

The future of feed intake regulation research

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ABSTRACT: Understanding the mechanisms involved in the control of feed intake and regulation of energy balance has increased greatly in recent years, thanks in part to the discovery of leptin, an event that ushered in a renaissance in research in this field. Over the last 5 yr, several other neuropeptides that affect feed intake and energy balance have been discovered, including cocaine- and amphetamine-regulated transcript, melanin-concentrating hormone, orexin/hypocretin, and agouti-related protein. In addition, new roles have been defined for previously discovered factors, such as galanin and neuropeptide Y. These recent advances have been possible because of new technologies, including cloning, transgenics, genomics, and bi-

oinformatics. For example, positional cloning techniques have been used to identify the genes for these peptides and factors and their receptors. Knowledge about specific transcription factor-binding motifs in promoter regions allows development of specific agents that alter gene expression. By using transgenic and cloning techniques, genes can be added or deleted, and transcription can be enhanced or suppressed to produce new animal models for studying interactions among factors. Over the next few years, the combination of microarray techniques and proteomics with sophisticated informatics tools will continue to provide fundamental insights into the complex physiological processes involved in feeding behavior and metabolism.

Key Words: Feeding behavior, Transgenic Animals

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Introduction

Advances in genetic manipulation technologies, such as targeted mutagenesis and gene cloning, have allowed investigators to better define the roles and mechanisms of a number of factors involved in feeding behavior and energy-balance regulation and to begin to define the interrelationships among these factors. To date, over 30 neuropeptides and neurotransmitters have been shown to be involved in the homeostatic mechanism for regulation of feed intake and energy balance. The ability to measure small changes in gene-expression levels and to inactivate specific genes has made it possible to identify where many of these factors fit into this feedback loop. In addition, genetic manipulation has been used to identify previously unknown regulators of energy balance, including intracellular processing enzymes and transcription factors. The picture that is evolving is that of a complex redundant system that functions under normal conditions to minimize the effects of short-term fluctuations in energy intake and expenditure.

Genetic and Transgenic Models and the Neurochemical Coding of Feeding Behavior

Genetic models of obesity have been extensively studied over the last 50 yr, particularly the *fa/fa* rat, the *ob/ob* mouse, and *db/db* mouse. Although obesity in these rodent models was known to be caused by single gene defects, the technology has only recently been available to determine the specific genes involved. The identification of the genes for leptin and its receptor was an important breakthrough and has led to a number of other discoveries in the field of feeding behavior and body weight regulation research.

Leptin

In 1994 and 1995, the leptin and leptin-receptor genes were shown to be the genes responsible for the obesity syndromes in the *ob/ob* mouse and in the *db/db* mouse and *fa/fa* rat (Zhang et al., 1994; Yamashita et al., 1997), respectively. Leptin and its receptors comprise the major pathway for relaying the metabolic state of adipose tissues, the primary site of leptin synthesis, to the brain. Leptin receptors have been localized in areas in the hypothalamus that contain other neuropeptides and neurotransmitters known to be involved in feeding behavior and energy-balance regulation. By using selective mutation of the genes that encode some

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of these neuropeptides, it has been possible to identify specific neuronal pathways involved in transduction of the leptin signal, as well as those involved more broadly in energy-balance regulation.

Neuropeptide Y

Neuropeptide Y (**NPY**) has been studied extensively for its effects on feeding behavior and energy-balance regulation. Central administration of NPY has been shown to cause hyperphagia, and, with chronic administration, obesity (Stanley et al., 1985; Miner et al., 1989; Billington et al., 1994). Synthesis and secretion of NPY are increased during food deprivation and weight loss (Sahu et al., 1992; Kalra et al., 1997; Kalra et al., 1999) and in several models of genetic obesity, including *ob/ob* mice, *db/db* mice and *fa/fa* rats (Sanacora et al., 1990; Wilding et al., 1992; Dryden et al., 1995). These and other findings have suggested an important link between leptin and NPY signaling. Unexpectedly, though, NPY knockout mice have normal feed intake and body weight and respond appropriately to food deprivation and leptin administration, findings that complicate the interpretation of NPY's role in energy-balance regulation (Erickson et al., 1996; Palmiter et al., 1998). Deletion of both NPY and leptin genes resulted in mice that had body weights and fat mass halfway between those of normal lean and *ob/ob* mice (Erickson et al., 1996). Thus, although leptin and NPY systems interact, the obesity that results from leptin deficiency does not seem solely due to lack of inhibition of NPY secretion, as had been suggested from earlier experiments. More recent studies involving targeted disruption of NPY receptor subtypes, as well as studies with agonists and antagonists to specific NPY receptor subtypes, are beginning to shed some light on the complexity within the NPY system. For example, disruption of the Y5 receptor gene resulted in development of late-onset obesity due to hyperphagia, and disruption of the Y1 receptor gene resulted in reduced metabolic rate, impaired insulin secretion, and down-regulation of uncoupling protein-2 (**UCP-2**) in white adipose tissue (Kushi et al., 1998; Marsh et al., 1998; Pedrazzini et al., 1998).

