The Molecular Genetics of Rodent Single Gene Obesities*

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Only three fundamental mechanisms can underlie the development of obesity: 1) relative increase in energy intake; 2) relative decrease in energy expenditure; and 3) preferential partitioning of ingested calories to fat storage. That any one of these defects is sufficient to cause obesity is demonstrated by the phenotypes of animals produced by specific transgenic manipulations as follows.

1) Disruption of the 5HT2c serotonin receptor results in hyperphagia and obesity (1). 2) Defective nonshivering thermogenesis (energy expenditure) is the major abnormality in obese mice with ablation of brown adipose tissue due to tissue-specific expression of the diphtheria toxin gene driven by the uncoupling protein promoter (2). 3) Partitioning of calories to adipose tissue is the sole metabolic abnormality in obese mice carrying a human GLUT4 transgene that is constitutively expressed exclusively in fat (3).

By virtue of their effects on critical regulatory pathways of energy homeostasis, the rodent single gene obesities represent complex admixtures of these mechanisms and provide important insights into the molecular physiology of weight regulation.

agouti Signaling Protein (ASP): yellow Mutation (Ay)

A deletion in the autosomal dominant yellow mutations in the agouti (A) gene (e.g. Ay, lethal yellow; A<sup>y</sup>, viable yellow; A<sup>r</sup>, sienna yellow; A<sup>o</sup>, intermediate yellow; A<sup>ho</sup>, hypervariable yellow; A<sup>op</sup>, intracisternal A particle yellow) are promoter mutations characterized by obesity, hyperphagia, hyperinsulinemia, hypercorticos- ternism, and increased linear growth (4). The degree of obesity correlates with the amount and intensity of the yellow hair pigment, which results from overexpression of the agouti gene. Yellow mice are distinguishable by coat color from normal littermates at birth, but their obesity does not develop until postweaning (6–8 weeks). Plasma insulin and corticosterone concentrations are not elevated until the obesity is manifest. Hyperphagia is a major contributor to the obesity, since food restriction to 80% of normal intake can decrease body weight and body fat content to near ad libitum levels (4). However, restriction to 60% of normal intake does not decrease fractional body fat content beyond the reduction induced by the restriction to 80% of usual calories, indicating that there are metabolic alterations which increase metabolic efficiency and/or partitioning to adipose tissue of the yellow mutants. How-

ever, unlike the Lep<sup>ob</sup> or Lep<sup>ob</sup> mice, the yellow mutants display no defect in temperature control under cold stress.

The agouti locus encodes ASP, a 131-amino acid peptide with a non-coding sequence that place agouti under the control of heter- ologous promoters, which drive its overexpression in usual (e.g. hair follicle) and ectopic (e.g. brain) sites (7).

ASP in the hair follicle acts to switch pigment synthesis from eumelanin (black) to pheomelanin (yellow) production by blocking the action of melanocortin-stimulating hormone (MSH) at its recep-
tor (MC1R). Obesity induction by the yellow mutations, however, does not require the MC1R receptor since A<sup>y</sup> e/e mice lacking extension (e) = Mc1r<sup>e</sup> (8) are still obese (and black) (9). In parabiosis (joined circulation) studies and transgenic experiments in which mice over-express ASP solely in the skin, animals do not become obese (10). However, transgenic mice ubiquitously overexpressing ASP mimic the phenotype of A<sup>y</sup> mice (11). Thus, the ectopic overexpression of ASP likely acts through autocrine or paracrine, rather than endo-

crine, mechanisms to produce its pleiotropic effects.

Since the Mc1r gene product is not required for A<sup>y</sup>-induced obesity, the ASP synthesized in the brains of mutant animals must be producing its effects by another pathway. Recently, the melano-
cortin 4 receptor (MC4R) in the brain has been shown to mediate effects on food intake. Knockout animals homozygous for a disruption of the Mc4r gene are as obese as A<sup>y</sup> animals (12, 13). The normal ligand(s) for this brain receptor is not known. However, ASP competes with high affinity against MSH at MC4R (4). Thus, it appears likely that some of the obesity-producing effects of ASP expression in the brain may be due to its interference with signal generation by MSH at MC4R (4). This receptor which normally acts to suppress food intake. ASP also appears to induce lipogenesis in adipocytes by enhancing insulin sensitivity via a pathway that increases intracellular calcium (6).

