIc is only expressed during embryonic and fetal stages in line with a role in development. Rearrangements of the HMGIc gene have been frequently detected in benign tumors of mesenchymal origin such as

lipomas. Truncation of HMGIc seems to be responsible for cell transformation. Indeed, ubiquitous expression of a truncated HMGIc protein in transgenic mice led to an increased development of WAT early in life and a high incidence of lipomas (83, 84). Conversely, mice with disruption of one or two alleles of the HMGIc gene are resistant to diet-induced obesity (85). Lack of HMGIc expression in leptin-deficient Lep^{ob}/Lep^{ob} mice resulted in a decrease in fat pad weights due to a decrease in fat cell number. No differences in gene expression were observed in adipocytes. Together, the data suggest that HMGIc has an important role in fat cell precursor proliferation and/or commitment.

LIPID AND GLUCOSE METABOLISM

Lipoprotein metabolism

Transgenic models have contributed to clarify the respective roles of many proteins involved in lipid metabolism (**Fig. 2**). Links have been established between fatty acid transport in lipoprotein particles and AT development. Transgenic mice expressing human apolipoprotein C-I (apoC-I) in liver show reduced amounts of visceral fat depots and a lack of subcutaneous AT (86). Similarly, VLDL receptor-deficient mice are resistant to genetic and diet-induced obesity (87). These data suggest that fatty acid delivery to AT is impaired. They are also consistent with the role of VLDL receptor as a docking protein for triglyceride-rich lipolysis and for the defective binding of apoC-I enriched lipoprotein particles to the receptor. Overexpression of apoA-II, the second most abundant component of HDL, leads to increased fat mass and promotes insulin resistance with reduced skeletal muscle uptake of glucose (88). This finding shows that a primary disturbance in lipoprotein metabolism can result in traits associated with insulin resistance. LPL, located on the capillary endothelium of extrahepatic tissues, catalyses the rate-limiting step in the hydrolysis of triglycerides from circulating chylomicrons and VLDL. Most LPL is found in AT and skeletal muscle, where the released free fatty acids are stored or oxidized, respectively. Hence, LPL plays a key role in fat partitioning and, through delivery of fatty acids to skeletal muscle, may play an important role in the genesis of insulin resistance as a result of competition between fatty acid and glucose. LPL deficient mice are normal at birth, but develop lethal hypertriglyceridemia within the first day of life (89). To directly assess the role of LPL in AT, LPL heterozygous knockout mice have been crossed with transgenic mice expressing human LPL in skeletal muscle and heart (90). Through backcross, mice expressing LPL exclusively in muscle were obtained. Growth and body composition were not altered by the lack of LPL in AT on a standard genetic background. However, when AT LPL deficiency was obtained on an Lep^{ob}/Lep^{ob} background, the rate of weight gain was decreased due to an impaired accumulation of lipid in AT.

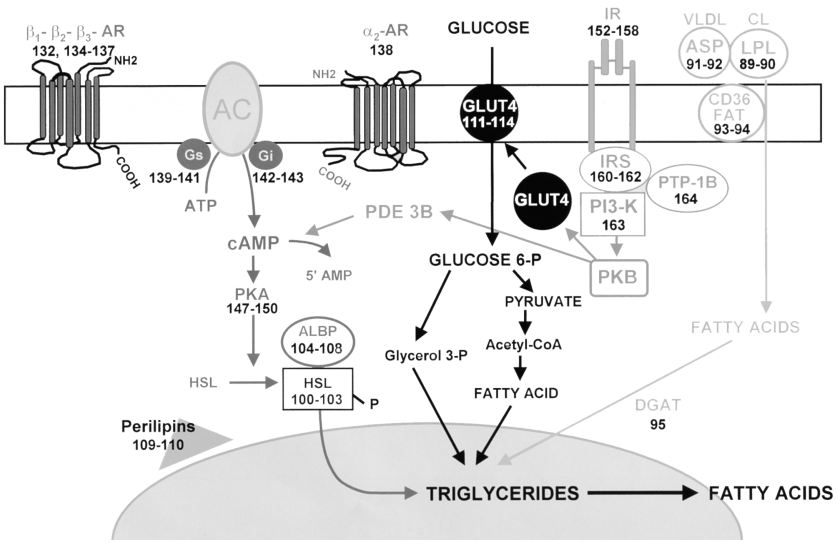

Fig. 2. Elements of signal transduction pathways involved in white adipocyte metabolism. Lipolysis: The β_1-, β_2-, β_3-, and the α_2-adrenergic receptors (AR) are, respectively, positively and negatively coupled to adenylyl cyclase (AC) and to cAMP production by heterotrimeric G proteins (Gs and Gi). cAMP produced by activation of adenylyl cyclase activates protein kinase A (PKA), which stimulates phosphorylation of hormone-sensitive lipase (HSL). Perilipins and adipocyte lipid binding protein (ALBP) are other members of the lipase complex influencing lipolytic capacity. Activation of HSL catalyzes the hydrolysis of triglycerides. Insulin antilipolytic effect is mediated by the activation of insulin receptor (IR), insulin receptor substrates (IRS), phosphatidylinositol 3-kinase (PI3-K), protein kinase B (PKB), and the phosphodiesterase 3B (PDE3B), which hydrolyzes cAMP into 5′AMP. Glucose transport: The glucose transporter 4 (GLUT 4) is translocated to the plasma membrane in response to insulin by a PI3-K-dependent pathway that involves PKB. Uptake of triglycerides: triglycerides contained in lipoprotein particles like chylomicrons (CL) and VLDL are hydrolyzed by LPL. The released fatty acids are re-esterified for storage as triglycerides by the diacylglyceroltransferase (DGAT). Fatty acids can also be formed from glucose (lipogenesis). Acylation-stimulating protein (ASP) and CD36/FAT represent proteins that are also in fatty acid uptake. References in parentheses correspond to transgenic overexpression or gene knockout of the proteins.

Triglyceride content was increased in skeletal muscle suggesting partial reallocation of dietary fat storage from AT to skeletal muscle. The chemical nature of the lipid stored in AT was markedly modified in LPL deficient mice, though data suggests that the development of fat stores in AT LPL deficient mice relies on endogenous fat synthesis. On a genetically obese background, this compensatory mechanism does not keep up with the massive weight gain programmed in Lep^{ob}/Lep^{ob} mice due to leptin deficiency.