Corticotropin-Releasing Factor

Corticotropin-releasing factor (**CRF**) has also been proposed as an important factor in energy-balance regulation, based on findings from pharmacological studies. It is also another good example of how genetic manipulation has been used to demonstrate complexity of physiological functions in energy-balance regulation. Injection of CRF into the brain, particularly into the hypothalamic paraventricular nucleus, decreased spontaneous feeding as well as feeding stimulated by a variety of means, and chronic administration of CRF resulted in weight loss (Levine et al., 1983; Morley, 1987; Schwartz et al., 1995). Conversely, blockade of

CRF action through the use of specific antagonists or immunoneutralization caused hyperphagia and inhibited stress-induced anorexia (Krahn et al., 1986; Hulseley et al., 1995). Because CRF is also the primary physiological regulator of ACTH secretion from the pituitary, using genetic manipulations to produce generalized CRF deficiency or oversecretion in mice has not provided helpful information about its role in energy-balance regulation. Transgenic mice with overexpression of CRF demonstrated an increase in anxiogenic behavior, increased secretion of corticosteroids, and Cushing's Syndrome-like obesity (Stenzel-Poore et al., 1992, 1994). Deletion of the CRF gene and the resulting deficiency of CRF caused reduced viability in mice due to insufficient levels of corticosteroids (Muglia et al., 1997).

Another approach to studying CRF's role in energy-balance regulation has been to create transgenic mice overexpressing CRF binding protein, either under control of a pituitary-specific promoter or under control of the more ubiquitous metallothionein-1 promoter. In both cases, feed intake was enhanced, consistent with a decreased availability of CRF (Burrows et al., 1998; Lovejoy et al., 1998).

Corticotropin-releasing factor mediates its actions via two receptor subtypes, CRF-1 and CRF-2. The CRF-2 receptors are thought to be primarily responsible for CRF's effects on feeding behavior and energy balance (Martinez et al., 1998). Transgenic mice with CRF-1 receptor deficiency had reduced adrenal response to stress and decreased anxiety levels, indicating the role of this receptor subtype in stress-related reactions (Smith et al., 1998; Turnbull et al., 1999). Recently, CRF-2-deficient mice have been produced by targeted mutation of the CRF-2 gene (Kishimoto et al., 2000). The males, but not females, were shown to have enhanced anxiety without changes in hypothalamic-pituitary-adrenal axis activity. Neither males nor females showed any changes in feeding behavior, thus calling into question the role of CRF-2 receptors in energy-balance regulation. However, urocortin, an endogenous peptide that preferentially binds CRF-2 receptors, has been shown to reduce feed intake and to promote weight loss without affecting the stress response (Asakawa et al., 1999). Thus, further investigation into the role of the CRF receptors and their endogenous ligands is needed to determine the importance of this system in feeding behavior and energy-balance regulation.

Pro-Opiomelanocortin-Derived Peptides

A number of peptides derived from pro-opiomelanocortin (**POMC**) seem to be involved at several levels in energy-balance regulation. The opioid system, including β -endorphin, dynorphins, and enkephalins and their receptors (μ -, κ -, and δ -opioid receptors), seems to be involved in stimulation of feeding (Baile et al., 1986). Transgenic mice have been produced in which a specific opioid peptide or receptor has been either deleted or

overproduced, but the focus of these studies has been on their roles in anxiety, pain, or reward, and not feeding behavior or energy balance (Matthes et al., 1996; Rubinstein et al., 1996; Kieffer, 1999). Thus, how these peptides fit into the feeding circuit has not yet been fully explored.

The melanocortin family of peptides is also derived from POMC. One such peptide, α -melanocyte-stimulating hormone, has been shown to inhibit feeding in rats and mice (Fan et al., 1997), an effect that is mediated primarily by a specific melanocortin receptor subtype, MC-4 (Marsh et al., 1999). Studies of obese mice with the naturally occurring A^y autosomal dominant mutation, caused by overexpression of agouti peptide, led to the discovery of a similar peptide that is expressed only in the arcuate nucleus of the hypothalamus, agouti-related peptide (**AGRP**) (Ollmann et al., 1997; Wilson et al., 1999). Both agouti peptide and AGRP are specific and potent antagonists of MC-4 receptors, and transgenic mice overexpressing either agouti peptide or AGRP are phenotypically identical to the naturally occurring A^y mutant obese mice. Obesity can also be produced by targeted deletion of the MC-4 receptor gene (Huszar et al., 1997). These findings suggest that AGRP and the MC-4 receptor play an important role in energy-balance regulation. The finding that MC-4 receptor-deficient mice were resistant to leptin administered either centrally or peripherally suggests that the MC system is downstream of leptin (Marsh et al., 1999). However, targeted disruption of the MC-4 gene in *ob/ob* mice had an additive effect on weight gain and adiposity (Boston et al., 1997); thus, leptin's effects on feeding and adiposity do not appear to be mediated exclusively through changes in melanocortin signaling.