ASIP (gene symbol for the human homolog of agouti) maps to 20q11.2 in humans and is normally expressed at low levels in skin, adipose tissue, testes, ovary, heart, liver, and kidney, suggesting that it may have roles in humans which are different from the mouse (14). Whether ASIP is normally expressed in human brain is not known. ASIP has not yet been directly linked to human obesity (15) or to variation in skin pigmentation or hair color (16).

agouti-related protein (AGRP), also known as agouti-related protein (16q22 in human; 16q12 in mouse), is a 132-aa protein 25% identical to agouti, is normally expressed primarily in the adrenal and the arcuate nucleus of the hypothalamus. The gene is overexpressed in Lep<sup>ob</sup> and Lep<sup>ob</sup> homozygotes, and AGRP selectively antagonizes a-MSH binding to MC3R and MC4R. Thus, AGRP acting via MC3Rs is a potential distal mediator of leptin effects on energy homeostasis (17, 18).

Carboxypeptidase E (Cpe), Fat Mutation

The distinguishing characteristic of mice homozygous for the Cpe<sup>mt</sup> mutation is early and severe hyperproinsulinemia (19), which is evident as early as 4 weeks of age (20). These animals display transient hyperglycemia, which is characteristic of the Lep<sup>ob</sup> and Lep<sup>ob</sup> mutants, but no hypercorticos- ternism (21). Obesity development progressively, starting between 8 and 12 weeks of age, with females becoming obese earlier than males. The specific metabolic/behavioral mechanism(s) by which these mice become obese is not known.

Cpe is required for the excision of paired dibasic residues remain-
ing at the C terminus of peptide prohormone intermediates such as proinsulin. Naggett et al. (19) identified a single S202P mutation in Cpe in the Cpe<sup>mt</sup> mouse that abolishes virtually all activity of the enzyme and accounts for the elevation in plasma proinsulin (19). Sites specific mutagenesis to introduce the S202P into wild-type Cpe resulted in no CPE activity above background. Residual carboxypeptidase activity from the presence of other carboxypeptidases apparently prevents the loss of CPE activity from resulting in lethality (21).

The molecular mechanism by which inactivation of CPE leads to obesity in these animals is unclear. Transgenic replacement of CPE activity in the islets of Cpe<sup>mt</sup> animals does not alter the obesity,
indicating that the obesity is not caused by the hyperproinsulinemia per se (21). The putative role of CPE in processing other prohormones and proneuropeptides such as neurotensin, GLP, MSH, POMC, TRH, CCK, gastrin, CRF, and melanin-concentrating hormone suggests possible mechanisms for the obesity in Cpefat animals via effects on these neuropeptides that mediate food intake and energy expenditure. The enzyme also acts as a secretory pathway sorting receptor for pro-opiomelanocortin and probably other endocrine proteins and proinsulin (22), and derangement in such sorting is another potential mechanism for the protein phenotypic manifestations of the Cpefat mutation.

Although no instance of human obesity related to sequence variation in CPE (4q32) has yet been identified, obesity due to compound heterozygosity for mutations in prohormone convertase 1 has been recently described in a 47-year-old woman (23). Prohormone convertase 1 cleaves prohormones at pairs of basic amino acids, leaving C-terminal basic residues, which are then excised by CPE. The phenotype of this individual, moderate obesity, hyperproinsulinemia, infertility, and lack of hypercortisolism, is very similar to that seen in the Cpefat mouse. Thus, the CPE pathway has been implicated in the control of body weight in humans.

**Tubby Mutation (tub)**

The tubby (tub) mouse does not develop obesity until at least 12 weeks of age, and when the obesity is much milder in degree than any of the other rodent single gene mutations (24). Hyperproinsulinemia is initially mild but progresses steadily until insulin concentrations are 10–20-fold higher than in lean mice with pancreatic islet hyperglycemia/hyperplasia. Hyperglycemia is never present. In fact, older tubby mice have blood glucose concentrations somewhat lower than lean control mice (20). Similar to fat, tub does not elicit hyperadrenocorticism (21). The physiologic mechanisms for the obesity in these animals is not known. The tubby gene product shows 62% amino acid similarity to a putative phosphodiesterase but is actually be a member of a novel protein family. Based upon the associated phenotypes of retinal degeneration and degeneration of the organ of Corti and the ganglion cells of the cochlea at the earliest age examined (3 weeks), it has been hypothesized that lack of the tubby gene product may result in apoptosis of neural cells (24, 25). tub is expressed at high levels in the hypothalamus, a region of the brain that plays a critical role in control of food intake and energy expenditure (25). Apoptosis of specific cells within the ventromedial nucleus might recapitulate the phenotype of increased adiposity observed when these nuclei are physically or chemically ablated (26). Allelic variation at the tub locus may also play a role in dietary-induced obesity, since one of the mouse quantitative trait loci for this phenotype maps to the genetic interval that contains tub (25, 27).