Fatty acid transport and metabolism

Acylation-stimulating protein (ASP) is a cleavage product of complement C3 produced by the adipocyte that promotes fatty acid reesterification and inhibits lipolysis. Knockout of the C3 gene has provided a model of ASP deficiency (91, 92). The knockout mice show a delay in postprandial triglyceride clearance and an increase in plasma nonesterified fatty acid levels. A moderate decrease in fat depot weights is observed on both high and low fat diets. The data suggest that ASP may play a role in fat partitioning. Another potential gatekeeper of fatty acid entry into adipose cells is the transporter CD36/fatty acid transporter (FAT) which is expressed in tissues with a high metabolic capacity for fatty acid such as AT, skeletal muscle, and heart. The adipocytes of CD36 null mice lack the high affinity component of long chain fatty acid transport observed in wild-type fat cells (93). Furthermore, there is a defective in vivo uptake of fatty acid in AT and skeletal muscle, which results in impaired triglyceride synthesis in the two tissues (94). The defective fatty acid esterification is most likely due to a limiting supply of acylCoA that impairs conversion of diglyceride to triglyceride at the level of diacylglycerolacyltransferase (DGAT), suggesting a regulatory role for this enzyme in vivo. The importance of DGAT was recently assessed through gene targeting (95). Mice lacking DGAT are viable and fertile. The animals are capable of synthesizing triglycerides and have normal body weight on a standard chow diet. The fat pad weights are slightly lower than in wild-type control mice. However, DGAT-deficient mice are resistant to diet-induced obesity, which appears to be due to increased energy expenditure. The mechanism underlying the changes in metabolic rate is unclear. It does not result from increased lean body mass or changes in cold-induced thermogenesis. Puzzlingly, the study also shows that triglyceride synthesis can occur without DGAT. This suggests the existence of another enzyme with DGAT activity. Indeed, such an enzyme has recently been characterized and may partially compensate for the lack of DGAT (96). Increased fatty acid oxidation may also lead to reduced fat storage. This is best exemplified by the phenotype of ACC2 deficient mice (97). The lack of ACC2 leads to a reduction of malonylCoA levels in heart and skeletal muscle and increased fatty acid oxidation in these tissues. The ACC2 null mice consume more food than wild-type mice, yet have a reduction in fat pad sizes.

Adipose tissue lipolysis

Hormone-sensitive lipase (HSL) is classically considered as the key enzyme catalyzing the rate-limiting step of AT lipolysis (98, 99). This view is supported by numerous biochemical, physiological, and clinical studies. However, recent data from HSL deficient mice led to a reassessment of the role of HSL in WAT and BAT fat mobilization (100, 101). Catecholamine-induced lipolysis is markedly blunted as expected, but basal (or unstimulated) lipolysis is unaltered in isolated adipocytes suggesting the existence of a lipase different from HSL. Although no major change in the weight of fat pads was observed, lipid metabolism was altered in the knockout mice. WAT from HSL deficient mice accumulated diglycerides demonstrating that the enzyme catalyzed the rate-limiting step in diglyceride catabolism (102). During fasting, i.e., when the enzyme activity is maximal in wild-type animals, $HSL^{-/-}$ mice showed decreased plasma free fatty acid and triglyceride levels (103). Alteration of triglyceride-rich lipoprotein metabolism was due to a downregulation of VLDL synthesis in liver and an upregulation of LPL activity in skeletal muscle and WAT.

Proper activation of lipolysis also relies upon proteins that are not directly involved in the catalytic process. ALBP/aP2 is an intracellular fatty acid-binding protein highly expressed in adipocytes. Its interaction with HSL N-terminal region may avoid local accumulation of fatty acid during lipolysis and prevent their deleterious effects. It would also allow the fatty acids to be shuttled out of AT. Consistent with such a role for ALBP is the observation that ALBP-null mice exhibit a decreased lipolytic capacity (104, 105). The ALBP-deficient mice show minor alterations of lipid metabolism under a standard chow diet as a consequence of functional compensation by the keratinocyte fatty acid binding protein (106, 107). However, the lack of ALBP protects against hyperinsulinemia and insulin resistance in high-fat-diet-induced or genetically obese mice (106, 108). Access to the lipid droplet constitutes another potential mechanism for the control of lipolysis. Perilipins are proteins covering the large lipid droplets in adipocytes. They shield stored triglycerides from cytosolic lipases. It has been hypothesized that, upon phosphorylation, perilipins allow access to the lipid droplet and thereby allow lipases to interact with their substrates. In two independent studies, ablation of perilipin resulted in mice with decreased fat mass and increased lean body mass (109, 110). The mice are resistant to diet-induced obesity. Moreover, double mutant $Lepr^{db/db}/Plin^{-/-}$ mice are protected against the obesity phenotype due to mutation in the leptin receptor. No hepatic steatosis or alteration of the lipid profile was observed, which might be due to the increased metabolic rate of the mutant animals. Basal lipolysis is increased in perilipin-deficient adipocytes, which is in line with a role of perilipin as a suppressor of lipolysis in quiescent cells. However, the results of β-adrenergic stimulated lipolysis differ between the two studies. Martinez-Botas et al. observed that the basal lipolysis in $Plin^{-/-}$ mice was similar to the maximal lipolytic capacity of wild-type fat cells and that there was no further stimulation by a β-adrenergic agonist (109). The data suggest that, without perilipin, adipocytes have a permanent lipolytic drive. In contrast, Tansey et al. report that the in-

crease of glycerol and free fatty acid release induced by a β-adrenergic agonist was markedly blunted in Plin$^{-/-}$ adipocytes, which would indicate that perilipin is a necessary cofactor for full lipolytic stimulation (110). The reasons for the discrepancy are unclear. However, the issue needs to be solved as it implies different functions for perilipin. If perilipin does suppress basal and stimulated lipolysis, one can envisage a futile cycle of lipogenesis and lipolysis in Plin$^{-/-}$ animals that could contribute to the increased metabolic rate. Alternatively, the increased metabolic rate may derive from the increased lean body mass, which is an intriguing aspect of the phenotype, as perilipin is not expressed in skeletal muscle.

Glucose metabolism

The glucose transporter 4 (GLUT4) is the major transporter in tissues in which glucose uptake is stimulated by insulin such as skeletal muscle and WAT. A decrease in GLUT4 level might therefore be responsible for the insulin resistance observed in type 2 diabetes. Skeletal muscle accounts for most of the mass of insulin-responsive tissues. Hence, it has been postulated that in vivo alterations in glucose disposal is due to skeletal muscle. Indeed, GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes, which is prevented by transgenic complementation of GLUT4 in skeletal muscle (111, 112). However, the amount of GLUT4 is not decreased in muscle cells in diabetic people whereas it is in their fat cells. Transgenic techniques have therefore been used to modify the level of GLUT4 expression in WAT. GLUT4 overexpression in WAT and BAT was achieved using the aP2/ALBP promoter linked to a genomic fragment encompassing all coding exons of the GLUT4 gene (113). In vivo glucose tolerance is enhanced in transgenic mice. The 6- to 9-fold increased GLUT4 expression in WAT resulted in increased basal and insulin-stimulated glucose transport. Interestingly, young transgenic mice showed increased fat mass resulting from an increase in fat cell number without a change in fat cell size. However, in old female mice, adipocyte size increases. To gain further insight into the role of adipose GLUT4, inactivation of the GLUT4 gene was performed selectively in WAT and BAT by crossing mice with a floxed GLUT4 allele with transgenic mice expressing the Cre recombinase under the control of the ap2/ALBP promoter (52). GLUT4 levels were reduced by more than 70% in BAT and WAT without change in GLUT1 expression. GLUT4 expression was preserved in skeletal muscle and heart. No apparent growth retardation or cardiac abnormalities were observed in contrast to mice totally deficient in GLUT4 (114). Unlike overexpression, GLUT4 targeting in AT does not affect body weight or fat mass of mice eating a standard chow diet. Reduced basal and markedly blunted insulin-stimulated glucose uptake was observed in isolated adipocytes but not in skeletal muscle ex vivo. In vivo, the animals were intolerant to glucose and resistant to insulin. Insulin-stimulated whole-body glucose uptake was decreased. As expected, in vivo insulin-stimulated glucose transport was reduced in WAT and BAT but, surprisingly, the impairment was also observed in skeletal muscle despite normal GLUT4 expression. Moreover, the ability of insulin to suppress hepatic glucose production was blunted. The data clearly suggest that impaired expression of GLUT4 in AT may lead to insulin resistance in WAT but also in skeletal muscle and liver leading to glucose intolerance and hyperinsulinemia. This provocative discovery renews the interest in the role of WAT GLUT4 in the development of type II diabetes but also questions the transmission of impaired insulin action from AT to skeletal muscle and liver. Fat cell-secreted products such as leptin, tumor necrosis factor α (TNFα) and fatty acids were excluded as potential culprits. Other adipocyte-derived molecules such as adiponectin could be involved.