Melanin-concentrating Hormone

Another peptide that has been identified through genetic manipulation as having an important role in energy-balance regulation is melanin-concentrating hormone (**MCH**). Pharmacological studies indicated that, compared with NPY, MCH had only a weak orexigenic effect when administered centrally, and chronic administration had no effect on weight gain (Rossi et al., 1997). However, in contrast to NPY gene deletion, MCH gene deletion resulted in decreased feed intake, body weight, and adiposity, and increased metabolic rate (Shimada et al., 1998), thus suggesting a physiological role for MCH in energy-balance regulation. Several studies have been carried out to investigate the interaction between leptin and MCH systems. In one study, leptin administered peripherally in *ob/ob* mice decreased feed intake and body weight, but unexpectedly resulted in increased hypothalamic levels of MCH and MCH mRNA (Huang et al., 1999). In another study, however, leptin administered centrally in rats decreased MCH levels (Sahu, 1998b). Leptin administration has also been shown to block MCH-induced feeding (Sahu, 1998a). It has been suggested that leptin acts

postsynaptically to prevent MCH-induced feeding (Sahu, 1998a), but further work will be necessary to define the interrelationships between the leptin and MCH pathways.

Cocaine- and Amphetamine-Regulated Transcript

Cocaine- and amphetamine-regulated transcript (**CART**) peptides are some of the most recently identified peptides thought to be involved in feeding behavior. The discovery of CART peptides also illustrates how some of the newer technologies in genomics and proteomics have been used to identify physiologically important peptides. Cocaine- and amphetamine-regulated transcript mRNA were identified on the basis of their increase in the brain following cocaine or amphetamine treatment in rats (Douglass et al., 1995). Once the mRNA sequence was known, it was possible to predict the amino acid sequence of the protein product. The amino acid sequence indicated that it had characteristics in common with other well-known peptide neurotransmitter precursors, even though the CART mRNA sequence was not homologous to any other mRNA (Gautvik et al., 1996; Strand, 1999). The gene for CART peptides has now been characterized in both humans and mice (Douglass and Daoud, 1996; Adams et al., 1999), along with several CART peptides that are produced through posttranslational modifications (Kuhar and Yoho, 1999).

The CART peptides are localized in specific areas of the brain, including those associated with reinforcement and reward (Koyle et al., 1998), stress and endocrine regulation (Kristensen et al., 1998), sensory processing (Koyle et al., 1998), and feeding (Elmquist et al., 1999). There is good evidence that CART peptides play an important role in the control of feed intake and that CART and leptin pathways are linked. Injection of CART peptides into the cerebral ventricular system decreased feeding, and injection of CART antibodies stimulated feeding (Lambert et al., 1998). The CART peptides are colocalized with leptin receptors in hypothalamic neurons, both *fa/fa* rats and *ob/ob* mice have reduced levels of CART mRNA in the arcuate nucleus, and administration of leptin to *ob/ob* mice increased CART mRNA (Elias et al., 1998; Kristensen et al., 1998). Because CART peptides have functions in other physiological systems, the relative importance of its effects on feeding is not yet known. However, once specific CART receptors have been identified, it will be possible to explore their roles in feeding behavior and energy-balance regulation more fully.

Malonyl-Coenzyme A

Recently, findings from a study investigating the effects of a synthetic inhibitor of fatty acid synthase (**FAS**), C75, have demonstrated a potent and dose-dependent suppression of feed intake and loss of body weight (Loftus et al., 2000). This effect was independent

of leptin, and it seemed to be mediated through suppression of NPY expression in the hypothalamus. The mechanism for the inhibition of feeding seems to be related to levels of malonyl CoA, the substrate of FAS, in specific areas of the hypothalamus. Thus, malonyl CoA levels in hypothalamic neurons may act as a signal of fuel status.

Genetic Modification of Adipose Tissue

Genetic manipulation has been used to identify specific intracellular proteins and signaling molecules involved in energy-balance regulation. Many of these include factors involved in adipose tissue function. An early example was the use of a transgenic toxigene approach to ablate brown fat in mice, resulting in development of obesity due to reduction in thermogenesis (Lowell et al., 1993; Klaus et al., 1998). Targeting genetic modifications to adipocytes through the use of adipocyte-specific promoters, such as the aP2 gene promoter, has led to other important findings about adipocyte function. Transgenic mice overexpressing β -1 adrenergic receptors (**β 1-AR**) in adipocytes were shown to have reduced adipose tissue mass and resistance to diet-induced obesity (Soloveva et al., 1997). Mice with targeted disruption of β 3-AR had reduced energy expenditure, increased adipose tissue mass, and increased feed intake, but also had upregulation of β 2- and β 1-AR gene expression, indicating an important interaction among adrenergic receptor subtypes in adipose tissue (Susulic et al., 1995).