The human tub homolog is 94% identical to the mouse gene at the amino acid level with particularly high conservation within the first 260 amino acids. The human homolog maps to 11p15 in a region of synteny homology with mouse T (28). To date, no mutations in the human homolog have been reported.

**Leptin (Lep) and Leptin Receptor (Lepr) Mutations**

Considering that the Lepob and Lepob (or Lepob) mutant rodents share a mutation in a ligand (ob)-receptor (db/fa) pair, it is not surprising that these mutations produce identical phenotypes when carried on the same inbred strains (28). The earliest manifestation of these mutations is a defect in thermogenesis detectable within the first few days of life as a lower core temperature and a more rapid decline in body temperature upon cold stress (29). At 2 weeks of age, somatic fat mass is increased (30, 31). The mutants are obese and hyperphagic at the time of weaning (21 days). High concentrations of circulating corticosterone are a hallmark of the Lepob and Lepob phenotypes. Adrenalectomy can arrest the progression of the obesity and the diabetes (26), whereas extremely low doses of corticosterone after adrenalectomy are sufficient to bring back the full-blown obesity/diabetes syndrome (32, 33). It is unclear whether glucocorticoid or mineralocorticoid receptors, or both, convey the striking steroid dependence of the obese phenotype in these animals and most of the other genetic and experimental models of rodent obesity (26, 34).

**OB Protein (Leptin)**

Leptin is synthesized as a 167-amino acid protein and secreted from adipose tissue after excision of a 21-aa signal peptide (35, 36). For reasons indicated below, leptin is a plausible afferent signal of somatic fat stores. Leptin has a cytokine-like predicted tertiary structure, which includes 4 α-helices, 2 β-sheets, and a single disulfide bond between cysteines 96 and 146 (37).

Replacement of leptin in Lepob homozygous mice via either intraperitoneal or intracerebroventricular injection results in weight loss, decreased food intake, and increased physical activity. These effects are immediately reversed upon termination of leptin administration (38–40). Leptin treatment of normal mice, with or without diet-induced obesity, also decreases adipose tissue mass, whereas Lepob mice are unaffected by leptin treatment, suggesting that they are resistant to the hormone as originally suggested by Coleman’s parabiosis experiments (28).

Intracerebroventricular administration of leptin in Lepob mice rectifies systemic glucose disposal to a degree not fully accounted for by weight loss (41), suggesting that leptin may affect peripheral insulin sensitivity by neurally mediated effects. Intraperitoneal administration of leptin to non-obese male mice during starvation normalizes many of the endocrine changes (e.g., decreased thyroxine and testosterone, increased corticosterone), which occur as a result of food deprivation (42), but does not significantly alter the rate of weight loss. Administration to female mice accelerates the onset of sexual maturity (43, 44).

The human leptin gene (LEP) is 85% identical to the murine gene at the amino acid level (36) and maps to 7q31.3 as predicted based on synteny with proximal mouse chromosome 6 (45). Extreme obesity in humans has been linked to genetic markers near LEP (46–48). A definite role for leptin in human energy balance was recently confirmed by the demonstration of homozogosity for a frameshift mutation in LEP in two cousins (2 and 8 years old) with massive, early onset obesity (49). The phenotypes of the two children (from a consanguineous pedigree) differ in some very interesting ways from those seen in ob/ob mice. For example, the children do not show evidence of stunting, reduced lean body mass, or hypothermia. Such mutations are, however, apparently rare since earlier direct coding sequence analysis of a large number of human subjects has failed to identify any functionally significant coding sequence variants in this gene (50–52).

Plasma leptin concentrations (normal, 3–15 ng/ml) are increased in obese humans (53) and some rodents (other than Lepob) (54, 55) in proportion to body fat mass. Within a given fat depot, leptin mRNA expression is proportional to adipocyte volume (56). However, there is no evidence of a primary difference between obese and never-obese humans in rates of leptin production per unit of fat mass or of leptin clearance (57). Subcutaneous adipose tissue appears to produce more leptin than intraabdominal fat (58).