Glucose metabolism through the hexosamine pathway has been hypothesized to mediate some of the toxic effects of hyperglycemia. The first and rate-limiting enzyme of the pathway is glutamine:fructose-6-phosphate amidotransferase which catalyses the formation of glucosamine-6-phosphate. Overexpression in skeletal muscle and AT using the GLUT4 promoter leads to whole body insulin resistance with a defect in GLUT4 translocation that impairs glucose uptake in skeletal muscle (115, 116). The animals are hyperleptinemic due to increased expression of the leptin gene in WAT (117). This in vivo result is consistent with in vitro data, which suggest that the end product of the hexosamine pathway, UDP-*N*-acetyl glucosamine, modulates leptin gene transcription through the O-linked glycosylation of the transcription factor Sp1 (118). The hexosamine pathway may serve as a glucose-sensing device linked to leptin expression and glucose disposal.

Thermogenesis

The thermogenic function of BAT results from the expression in this tissue of the uncoupling protein UCP1. UCP1 is expressed in the inner membrane of the mitochondrion where it promotes the dissipation of the proton electrochemical gradient across the inner mitochondrial membrane. The activation of this pathway results in energy dissipation as heat instead of ATP synthesis and is involved in the regulation of body temperature and body weight. Mice lacking UCP1 show no difference in resting metabolic rate but have blunted β-adrenergic stimulated oxygen consumption (119). In the initial study on mice with a mixed 129/SvPas and C57BL/6J background, 85% of the animals were sensitive to cold, showing the pivotal function of UCP1 for thermogenesis but also suggesting that variations in the genetic background could alter cold sensitivity. Through backcross, UCP1-deficient mice congenic on 129/SvImJ and C57BL/6J backgrounds were obtained (8). The animals showed a profound cold-sensitive phenotype. However, mice on a (129/SvImJ × C57BL/6J) F1 hybrid background were cold resistant despite the absence of UCP1. The cold resistance on the hybrid background is a dramatic example of heterosis or hybrid vigour. The compensation does not come from the UCP1 homologs, UCP2 and UCP3, which are expressed in BAT. Determination of the unknown alternate mechanisms leading to protection against cold in mice without UCP1

may be of great interest for adult humans that express very little BAT. Unlike partial ablation of BAT (see below), UCP1 deficiency does not cause hyperphagia or obesity, suggesting that other factors in BAT are responsible for diet-induced thermogenesis.

Targeted disruptions of the ubiquitously expressed UCP2 and BAT and skeletal muscle-specific UCP3 do not result in alterations of WAT development and response to cold (120–123). However, ectopic expressions of UCPs in WAT and skeletal muscle have provided models of resistance to obesity. Expression of UCP1 in WAT of C57BL/6J mice was achieved through the use of the aP2/ALBP promoter (124). UCP1 level represented 2–10% of the level normally expressed in BAT. In BAT, the overall level of UCP1 was nearly identical in transgenic and wild-type mice. On a standard chow diet, differences in body weight appeared after 6 months of age. The first effect of the transgene was a reduction of the subcutaneous fat pad. At 1 year of age, most fat pads were diminished with the noticeable exception of gonadal fat pads. This regional difference was not due to differences in the level of transgene expression. To determine whether the transgene could alter the development of obesity, the aP2-UCP1 transgenic mice were crossed with the genetically obese C57BL/6J-A^{vy}/+ mice. Expression of the transgene markedly reduced fat masses in A^{vy} mice. As in C57BL/6J mice, the effect was more pronounced in subcutaneous AT. Similarly, transgenic C57BL/6J mice were resistant to diet-induced obesity despite food intake comparable to that of non-transgenic animals (125). These data show that expression of UCP1 in WAT protects against genetic and dietary obesity with a more pronounced effect in subcutaneous fat depots compared with intraabdominal fat pads. The mechanisms underlying the phenotype of the aP2-UCP1 mice have been intensively studied. Energy dissipation was depressed in BAT whereas WAT expressing UCP1 showed increased oxygen consumption (126). Despite the inherently low respiratory capacity of WAT, the increase in thermogenesis conferred by UCP1 in all depots may contribute to a slight shift in energy balance leading to a decreased weight gain. However, alternative mechanisms may be involved. Fatty acid synthesis was decreased in aP2-UCP1 mouse WAT as a consequence of UCP1-mediated uncoupling of oxidative phosphorylation (127). Targeting UCP1 into skeletal muscle also resulted in a mouse model resistant to obesity (128). UCP1 levels in muscle were 1% of BAT levels. Fed a chow diet, the transgenic mice weighed less. Furthermore, they were resistant to diet-induced obesity as a result of increased resting metabolic rate. With both types of diet, the transgenic mice had better glucose tolerance and insulin sensitivity than control mice. Decreased competition between fatty acid and glucose in transgenic mice fed a high-fat diet does not seem to contribute to the improved glucose tolerance since, as observed in endurance-trained athletes, there is enhanced expression of LPL and increased triglyceride content in skeletal muscle. The improved skeletal muscle glucose transport may therefore result from increased uncoupling of the respiratory chain as shown in cellular models. Strong overexpression of human UCP3 in skeletal muscle results in a similar phenotype, except that the animals, despite their body weights, which are lower than their non-transgenic littermates, are hyperphagic (129). Hence, whereas neither UCP1 nor UCP3 knockout mice have an increased susceptibility to obesity, overexpression of UCP1 and UCP3 can prevent obesity. Compensatory mechanisms may explain the lack of response in knockout models. It remains to be seen whether pharmacological strategies targeting UCPs, either through increased expression or modulation of their activities, influence energy balance in humans.

Increased thermogenesis may also come from an unexpected route. Inactivation of a translational inhibitor, the eukaryotic translation initiation factor 4E-binding protein 4E-BP1, leads to high metabolic rate and small white fat pads. WAT of the knockout animals expresses UCP1 (130). The upregulation of the thermogenic protein may, in part, result from the increased translation of PPARγ coactivator 1, a transcriptional coactivator involved in mitochondriogenesis and adaptive thermogenesis. Besides providing a new model of leanness and obesity resistance, this study reveals the importance of translation in the regulation of energy expenditure.