Adipose tissue mass has also been altered through manipulation of uncoupling protein (**UCP**) genes and the *glucose transporter-4* (**GLUT-4**) gene. Overexpression of GLUT-4 in adipose tissue of transgenic mice resulted in obesity caused solely by adipocyte hyperplasia (Gnudi et al., 1996). Increased adipose tissue expression of UCP-1 resulted in decreased subcutaneous fat in mice fed a high-fat diet and prevented development of obesity in A^y genetically obese mice (Kopecky et al., 1995, 1996). Surprisingly, though, inactivation of the UCP-1 gene did not cause obesity, possibly as a result of increased expression of UCP-2 (Enerback et al., 1997).

A variety of other cellular components have been identified as having a role in regulation of adipose tissue mass through the use of targeted deletion of genes or enhancement of gene expression. For example, deletion of the gene for the RII subunit of protein kinase A resulted in reduced adipose tissue mass and resistance to dietary-induced obesity (McKnight et al., 1998). Likewise, disruption of HMGIC, a protein primarily expressed in undifferentiated mesenchymal cells, reduced obesity caused by leptin deficiency in *ob/ob* mice (Anand et al., 2000). Transgenic mice having no white adipose tissue were generated by targeting adipose tissue expression of A-ZIP, a protein that prevents DNA binding of certain transcription factors (C/EBP and Jun families) (Moitra et al., 1998). These mice also had many biochemical defects and reduced viability.

Genetic Modification in Food-Producing Animals

The use of transgenic technologies to alter production traits in livestock holds tremendous promise for the future of the animal-production industries; however, there are major limitations to overcome, including insufficient information about which genes to target and the low efficiency and high cost of producing transgenic livestock. Information about factors involved in energy-balance regulation that has been obtained from studies of transgenic mouse models can suggest certain directions for experiments in livestock, but currently there are few actual examples where this has been done. Recently, transgenic pigs with a zinc-inducible porcine GH gene have been produced (Nottle et al., 1999). Expression of GH in these pigs is regulated by zinc content in the feed, thereby reducing the potential development of adverse effects of continuously high GH levels. These pigs have increased growth rate, feed efficiency, and muscle-to-body fat ratio.

A naturally occurring genetic mutation in certain breeds of cattle is responsible for double muscling, or muscle hyperplasia. This trait has been reproduced in transgenic mice with targeted deletion of growth-differentiating factor-8 (also known as myostatin), an inhibitor of muscle growth (McPherron et al., 1997). Because the myostatin gene has been mapped to the same locus as that for muscle hypertrophy in cattle (Smith et al., 1997), myostatin could be a potentially useful target for genetic manipulation in other breeds of cattle, as well as in other meat-producing animals.

The Future: Genetic Modification, Genomics, and Proteomics

At the cutting edge of research into many physiological and pathophysiological processes are the technologies employed in genomics and proteomics. Genomics, the study of all genetic information, is expected to lead to the development of gene-based therapeutics, small-molecule drugs, and diagnostic tests for the detection of genetic conditions. For example, genotyping is used to help determine which genes are responsible for specific inherited traits. Identification of these genetic markers provides a means of understanding the mechanisms behind certain inherited traits. It has long been known that traits of economic importance such as litter size in swine, growth efficiency, lean-meat yield, and meat quality and palatability in both cattle and swine are genetically determined. As much as 50% of the variation in these traits can be explained by the specific combinations of genes that define each animal's genome. The remaining variation is due to preharvest management and environmental influences, including animal health, feed quality and availability, and post-harvest processing.

The discovery and use of single nucleotide polymorphisms in genes linked to specific traits and inherited