Circulating concentrations of leptin and adipose tissue leptin mRNA expression are reduced by fasting and restored to normal levels by feeding in rats (59). These changes are seen within a few hours in rodents and in 12–36 h in humans (60).

Whereas systemic administration of leptin to Lepob mice restores insulin-sensitive glucose disposal (41), insulin resistance to glucose disposal in obese humans occurs in the presence of high ambient leptin. Thus, both total leptin deficiency and extreme leptin excess are associated with resistance to insulin action. In human hepatocytes, leptin attenuates certain insulin-induced processes such as tyrosine phosphorylation of IRS-1, association of GRB2 with IRS-1, and down-regulation of gluconeogenesis (61). The findings in humans suggest that leptin may mediate some of the effects of obesity on insulin resistance.

Recombinant leptin inhibits basal insulin release in perfused pancreata of Lepob mice but not Lepob (LEPR-deficient) Zucker rats (62). The mechanism for this effect may be via leptin-mediated reduction in the amount of “lipotopic” intracellular triglycerides in the islets of Langerhans (63). Thus, leptin may also play a role in maintaining islet metabolic integrity.

It is not yet clear whether leptin must enter the cerebrospinal fluid to affect the regions of the brain that receive its putative signal.
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regarding fat mass. One of the circumventricular organs (vascular elements lacking the blood brain barrier) is located in the median eminence, just below the arcuate nucleus of the hypothalamus. The arcuate, which mediates aspects of ingestive behavior, projects axons to the median eminence, and it is possible that arcuate cell bodies are exposed directly to the circulation. In non-obese humans and animals, cerebrospinal fluid leptin concentration is about 5% of the plasma concentration, whereas in obese individuals, the ratio of cerebrospinal fluid to plasma leptin concentration is diminished, apparently due to saturation of the transport system at a plasma leptin concentration of approximately 25 ng/ml, well below the circulating concentration of leptin in most obese individuals (64). The resulting curvilinear relationship between plasma and cerebrospinal fluid leptin concentrations has been proposed as the mechanism for apparent “leptin resistance” of the obese.

Leptin’s effects on body weight appear to be mediated primarily via effects on the hypothalamus (38). Some of leptin’s effects on energy homeostasis are apparently conveyed by suppression of expression or action in the arcuate nucleus (41, 65) of neuropeptide Y (NPY), a potent stimulator of food intake. However, since mice without the Npy gene also respond to the anorexigenic effects of leptin (66) and since animals doubly mutant for Lep db and Npy are still obese (∼50% of Lep db with intact Npy) (66, 67), this neuropeptide cannot be the sole conveyer of leptin’s effects on energy balance. CRH (68) and AGRP (17) are examples of other candidates.

A novel splice donor site in the 3’-untranslated region of the penultimate exon of the gene has been described (74) with differential splicing of the gene in fa/fa rats. Transient transfection studies of a mouse Lep cDNA containing the Q269P mutation showed decreased cell surface binding of leptin with a normal affinity constant for leptin (82). Since it has been reported that there was no difference between leptin binding by frozen brain sections from lean and fa/fa rats (84), it appears that the Lepr db mutation affects binding and/or transport of the receptor to the cell surface. The Koletsky (fa) mutation is allelic to fa. Lepr db is a point mutation in Lepr causing a premature stop at codon 763 (TAT → TAG, Tyr → Stop) (82, 85).

The existence of this array of mutations in the leptin receptor in rodents clearly provides a rich resource for structure-function analysis of this protein. For example, the fa mutation may result in partial deficiency of LEPR, the ligand receptor of leptin, whereas fa/db is null.

The human leptin receptor protein is 78% homologous to the mouse gene at the amino acid level (69), and the gene (Lepr) maps to 1p31. There is an inversion of the gene order found in the mouse and rat (Pgm-Lepr-C8b) in humans (OBR-PGM-C8B) (86). Linkages of markers flanking LEPR to acute insulin release (87) and obesity (88) have recently been reported. Several polymorphisms within LEPR have been reported in both obese and normal weight humans (89–92), but the functional effects of these polymorphisms have yet to be determined. No human subject has yet been described with an unambiguous loss-of-function mutation in LEPR.