ENDOCRINE RESPONSES AND SIGNAL TRANSDUCTION

Adrenergic receptors

Among the nine pharmacologically and genetically distinct adrenergic receptors (AR), four are expressed in adipocytes (Fig. 2). The α_2- and β-ARs have opposite signal transduction pathways and are known to participate in the regulation of BAT and WAT development and metabolism (131). In addition to variations in receptor number, lipolytic rates in AT are thought to be affected by the expression of particular β-AR subtypes and by the ratio of α_2/β-AR. Transgenic mice overexpressing the human β_1-AR have been generated (132). The aP2/ALBP promoter was used to specifically target the transgene to differentiated white and brown adipocytes (16). Transgenic mice gained weight more slowly and had reduced adipose stores compared with transgenic littermates, especially in response to a high fat diet. Moreover, brown adipocytes appeared in the subcutaneous white fat pads. The in vivo phenotypic effects are in agreement with the in vitro responses to β_1-AR stimulation in isolated fat cells, i.e., increased lipolytic activity of the adipocytes and greater energy expenditure through heat production by the additional population of brown adipocytes.

In rodents, the β_3-AR is expressed in fat at a much higher level than β_2 and β_1-AR (133) and has therefore been proposed to be the major regulator of adrenergic responses in spite of its lower affinity for endogenous catecholamines. Moreover, β_3-AR selective agonists have been proposed as potential anti-obesity drugs based on their impressive effects on energy expenditure, insulin levels,

and food intake in rodents. To study the physiological relevance of β_3-AR, mice lacking the receptor were generated (134, 135). Surprisingly, β_3-AR$^{-/-}$ mice show only a modest tendency to become overweight even when fed a high fat diet. However, a rise in total body fat was observed. Decreased action of the β_3-AR was once thought to be responsible for the development of obesity. It is clear now that the absence of the receptor is not sufficient. Specific expression of β_3-AR in BAT or BAT and WAT confirmed that the expression of β_3-AR was indispensable in white and brown adipocytes to rescue the effects of β_3-AR agonists on oxygen consumption, insulin secretion, and food intake and that β_3-AR in other tissues were not required (136). White adipocytes appeared to be predominantly involved in the β_3-AR agonist effects on insulin levels and food intake while effect on oxygen consumption was associated to brown adipocytes.

Although β_3-AR agonists have been described as potent anti-obesity drugs in rodents, their relevance remains questionable in humans. Rodents have large amounts of thermogenic brown adipocytes and high levels of β_3-AR in WAT compared with humans. To understand the differences in β_3-AR sites of expression, fragments of human genomic DNA encompassing the β_3-AR gene were microinjected into β_3-AR knockout mouse oocytes (137). The transgenic mice were used to identify tissues where the human β_3-AR promoter/enhancer is active and to delineate the *cis* acting regions involved in tissue-specific expression. Human β_3-AR mRNA was expressed only in brown adipocytes with little or no expression in WAT, liver, stomach, small intestine, skeletal muscle, or heart. This strategy can prove very useful for the precise mapping of *cis*-acting elements conferring tissue-specific expression and for the study of physiological or pharmacological mechanisms controlling human β_3-AR gene expression.

In human white adipocytes, the β-adrenergic response to catecholamines can be totally counteracted by the α_2-adrenergic pathway. A large body of evidence indicates that the ratio of α_2/β-AR in different fat pad depots affects the lipolytic rate and is closely associated with the enlargement of AT in obese subjects (131). Because of the high levels of β_3-ARs and the very low expression of α_2-ARs in WAT, rodents do not mimic human adrenergic receptivity. To assess the importance of the α_2/β-AR balance in vivo, gene targeting and transgenic approaches were combined to create mice with increased α_2/β-AR balance in AT (138). Using the aP2/ALBP promoter, expression of human α_2A-AR was targeted in the β_3-AR null mouse AT. Such "human-like" mice developed high-fat-diet-induced obesity associated to adipocyte hyperplasia rather than hypertrophy. None of the plasma parameters such as glucose or insulin levels were modified in obese mice while a slight increase in leptin levels was observed. In these conditions, the lack of insulin resistance could be associated to adipocyte hyperplasia. No apparent phenotype was noticed in mice expressing both β_3- and α_2-ARs, clearly demonstrating that the obese phenotype required the interactions between two genes and diet, i.e., the presence of α_2-ARs, the absence of β_3-ARs, and a high fat diet.

GTP binding proteins

When considering the control of lipolysis in adipocytes, the activity of adenylyl cyclase and consequently the levels of intracellular cAMP are crucial for HSL activation. Key elements involved in the transmembrane control of adenylyl cyclase activity are heterotrimeric GTP-binding proteins because of their capacity to be coupled with multiple receptors. The Gαs and Gαi subunits are able to stimulate or inhibit adenylyl cyclase activity, respectively. Gαs deficiency provokes embryonic lethality (139). In heterozygous animals, a different phenotype is observed depending on the sex of the transgenic genitor. Tissue-specific imprinting of the Gαs gene has been reported in numerous tissues, including WAT and BAT. A decreased fat mass is observed in +/p− mice from heterozygous male genitors, while m−/+ mice from heterozygous female genitors become obese in the early adulthood (140). The effects on energy metabolism also depend on parental inheritance, i.e., +/p− mice are hypermetabolic and +/m− mice are hypometabolic. It is likely that decreased adipocyte Gαs expression in +/m− mice leads to obesity because cAMP stimulates lipolysis and thermogenesis in mouse WAT and BAT. The explanation of the phenotype described in +/p− mice remains unclear although a rise in sympathetic nervous system activity could contribute to the phenotype. A better insulin sensitivity is observed in lean males and, surprisingly, in obese females (141).

The inhibitory pathway of adenylyl cyclase has also been genetically modified by an inducible RNA antisense strategy directed against the Gαi2 subunit (33, 34, 142). The PEPCK promoter was used for its capacity to drive the transgene in liver and fat and to be inducible by cAMP. The expression of the antisense RNA induced an increase in basal cAMP levels, no modifications of the β-AR stimulatory pathway, and severe blunting in the response to the inhibitory agonists such as adenosine A1. Transgenic mice in which the transgene becomes active at birth by a rise in basal levels of cAMP displayed a smaller liver and less AT. The animals developed type 2 diabetes with hyperinsulinemia, impaired glucose tolerance, and resistance to insulin. This data suggesting that Gi mimics insulin action was confirmed by targeted expression of the GTPase-deficient constitutively active Gαi2 in fat and muscle (143). The same animals were used to define protein-tyrosine phosphatase 1B (PTP1B) as a step in the cellular pathway leading to Gαi2-mediated enhancement of insulin signaling (142, 144).

Although the Gαq-protein kinase C signaling pathway has only been poorly documented in AT, a similar antisense strategy has been used to impair Gαq expression (145). Gαq deficiency in liver and AT at birth was associated to increased body and fat masses and reduced lipolytic response. Since α_1-ARs are coupled to phosphoinositide hydrolysis and protein kinase C activation, the authors suggested a potential role for α_1-ARs and intracellular Ca^{2+} in the inhibition of lipolysis in agreement with other studies (146).