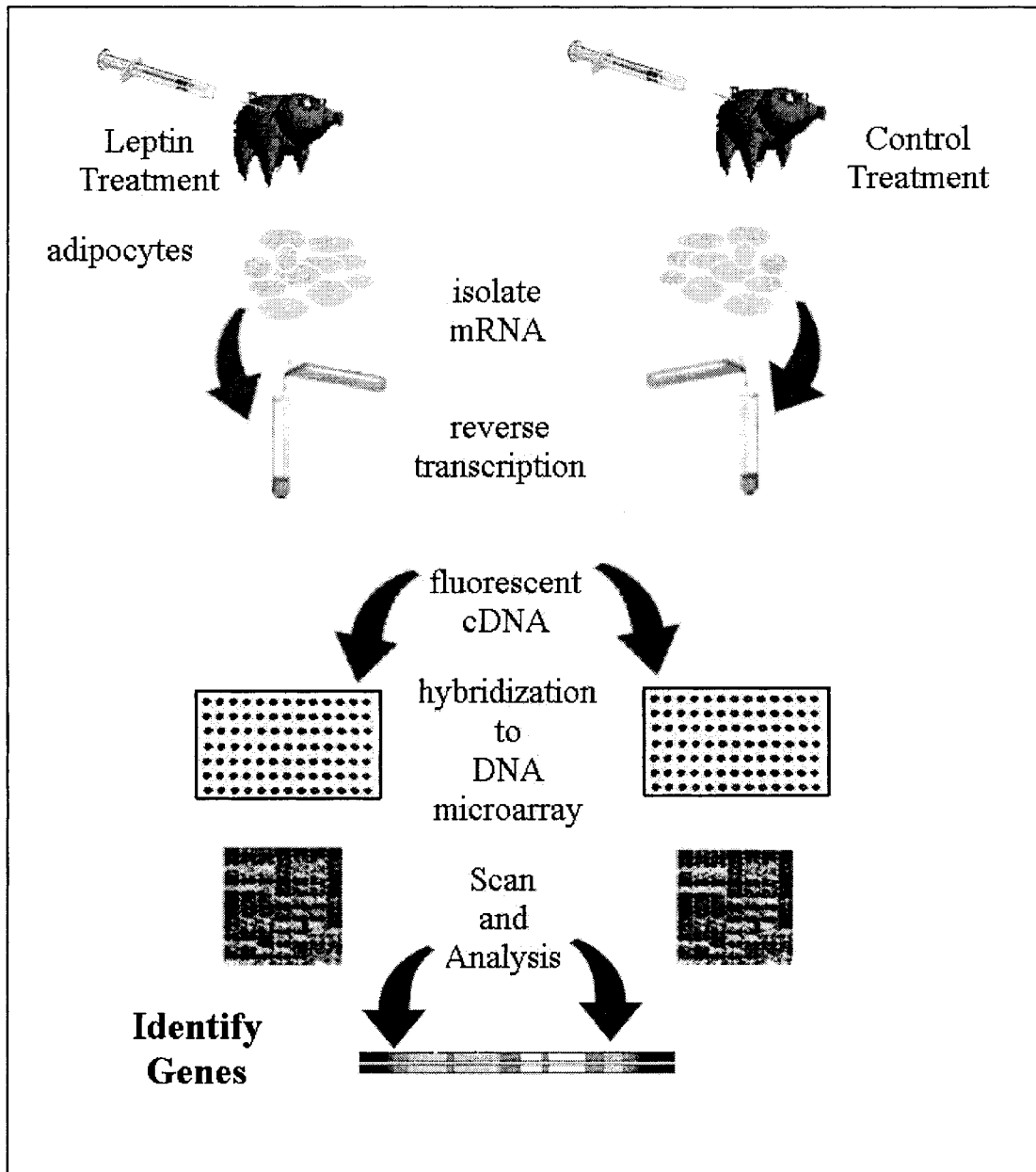


Figure 1. Comparative gene expression analysis

diseases have provided an important impetus for genomic research. Single-nucleotide polymorphisms represent natural genetic variability occurring at high density in the genome, and their properties and density make them useful markers or tools for identifying trait-associated genes in as yet uncharacterized parts of the genome.

Methods currently employed for detection of genetic variability, such as sequencing, are often complex, costly, and inaccurate. However, the development of enzymatic mutation detection assays has greatly accelerated the process of gene discovery and characterization (Ingnas et al., 2000). With these assays, mismatches can be localized within two to four codons of the actual position, thus reducing the length of the DNA

fragment that must be sequenced for confirmation and greatly simplifying evaluation of the final sequencing data.

Genetic-modification techniques have led to tremendous growth in information about the physiology of feed intake and energy-balance regulation; however, some of the strategies used in the past have been inadequate for deciphering the various roles of factors involved. More recent advances in gene-targeting techniques offer the possibility of controlling gene expression in both time- and site-specific ways. For example, the use of tissue-specific promoters, such as the adipocyte-specific promoter aP2, allows genetic modifications to be limited to specific cell types. Likewise, specific control elements that regulate the activity of transgenes can be used to