OB Receptor (Leptin Receptor)

The leptin receptor (Lepr) was cloned from a mouse choroid plexus cDNA expression library by screening the library with a leptin-like phosphatase fusion protein (69, 70). Mapping of the leptin receptor to the minimal 600-kilobase pair interval on mouse chromosome 4 and rat chromosome 5 containing, respectively, the diabetes (db) and Zucker fatty (fa) mutations and evidence of a partial duplication of the receptor in the db/db mutation strongly suggested that Lepr was the gene mutated in the diabetes mouse and Zucker fatty rat (71).

Sequence analysis of the Lepr cDNA indicated strong homology to gp130 and membership within the cytokine receptor superfamily, which includes the growth hormone receptor (69). Like most cytokine receptors, the leptin receptor has one stretch of hydrophobic amino acid residues (mouse LEPR amino acids 840–861) presumed to be the single transmembrane region. The extracellular region of Lepr (839 aa) has two cytokine receptor motifs (aa 251–325 and aa 551–627), which are suggested to be ligand binding domains (72). The intracellular region has two domains for binding to signal-transducing molecules. One region (aa 686–876) is near the membrane-spanning stretch and has a sequence sufficient for docking of kinases of the JAK family. Another region, near the C terminus of the longest isoform (1162 aa), has a sequence (1135YMPQ1141) that is sufficient (XXQ) for STAT3 binding (73).

At least five differentially spliced mRNAs for the mouse Lepr gene have been described (74) with differential splicing of the various 3’-terminal exons responsible for the heterogeneity.

The leptin receptor gene is expressed in multiple tissues. The long isoform represents the majority of Lepr transcripts in the brain and hypothalamus (up to 36% of all Lepr mRNA in the hypothalamus). Almost all other tissues express minor amounts (less than 10% of all Lepr mRNA) of the long isoform (75). The Lepr db mutation is due to a point mutation (74, 76) that generates a novel splice donor site in the 3’-untranslated region of the penultimate exon of the gene. The resulting frameshift affects only the longest isoform, showing that a deficiency of just this isoform is sufficient to produce the obesity/diabetes syndrome. The db/db mutation is due to a 17-base pair deletion in exon 11 (77).

In transient co-transfection experiments, the long isoform has been found to stimulate STAT1, STAT3, and possibly STAT5 (70). In addition, the mutant receptor appears to cause ligand-independent activation of STAT1 and STAT3 as well as impaired ligand-mediated STAT activation. Constitutive activation of STAT3 (in Lepr db) may desensitize the distal leptin-signaling cascade (78). Only STAT3 activation in hypothalamus has been detected when leptin is administered in vitro to mice (79). The intact long form is, however, not required for activation of all STATS, since STAT5B activation is achieved only when one small portion of the membrane’s proximal portion of the receptor is intact (80). Signal transduction (not necessarily JAK-STAT mediated) has been demonstrated in vitro with one of the short forms (Ra) (81).

The rat fatty mutation (Lepr db) is a point mutation of codon 269 (CAG → CCG, Q269P), which is within the first cytokine motif of the receptor (72, 82, 83). There are no alterations in mRNA levels of Lepr in fa/fa rats. Transient transfection studies of a mouse Lepr cDNA containing the Q269P mutation showed decreased cell surface binding of leptin with a normal affinity constant for leptin (82).

The cloning of these rodent mutations and initial characterization of the molecular bases for their effects on somatic energy stores constitute a major advance in our understanding of regulation of body weight in mammals. Access to these molecules will aid in the identification of additional components of these pathways.

It is tempting to think of these genes as belonging to a single pathway that controls body weight. This is almost certainly not the case. If we were to expect the phenotypes of all of the mutant animals to be much more similar (as they are for db and db/db), we would now have to focus on: 1) defining the manner in which these genes interact with other known components of the regulation of energy intake (such as the neuropeptides NPY, AGRP, CCK, CRF, melanin-concentrating hormone, GLP1, energy expenditure (sympathetic nervous system and uncoupling proteins), and energy partitioning (LPL, HSL, GLUT4, insulin); and 2) identifying all of the molecules that participate in these processes.

What is the relevance of these genes to understanding human obesity? Currently, instances of human obesity-attributable mutations in LEP and the CPE pathway have been identified. It is very likely that such examples also exist for the other genes discussed. However, the role of these genes in the less extreme forms of human obesity will likely be through subtle variation in the regulation of their expression rather than gross defects in their function (93). Efforts are currently under way to look for sequence variants in exons and regulatory elements, which may (alone or more likely in concert) play a role in the control of body weight.

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