Protein kinase A

Surprisingly, adipocyte adenylyl cyclase, one of the major components of transmembrane signaling associated

with the control of lipolysis has not been investigated using transgenic techniques. Stimulation of the lipolytic cascade involves the phosphorylation of HSL by the cAMP-activated protein kinase, which is composed of two regulatory and two catalytic subunits. Among the four regulatory subunit genes, the RIIβ isoform is abundant in BAT, WAT, and brain. Targeted disruption of the RIIβ subunit produces lean mice resistant to obesity when fed a high fat diet (147–149). In both brown and white adipocytes, a compensatory rise in the RIα subunit has been described. This isoform switch is associated with an increased UCP1 expression in BAT and enhanced basal lipolysis in WAT due to the higher binding capacity of RIα to cAMP. The disruption of both RIβ and RIIβ genes leads to the same preservation of cAMP-dependent regulation by the compensatory rise in RIα protein half-life without a change in gene transcription. However, the ability of β-AR agonists to stimulate lipolysis is strongly compromised in WAT. Finally, RIα null mice show early embryonic lethality with severe developmental abnormalities confirming RIα modulation as an essential mechanism in the safeguard of pleiotropic cAMP cellular responses (150).

Insulin and insulin signaling

Insulin is the primary hormone to inhibit lipolysis and is responsible for signaling the storage and utilization of glucose. The application of transgenic mouse technology to the study of diabetes has been extensively reviewed (151). Here, we will focus on the genetically modified animals exhibiting AT phenotypes. Targeted disruption of the insulin receptor (IR) gene leads to diabetic ketoacidosis and marked post-natal growth retardation. Pups die within 7 days of birth (152). Studies performed on white dermal AT in newborn mice lacking IR ($IR^{-/-}$ mice) showed an important decrease of fat amount compared with the $IR^{+/-}$ or $IR^{+/+}$ mice with a reduction of fat cell volume rather than modification of fat cell number. Moreover, the lack of IR was not associated with impairment of adipocyte differentiation (153). The lethal phenotype observed due to the pleiotropic effects of IR activation led to the development of selective invalidation of the IR gene using the Cre-LoxP strategy (154). Mice with an exon 4 flanked by LoxP sites have been produced. The animals were available for breeding with any other line expressing Cre recombinase under the control of a tissue specific promoter/enhancer. Such a strategy led to tissue-specific invalidation of IR in skeletal muscle, pancreatic β-cells, liver, brain, and very recently in BAT (not yet in WAT) with, respectively, the creatine kinase, insulin, albumin, nestin, and UCP1 promoters (154–158). Among the different tissue-specific knockouts of the IR, two have consequences on AT. The muscle specific invalidation of IR exhibits several of the metabolic alterations seen in type II diabetes, such as an increased fat mass, altered glucose transport, elevated blood triglyceride, and free fatty acid levels. However, there was no alteration of glucose tolerance in this model. Mice lacking IR in BAT exhibit profound brown fat atrophy and reduction of lipogenic gene expression.

The IRS proteins (IRS-1, IRS-2, IRS-3, and IRS-4) are the major substrates of the IR (159). To clarify the role of the different IRS proteins, targeted disruption of each IRS and double deficient mice were generated. The roles of IRS-1 and IRS-2 in adipocyte differentiation have been recently investigated. Brown adipocyte cell lines derived from IRS-1 knockout mice showed 60% to 90% decrease in differentiation and lipid accumulation. Different adipogenic markers (PPARγ, C/EBPα, FAS, UCP1, GLUT 4) were also significantly decreased. Moreover, there was a defect in phosphatidylinositol 3-kinase (PI3-K) activation in the IRS-1 knockout cells associated with decreased protein kinase B activation (160). Therefore, IRS-1 appears to be an important mediator of brown adipocyte differentiation. Since the lack of IRS-1 can be compensated by IRS-2, heterozygous mice were intercrossed for each of the two null alleles in order to generate wild-type, IRS-$1^{-/-}$, IRS-$2^{-/-}$, and IRS-$1^{-/-}$ IRS-$2^{-/-}$ mice. The abilities of IRS-$1^{-/-}$ and IRS-$2^{-/-}$ cells to differentiate into adipocytes were respectively 60% and 15% lower than wild-type cells. IRS-$1^{-/-}$ IRS-$2^{-/-}$ cells were completely unable to differentiate into adipocytes (161). Like in IRS-1 knockout cells, adipogenic markers and PI3-K activity were severely decreased in IRS-$1^{-/-}$ IRS-$2^{-/-}$ mice. Histological analysis of newborn IRS-$1^{-/-}$ IRS$2^{-/-}$ mice showed marked reduction in WAT but not in BAT mass. Even if IRS-1 and IRS-2 play an important role in adipocyte differentiation, the role of the different IRS proteins in AT metabolism needs further investigations. IRS-1 null mice were also used to show that, to activate PI3-K in response to insulin, the preferential subcellular locations of IRS-2 and IRS-3 were the low-density microsome fraction and the plasma membrane fraction, respectively. Moreover, in IRS-1 null mice, the antilipolytic effect of insulin was unaffected compared with wild-type mice, whereas lipolysis and HSL mRNA and protein was increased (162). The data raises the possibility that the actions of insulin may be linked to distinct subcellular locations of IRS proteins associated to PI3-K activity. The targeted disruption of the gene encoding the p85α regulatory subunit of the PI3-K was generated in order to elucidate the role of this enzyme in glucose homeostasis in vivo. Null mice show increased insulin sensitivity and hypoglycemia due to increased glucose transport in skeletal muscle and adipocytes (163). These results provide the first in vivo evidence that PI3-K contributes to glucose homeostasis.

The counteregulation of insulin signaling involves PTP-1B, which directly interacts with the activated IR. Targeted disruption of the gene encoding PTP-1B enhanced insulin sensitivity in mice. Moreover, a total lack of weight gain and insulin resistance was observed when the mice were fed a high fat diet (164).

Growth hormone

The effects of modified growth hormone (GH) expression have been studied in transgenic mouse, rat, and pig (165). In 1985, overexpression of the human growth hormone-releasing factor was obtained with the zinc-inducible metallothionein-I promoter (166). Among the expected ef-

fects on growth, human growth hormone-releasing factor-transgenic mice showed greater amounts of abdominal fat, higher levels of GH, leptin, and insulin (167). The inducible promoter has also been used to control GH expression in rats (168, 169). Zinc-induced GH overexpression induces a rise in insulin levels, which is abolished upon cessation of transgene expression. Repression of the transgene expression following a period of elevated GH levels led to the development of obesity when compared with nontransgenic or still activated transgenic mice. Large increase in epididymal and subcutaneous fat pad weights were associated to increased fat cell size and number. An alternative transgenic strategy has been used in rats (170, 171). A chimeric transgene comprising murine whey acidic protein and human GH was used to produce transgenic rats, which express human GH and secrete it into the blood. The continuously secreted human GH led to the inhibition of rat GH secretion and pulsatility and to a decreased overall mean plasma GH concentration. The transgenic rats showed severe obesity associated to a rise in plasma glucose, insulin, triglyceride, and free fatty acid levels. While plasma leptin is higher in transgenic rats, the animals exhibit an early onset of increased food intake due to a defect in leptin transport from peripheral blood to cerebrospinal fluid (172). Interestingly, treatment with recombinant human GH for 1 week to produce pulsatile secretion reduced the size of fat pads and restored normal weight gain. The data illustrate in vivo the opposite effects of long and short-term stimulation by GH observed in vitro on isolated adipocytes. The 762-bp proximal leptin promoter was recently used to drive GH expression by adipocytes and other cells. Female transgenic mice showed reduced body and fat pad weights, decreased expression of PPARγ, C/EBPα, and C/EBPβ and increased expression of Pref-1, an inhibitor of adipocyte differentiation. The data suggest that a rise in GH reduces adipogenesis (173). Although GH has been shown to induce differentiation of preadipocytes into adipocytes, the authors suggest that high GH levels induce in vivo resistance to the adipogenic action of insulin.