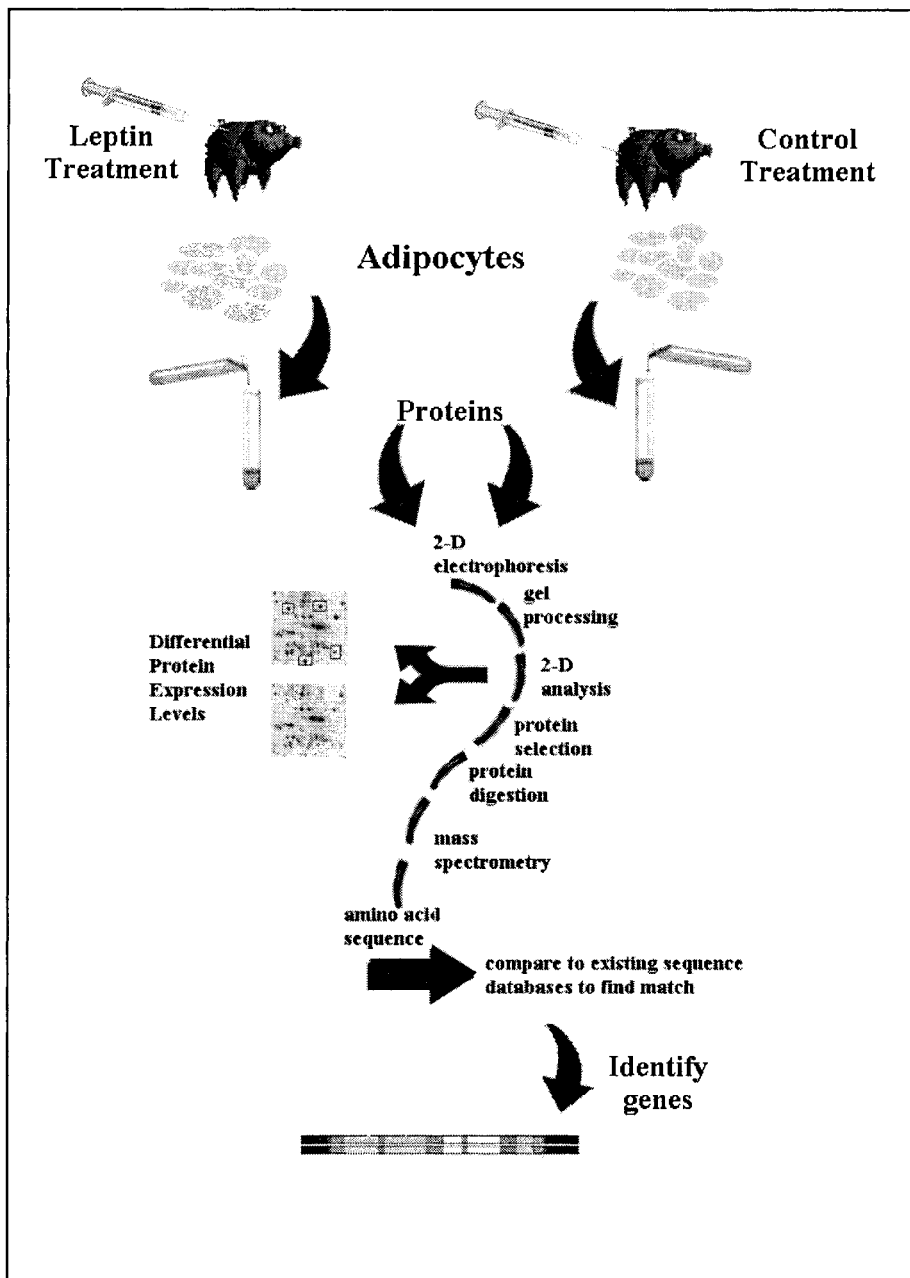


Figure 2. Comparative proteomic analysis can be used to determine treatment-related changes in protein expression, and subsequently identify which genes are involved.

induce or suppress gene expression. Temporal control of gene expression has been achieved through the use of a transcriptional transactivator that is responsive to the presence or absence of tetracycline (Gossen et al., 1995). The use of a zinc-inducible porcine GH gene in pigs (described above) is an example of application of this technique in animal production.

The creation of artificial chromosomes may provide a means of introducing gene clusters into the genome. Pronuclear injection of artificial chromosomes constructed in bacteria has been carried out in mice and resulted in germline transmission as well as expression of the transgene (Yang et al., 1997). This method could

be used to create cell lines that would provide nuclei for nuclear transfer, a technique that has already proven successful in a number of species, including cattle and sheep. However, there are still many technical hurdles to overcome in combining targeted genetic modification techniques with efficient use of nuclear transfer in livestock (Wilmut et al., 1997; Cibelli et al., 1998; Boquest et al., 1999)

Genomics is generating massive amounts of new information that is likely to take decades to be even partially understood and utilized. Interpretation and application of this information are limited in part by the lack of information about the control of gene expression,

translation, co- and posttranslation modifications, etc. The “proteome” is defined as the protein complement of the genome, including the different proteins occurring in an organism in space and time, such as the co- and posttranslational modifications of proteins. Proteomics, the study of these postgenomic steps, provides a means of identifying and characterizing disease-specific proteins, as well as proteins that play important roles in growth, reproduction and metabolism (Figures 1 and 2). Once these proteins are identified, they can be targeted for the development of both diagnostic tools and therapeutic treatments. The development, integration, and automation of large-scale analytical tools and the emergence of sophisticated informatics approaches are making this possible.

Through the marketing of a diverse array of genomics-based products in animal-production industries, research initiatives will support these industries to more efficiently produce a variety of products, including the following:

- Genome scans for the localization of genes influencing production efficiency and product quality (value);
- Marker-assisted selection programs to support breeding and marketing of food-producing animals possessing enhanced genetic merit for value;
- Diagnostic services to assist animal-production facilities to derive increased value from the improved management and marketing of the inherent genetic variation within these sectors;
- Genomics “tools” to facilitate the processes of gene discovery, patenting, and ownership; and
- The development of gene-based products targeted toward pharmaceutical applications for the improvement of value.