Insulin growth factors and associated proteins

The expression of the various components of the insulin growth factor (IGF) system comprising ligands (IGF-I/II), receptors (IGF-1R, IGF-2R), and soluble binding proteins (IGFBP1-6) is ubiquitous throughout intrauterine and postnatal development. Mice lacking either the IGF1 or IGF2 genes have intrauterine growth retardation and weigh approximately 60% less than their littermates. IGF1-$R^{-/-}$ pups have a reduced weight and die soon after birth while IGF2-R null phenotype is lethally associated with a fetal overgrowth syndrome. Mice lacking the liver IGF-I by conditional gene deletion have a dramatic reduction of serum levels of IGF-I without a marked modification of growth and development, suggesting that local tissue production such as that of AT may compensate for the lack of the liver protein (174). The major urinary promoter induced by male hormones at puberty (primarily in the liver) has been used to avoid the possible lethal effects of IGF-II overexpression in the fetus. High plasma levels of IGF-II were asociated with a dramatic reduction of fat mass (175) while a 3-fold increase in IGF-II levels failed to modify AT mass (176). Although IGF-I has also been suggested to play an important role in the preadipocyte differentiation process, the CMV promoter-dependent overexpression of IGFBP1 that partially impairs IGF action did not result in alterations of AT development (177, 178). However, when fed a sucrose-enriched diet, transgenic mice gained less body weight and exhibited smaller fat pads, and the adipocyte size and the mitogenic response were reduced when compared with wild-type mice. In contrast, IGFBP2 overexpression also reduced the bioavailability of IGF-I and failed to modify fat pad absolute or relative weight while the total body weight is reduced (179). Counter regulation or adaptive mechanisms involving the other IGFBP are suspected.

Estrogens

Estrogens are suspected to play an important role in WAT development and anatomical distribution but the mechanisms are unclear. WAT expresses estrogen receptor α, which is a member of the nuclear receptor superfamily. Evidence for a direct role of estrogen in fat mass regulation came from two complementary gene knockout studies. A mouse model of estrogen insufficiency was obtained by targeted disruption of the Cyp19 gene that encodes aromatase, the enzyme catalyzing the final step in C_{18} estrogen biosynthesis. Impaired action of the hormones was achieved through ablation of estrogen receptor α. In both models, the mice developed obesity and moderate insulin resistance (180, 181). Interestingly, the phenotype was observed in females but also in males, suggesting that estrogens are also important for WAT development in males.

Glucocorticoids

Patients with the Cushing's syndrome have increased systemic glucocorticoid levels which cause visceral obesity. However, most obese patients have normal blood levels of cortisol (182). Glucocorticoid action on target tissues depends not only on circulating hormone levels but also on local intracellular concentrations. Type 1 11β hydroxysteroid deshydrogenase (11β HSD-1) plays a role in glucocorticoid reactivation locally in visceral AT of obese subjects (183). Transgenic mice overexpressing the 11β HSD-1 in AT were created in order to increase glucocorticoid production exclusively within AT. Transgenic mice had increased adipose levels of corticosterone, developed visceral obesity, had pronounced metabolic complications and were hyperphagic (184). Local production of active glucocorticoids may play a role in the development of visceral obesity, and 11β HSD-1 appears to be a new potential drug target for the treatment of obesity.

Mineralocorticoids

A targeted oncogenesis strategy was used to study the regulatory mechanisms controlling mineralocorticoid hormone receptor expression in vivo (36). The receptor is

well known to mediate the effect of aldosterone on sodium reabsorption in kidney. Two alternative promoters have been characterized. Unexpectedly, fusion of the P1 promoter to the large tumor antigen of simian virus 40 led to the precocious development of malignant liposarcomas in brown AT. Cell lines were derived from the hibernomas and it could be shown that aldosterone participates in the very early induction of brown adipocyte differentiation (185).

Other hormones

Other hormones are involved as occasional regulators of AT development, such as lactogenic hormones during late pregnancy and lactation: prolactin and placental lactogen hormone, which act through the same receptor and are known to regulate fat metabolism in several species and particularly to stimulate lipolysis. Steady state levels of prolactin receptor mRNA are very low in mature adipocytes. However, in physiological situations such as pregnancy or lacation, prolactin upregulates its own receptors. The retroperitoneal AT of transgenic mice overexpressing the prolactin receptor is reduced while no difference is noticed in parametrial fat pads (186). By contrast, the absence of prolactin receptor in prolactin-receptor null mice is accompanied by a mild reduction in body weight mainly in females and a strong decrease in abdominal fat mass (187). Considering the very low expression of prolactin receptor in AT in standard physiological situations, the authors suggest indirect roles for the lactogens in AT growth and metabolism.

ADIPOCYTE SECRETIONS

Cytokines

The ability of AT to secrete proteins with endocrine functions has been fully recognized since the discovery of leptin. Leptin is mainly produced by adipocytes, and multiple lines of evidence indicate that its primary site of action is in the hypothalamus. Several studies have been performed to modify plasma leptin levels since it is considered as a major regulator of the body weight set point. Mice lacking leptin such as Lep^{ob}/Lep^{ob} mice are obese. Transgenic Lep^{ob}/Lep^{ob} mice expressing leptin under the control of aP2/ALBP promoter show a moderately obese phenotype (188). The infertility and several endocrine abnormalities associated to leptin deficiency are normalized. Chronic hyperleptinemia has been obtained using the same approach in normal mice (189). The mice exhibit low body weights at a young age then an increase in body weight, accumulation of adipose mass, and lipid-filled adipocytes are observed at older age (33–37 weeks). The mechanism of response of these two phases to sustained high levels of plasma leptin remains unknown. Forced expression of leptin by liver obtained with the serum amyloid promoter resulted in complete disappearance of WAT and BAT in mice (190). These skinny mice show increased glucose metabolism and insulin sensitivity in muscle and liver, accelerated puberty, and elevated blood pressure (191). The results led the authors to suggest that leptin is an adipocyte-derived antidiabetic hormone in vivo.

TNFα is overexpressed in a variety of experimental obesity models and is a potential candidate for obesity-induced insulin resistance, since knockout mice for either the gene encoding TNFα or the two TNFα receptors (p55 and p75) are protected from obesity-induced insulin resistance (192, 193) and exhibit lower plasma leptin levels (194). However, this hypothesis is still open to debate since results from Schreyer et al. (195) obtained in p55 and/or p75 TNFα receptor null mice do not support the concept. The lack of TNFα receptors did not improve insulin sensitivity or glucose tolerance of mice fed a high fat diet. The lack of receptors has been studied in genetically obese mice (Lep^{ob}/Lep^{ob}) (196). The absence of p55 improved insulin sensitivity while p75 deficiency did not modify insulin resistance. TNFα could also contribute to the defects of thermoregulation observed in BAT of genetic and dietary models of obesity, since Lep^{ob}/Lep^{ob} mice lacking either TNFα or its receptors exhibit increased β_3AR and UCP1 expression associated with a rise in multilocular functional brown adipocytes (197). Obesity-linked diabetes is associated with increased systemic and tissue concentrations of TNFα but also interferon γ and interleukin 6. These proinflammatory cytokines synergistically increase nitric oxide production through increased expression of inducible nitric oxide synthase (iNOS) in myocytes and adipocytes. It has been proposed that iNOS induction causes muscle insulin resistance since iNOS is increased in muscle and fat of genetic and dietary models of obesity. Accordingly, targeted disruption of iNOS protects against high fat diet induced insulin resistance (198). Lack of endothelial nitric oxide synthase (eNOS), which is expressed in skeletal muscle and vascular endothelium, appears to improve insulin sensitivity, since eNOS null mice exhibit fasting hyperinsulinemia, hyperlipidemia, and defects in insulin-stimulated glucose uptake (199).

Interleukin 6 is secreted from AT during non-inflammatory conditions in humans and raised levels of interleukin 6 are seen in obese subjects (200). Surprisingly, interleukin 6 deficient mice developed mature-onset obesity with mainly an increase in subcutaneous fat depot mass. The obesity was partly reversed by interleukin 6 replacement (201). In addition, metabolic perturbations (increased circulating triglycerides levels and decreased glucose tolerance) and leptin insensitivity were observed in obese mice. These data suggest an anti-obesity effect of interleukin 6, but mainly exerted at the level of the central nervous system.

Adhesion molecules

An additional class of gene involved in the regulation of AT mass has been described by Dong et al. (202). These genes encode receptors mediating leukocyte adhesion. Mice lacking leukocyte integrin αMβ2 (Mac-1) as well as those deficient for their receptor (intercellular adhesion molecule-1) become obese in old age when fed a chow diet and at a young age when fed a high fat diet. The

ADAM, a disintegrin and metalloproteinase protein family, is described as involved in a diverse array of biological and pathological processes including the cleavage of membrane anchored precursor of TNFα, thus upregulating production of TNFα. ADAMTS-1 (a member of ADAM protein family) identified by differential display and cloned from a cachexigenic colon carcinoma dramatically interferes with AT development since disruption of the gene encoding ADAMTS-1 resulted in AT malformation among various other growth retardations (203).

Angiotensinogen

The renin-angiotensin system is known to be of major importance in blood pressure regulation. Its contribution to AT development is also of interest. Adipocytes are able to secrete both angiotensinogen (AGT) and angiotensin conversion enzyme. Angiotensin II, acting through its membrane receptors (AT1 or AT2), contributes to preadipocyte proliferation and differentiation through induction of the FAS gene. Overexpression of AGT in mice, reached by using 5′ and 3′ flanking regions of the human gene, confirmed the expression of AGT in BAT and WAT (204). AGT null mice fed standard chow diet exhibit lower body weight and fat mass than wild-type mice while high fat diet induces a rise in fat mass but not in body weight (205). The partial rescue of AGT deficiency through specific expression in AT of rat AGT led to a return to normal plasma AGT level. The associated increase in body weight did not reach wild-type values. Finally, overexpression of AGT in wild-type mice provokes a rise in body weight (206). The presence or absence of AGT was correlated to the modification of adipocyte size, which is in agreement with in vitro studies suggesting the control of FAS by the renin-angiotensin system.

Detoxification molecules

WAT is involved in the storage of a lot of molecules, including toxics and metals. Metallothioneins (MT) have several roles in Zn and Cu detoxification by scavenging free radicals. As expected, mice with disrupted MT-I and MT-II genes are more sensitive to metal and oxidant toxicity but they also rapidly attain higher body mass with larger reserves of fat than wild-type mice (207). When obese, these mice show increased fat accretion, plasma leptin levels, LPL, and C/EBPα mRNA expression.

Lipids

Besides their direct involvement in the metabolic response of AT, lipids act as potent paracrine factors and appear to be interesting targets. Genetic manipulations of enzymes involved in lipid production or cell surface receptors have been reported. Since adipocyte production of prostaglandins influences adipogenesis, the consequences of the cyclooxygenase-2 gene disruption was investigated on AT development (208). Surprisingly, a specific phenotype is only present in heterozygous animals. Cyclooxygenase-$2^{+/-}$ mice showed increased fat mass and leptin production. The basal levels of prostaglandin E2 or 6-keto prostaglandin F1α were markedly reduced. The involvement of prostaglandin E2 in leptin release is hypothesized by the authors as responsible for the obesity of these mice. Lysophosphatidic acid, a lipid produced by adipocytes, is involved in preadipocyte proliferation and thus, AT development. In the preadipocyte, this bioactive phospholipid acts through membrane receptors called endothelial differentiation gene 2 (EDG2) to stimulate proliferation processes. Disruption of EDG2 (209) led to mice exhibiting a dramatic delay in terms of the development of various tissues and also of fat mass. However, since the EDG2 null mice phenotype is also associated with a suckling dysfunction, the direct link between reduced fat mass and absence of adipocyte EDG2 is still a hypothesis.

GENETIC ABLATION OF ADIPOSE TISSUES, LIPODYSTROPHY MODELS

Transgenesis strategies

Lipodystrophies represent a heterogeneous group of diseases characterized by alterations in body fat distribution and quantity and, in most cases, insulin resistance. Several transgenic mouse models have proved very useful for understanding the consequences of a lack of AT. The strategies used to engineer these mice encompass several interesting techniques used to specifically alter the quantity of a targeted tissue. The first studies used the diphtheria toxin A (DT-A) chain gene. To target expression in WAT and, to some extent, in BAT, the toxin gene was placed under the control of the aP2/ALBP promoter (31). Specific targeting of BAT could be achieved through the use of the UCP1 promoter (30). More recently, two novel lipoatrophy models were created. Because C/EBP family members have been shown to be important in the growth and differentiation of WAT, mice that express a dominant negative protein named A-ZIP/F under the control of the aP2/ALBP promoter were produced (210). Through heterodimerization, A-ZIP/F prevents DNA binding of b-ZIP transcription factors of the C/EBP and Jun families. Using a more conventional approach, mice with reduced WAT were obtained through the aP2/ALBP promoter-driven expression of a constitutively active form of ADD1/SREBP1c (nSREBP1c) (211). Because the factor has a positive action in adipogenesis in vitro, it came as a surprise to find that expression of a constitutively active form led to a decrease in fat abundance. The explanation may come from the down-regulation of genes essential for adipose differentiation, such as C/EBPα and PPARγ. As will be discussed below, lipodystrophy was also found in some gene knockout and mutant mouse models.