Conclusion

The development of transgenic technologies has provided new opportunities for studying the basic mechanisms involved in control of feed intake and energy-balance regulation. Just within the last few years, major advances have been made in our understanding of these systems, as well as our appreciation of their complexities. However, the techniques used to create genetic modifications are still relatively crude, and most have only been applied in mice. Important advancements in these technologies will allow combination of tissue-specific promoters with elements that provide for temporal control of gene expression. Evolving technologies of genomics and proteomics, combined with powerful data mining and analysis techniques being developed, offer the best opportunity for unraveling the details of complex physiological systems, such as those involved in control of feed intake and regulation of energy balance.

Implications

New technologies being developed in genomics and proteomics are bringing about revolutionary changes

in animal agriculture. Genomic research is providing information necessary for better understanding the physiology of feed intake and growth regulation and for developing customized genetics. As much as 50% of the variation in important traits, such as control of feed intake, can be accounted for by specific combinations of genes. Once identified, transgenesis can be used to modify genes responsible for growth characteristics, and animals can be selected for specific niche markets for enhanced value capture. In the future, accepted and efficient means of introducing new genes associated with important traits will be used to greatly improve the efficiency of production. As the world faces increasing population growth pressures, these new technologies will be used to develop solutions to the problem of providing sufficient, nutritious, and safe food in environmentally sound ways.

Literature Cited

- Adams, L. D., W. Gong, S. D. Vechia, R. G. Hunter, and M. J. Kuhar. 1999. CART: from gene to function. *Brain Res.* 848:137–140.
- Anand, A., and K. Chada. 2000. In vivo modulation of HMGIC reduces obesity. *Nat. Genet.* 24:377–380.
- Asakawa, A., A. Inui, N. Ueno, S. Makino, M. A. Fujino, and M. Kasuga. 1999. Urocortin reduces food intake and gastric emptying in lean and ob/ob obese mice. *Gastroenterology* 116:1287–1292.
- Baile, C. A., C. L. McLaughlin, and M. A. Della-Fera. 1986. Role of cholecystokinin and opioid peptides in control of food intake. *Physiol. Rev.* 66:172–234.
- Billington, C. J., J. E. Briggs, S. Harker, M. Grace, and A. S. Levine. 1994. Neuropeptide Y in hypothalamic paraventricular nucleus: a center coordinating energy metabolism. *Am. J. Physiol.* 266:R1765–1770.
- Boquest, A. C., B. N. Day, and R. S. Prather. 1999. Flow cytometric cell cycle analysis of cultured porcine fetal fibroblast cells. *Biol. Reprod.* 60:1013–1019.
- Boston, B. A., K. M. Blaydon, J. Varnerin, and R. D. Cone. 1997. Independent and additive effects of central POMC and leptin pathways on murine obesity. *Science* 278:1641–1644.
- Burrows, H. L., M. Nakajima, J. S. Lesh, K. A. Goosens, L. C. Samuelson, A. Inui, S. A. Camper, and A. F. Seasholtz. 1998. Excess corticotropin releasing hormone-binding protein in the hypothalamic-pituitary-adrenal axis in transgenic mice. *J. Clin. Invest.* 101:1439–1447.
- Cibelli, J. B., S. L. Stice, P. J. Golueke, J. J. Kane, J. Jerry, C. Blackwell, F. A. Ponce de Leon, and J. M. Robl. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* 280:1256–1258.
- Douglass, J., and S. Daoud. 1996. Characterization of the human cDNA and genomic DNA encoding CART: A cocaine- and amphetamine-regulated transcript. *Gene* 169:241–245.
- Douglass, J., A. A. McKinzie, and P. Couceyro. 1995. PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. *J. Neurosci.* 15:2471–2481.
- Dryden, S., L. Pickavance, H. M. Frankish, and G. Williams. 1995. Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats. *Brain Res* 690:185–188.
- Elias, C. F., C. Lee, J. Kelly, C. Aschkenasi, R. S. Ahima, P. R. Couceyro, M. J. Kuhar, C. B. Saper, and J. K. Elmquist. 1998. Leptin activates hypothalamic CART neurons projecting to the spinal cord. *Neuron* 21:1375–1385.
- Elmquist, J. K., C. F. Elias, and C. B. Saper. 1999. From lesions to leptin: Hypothalamic control of food intake and body weight. *Neuron* 22:221–232.