Depletion of white adipose tissue

The aP2-DT-A mice were the first transgenic mouse model of lipoatrophy (31). Neonatal lethality was observed in lines with a high expression of the transgene. Mice expressing lower DT-A levels had normal WAT amounts until up to two months of age. WAT depots begin to diminish at 5–6 months of age with extensive atrophy and necrosis of fat. Young transgenic mice were resistant

to obesity induced by monosodium glutamate and showed AT necrosis as in non-treated older animals. The mice were hyperphagic, developed a fatty liver and diabetes. In A-ZIP/F-1 and aP2-nSREBP1c mice, WAT development is impaired earlier in life with a more severe phenotype for the A-ZIP/F-1 mice compared with the aP2-nSREBP1c mice (210, 211). The mice show increased food intake and are hypermetabolic. Triglyceride content is increased in muscle and liver leading to hepatic steatosis. Plasma free fatty acid and triglyceride levels are increased. As expected from reduced fat stores, they show reduced plasma leptin levels. A salient feature of the mice is the development of diabetes with marked insulin resistance. They proved to be very valuable models to investigate the etiology of the metabolic disturbances associated with the lack of WAT. Surgical subcutaneous implantation of AT grafts reversed the hyperglycemia and lowered insulin levels in diabetic A-ZIP/F-1 mice (212). Whole-body and skeletal muscle insulin resistance were reduced demonstrating that diabetes is caused by the lack of AT. Other aspects of the phenotype, such as hyperphagia and hepatic steatosis, were improved. However, the improvement in triglyceride and free fatty acid levels was modest. A candidate to explain the phenotype of lipoatrophic mice is leptin since its production is dramatically reduced. In aP2-nSREBP1c mice, continuous infusion of low doses of recombinant leptin led to a major improvement in insulin sensitivity (213). The effect was independent of the action of leptin on food intake. In contrast, when leptin was infused at higher doses to A-ZIP/F-1 mice, only a moderate decrease of insulin and glucose levels was observed (214). The difference may come from the smaller amount of WAT in A-ZIP/F-1 mice. Recently, the A-ZIP/F-1 mice were crossed with transgenic "skinny" mice that overexpress leptin in the liver and, hence, are hypophagic and show increased insulin sensitivity (215). Interestingly, the double transgenic mice that lack AT and have elevated leptin levels show markedly improved insulin sensitivity. These data suggest that leptin may be useful in the long-term treatment of lipoatrophic diabetes.

Lipoatrophic mice were also used to investigate the site(s) of action of the thiazolidinediones (TZD), a novel class of antidiabetic agents. TZD improve insulin sensitivity through increased glucose utilization in muscle and, at higher doses, through inhibition of hepatic glucose production. Liver and muscle have low levels of PPARγ, the candidate nuclear receptor to mediate TZD effects. Thus, WAT is viewed as a good target as it is the only insulin-responsive tissue with a high level of PPARγ. However, aP2-DT-A mice treated with troglitazone show improved glucose tolerance, suggesting that the action of the TZD is independent of WAT (216). The question was re-examined in the more severely lipoatrophic A-ZIP/F-1 mice (217). Rosiglitazone or troglitazone treatment did not reduce glucose or insulin levels. However, TZD improved the lipid profile of mice and exacerbated hepatic steatosis. Furthermore, whole-body fat oxidation was increased. Taken together, the data suggest that a minimum amount of WAT is required to mediate the antidiabetic effect of TZD, as observed in aP2-DT-A mice. The effect on circulating triglycerides may come from the markedly upregulated expression of PPARγ in liver of A-ZIP/F-1 mice.

Depletion of brown adipose tissue

Partial ablation of BAT with decreased UCP1 content was achieved in UCP1-DT-A mice (30). This is not a model of human lipodystrophy sensu stricto since BAT is present in minute amounts in adult humans. However, they allowed new insights into the function of BAT on energy balance in rodents. The animals show decreased energy expenditure and hyperphagia leading to obesity. Transgenic mice are more susceptible to diet-induced obesity than control mice. As they age, they develop diabetes and are hypertriglyceridemic (218). The adjustment of food intake to environmental temperature is defective in UCP1-DT-A mice. When the animals are raised at thermoneutrality, obesity and hyperphagia are prevented, highlighting the importance of functional BAT in diet-induced thermogenesis (219). Interestingly, the transgenic mice present cardiovascular abnormalities reminiscent of human obesity, such as hypertension and left ventricular hypertrophy with eccentric remodeling and fibrosis (220).

Other models

Unexpectedly, targeted disruption of the gene encoding lysosomal acid lipase results in a very severe depletion of WAT and BAT (221). The enzyme hydrolyzes cholesterol esters and triglycerides that are delivered to lysosomes via the LDL receptor or other receptors. The connection between the lack of the lipase and AT depletion is still unknown. The severity of the phenotype with massive accumulation of triglyceride and cholesterol esters in several organs and short life span may preclude a detailed analysis. Studies of adipocyte precursor differentiating capacity and of the phenotype of AT-specific gene knockout mice may help to determine whether lysosomal lipase has a critical role in AT development. Although not obtained from transgenic manipulation, it is worth mentioning that the gene responsible for the lipodystrophy observed in the fatty liver dystrophy mutant mice has recently been characterized (222). The gene encodes a nuclear protein named lipin 1, which is expressed at high levels in WAT and is induced during adipocyte differentiation, suggesting a role in WAT development.

CONCLUSIONS

Major advances have been made in the understanding of AT development through the analysis of genetically modified animal models. In the past few years, numerous transgenic models have been generated to study the physiology of AT and the development of obesity or the preservation of leanness. Through targeted expression and gene invalidation, the role of suspected key proteins in energy homeostasis, endocrine/paracrine regulation, and adipogenesis could be demonstrated in vivo. However, the

limitations of complete gene invalidation are obvious. Compensatory mechanisms may mask the phenotype as suggested by data on UCP1 and HSL knockout mice (8, 100). Modern transgenic techniques with spatiotemporal control of gene expression will undoubtedly prove very useful as shown for adipocyte ablation of RXRα (53). In vivo targeted regulation of gene expression by engineered transcription factors, as recently done in 3T3-L1 adipocytes to repress PPARγ2 expression (223), may proved very informative. Control of gene expression in terms of quantities and/or kinetics will provide a more powerful approach to delineate the pathophysiological events related to AT development. For example, precise control of AT leptin expression in leptin-deficient Lep^{ob}/Lep^{ob} mice (188) may help to define the thresholds necessary for the different physiological actions of the hormone.

At first glance, one could consider that systemic invalidation or overexpression of gene candidates will unravel all aspects of AT development. However, considering genes one by one in a multigenic pathology such as common obesity represents the tip of the iceberg. The combination of genetic and environmental modifications has begun to reveal convergent pathways such as the obesity-prone association of β_3-AR knockout, α_2-AR AT expression, and high-fat diet (138). Other experimental strategies based on the quantitative trait locus analysis of genetic variations among inbred strains have been used to reveal the involvement of unsuspected genes in brown adipocyte induction within white AT (224). Such an approach combined with improved physiological techniques and functional genomics (e.g., DNA microarrays) could be used on transgenic animals to characterize modifier genes that influence transgene-dependent phenotypes (225). Detailed analysis of the existing models lacking an apparent phenotype using these techniques could help to unmask new pathways contributing to the development of obesity. Undoubtedly, transgenic studies will favor the design and development of new drugs aimed at combating an excess of AT or at preventing its deleterious effects.

The authors gratefully acknowledge Institut de Recherches Servier, Institut Roche sur l'Obésité, Laboratoires Clarins, and Produits Roche France for their support.

Manuscript received 30 November 2001 and in revised form 8 February 2002.

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