- Enerback, S., A. Jacobsson, E. M. Simpson, C. Guerra, H. Yamashita, M. E. Harper, and L. P. Kozak. 1997. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature (Lond.)* 387:90–94.
- Erickson, J. C., G. Hollopeter and R. D. Palmiter. 1996. Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science* 274:1704–1707.
- Fan, W., B. A. Boston, R. A. Kesterson, V. J. Hruby, and R. D. Cone. 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature (Lond.)* 385:165–168.
- Gautvik, K. M., L. de Lecea, V. T. Gautvik, P. E. Danielson, P. Tranque, A. Dopazo, F. E. Bloom, and J. G. Sutcliffe. 1996. Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. *Proc. Natl. Acad. Sci. U S A* 93:8733–8738.
- Gnudi, L., P. R. Shepherd, and B. B. Kahn. 1996. Over-expression of GLUT4 selectively in adipose tissue in transgenic mice: implications for nutrient partitioning. *Proc. Nutr. Soc.* 55:191–199.
- Gossen, M., S. Freundlieb, G. Bender, G. Muller, W. Hillen, and H. Bujard. 1995. Transcriptional activation by tetracyclines in mammalian cells. *Science* 268:1766–1769.
- Huang, Q., A. Viale, F. Picard, J. Nahon, and D. Richard. 1999. Effects of leptin on melanin-concentrating hormone expression in the brain of lean and obese lepob/lepob mice. *Neuroendocrinology* 69:145–153.
- Hulsey, M. G., C. M. Pless, and R. J. Martin. 1995. ICV administration of anti-corticotropin-releasing factor antisense oligonucleotide: effects on feeding behavior and body weight. *Regul. Pept.* 59:241–246.
- Huszar, D., C. A. Lynch, V. Fairchild-Huntress, J. H. Dunmore, Q. Fang, L. R. Berkemeier, W. Gu, R.A. Kesterson, B.A. Boston, R.D. Cone, F.J. Smith, L.A. Campfield, P. Burn, and F. Lee. 1997. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88:131–141.
- Inganas, M., S. Byding, A. Eckersten, S. Eriksson, T. Hultman, A. Jorsback, E. Lofman, F. Sabounchi, U. Kressner, G. Lindmark, and N. Tooke. 2000. Enzymatic mutation detection in the P53 gene. *Clin Chem* 46:1562–1573.
- Kalra, S. P., M. G. Dube, S. Pu, B. Xu, T. L. Horvath, and P. S. Kalra. 1999. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr. Rev.* 20:68–100.
- Kalra, P. S., M. G. Dube, B. Xu and S. P. Kalra. 1997. Increased receptor sensitivity to neuropeptide Y in the hypothalamus may underlie transient hyperphagia and body weight gain. *Regul. Pept.* 72:121–130.
- Kieffer, B. L. 1999. Opioids: First lessons from knockout mice. *Trends Pharmacol. Sci.* 20:19–26.
- Kishimoto, T., J. Radulovic, M. Radulovic, C. R. Lin, C. Schrick, F. Hooshmand, O. Hermanson, M. G. Rosenfeld, and J. Spiess. 2000. Deletion of *crrh2* reveals an anxiolytic role for corticotropin-releasing hormone receptor-2. *Nat. Genet.* 24:415–419.
- Klaus, S., H. Munzberg, C. Truloff, and G. Heldmaier. 1998. Physiology of transgenic mice with brown fat ablation: Obesity is due to lowered body temperature. *Am. J. Physiol.* 274:R287–293.
- Kopecky, J., G. Clarke, S. Enerback, B. Spiegelman, and L. P. Kozak. 1995. Expression of the mitochondrial uncoupling protein gene from the *ap2* gene promoter prevents genetic obesity. *J. Clin. Invest.* 96:2914–2923.
- Kopecky, J., M. Rossmeisl, Z. Hodny, I. Syrový, M. Horakova, and P. Kolarova. 1996. Reduction of dietary obesity in *ap2-Ucp* transgenic mice: Mechanism and adipose tissue morphology. *Am. J. Physiol.* 270:E776–786.
- Koylu, E. O., P. R. Couceyro, P. D. Lambert, and M. J. Kuhar. 1998. Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain. *J. Comp. Neurol.* 391:115–132.
- Krahn, D. D., B. A. Gosnell, M. Grace, and A. S. Levine. 1986. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. *Brain Res. Bull.* 17:285–289.
- Kristensen, P., M. E. Judge, L. Thim, U. Ribel, K. N. Christjansen, B. S. Wulff, J. T. Clausen, P. B. Jensen, O. D. Madsen, N. Vrang, P. J. Larsen, and S. Hastrup. 1998. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature (Lond.)* 393:72–76.
- Kuhar, M. J., and L. L. Yoho. 1999. CART peptide analysis by Western blotting. *Synapse* 33:163–171.
- Kushi, A., H. Sasai, H. Koizumi, N. Takeda, M. Yokoyama, and M. Nakamura. 1998. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor-deficient mice. *Proc. Natl. Acad. Sci. U S A* 95:15659–15664.
- Lambert, P. D., P. R. Couceyro, K. M. McGirr, S. E. Dall Vechia, Y. Smith, and M. J. Kuhar. 1998. CART peptides in the central control of feeding and interactions with neuropeptide Y. *Synapse* 29:293–298.
- Levine, A. S., B. Rogers, J. Kneip, M. Grace, and J. E. Morley. 1983. Effect of centrally administered corticotropin releasing factor (CRF) on multiple feeding paradigms. *Neuropharmacology* 22:337–339.
- Loftus, T. M., D. E. Jaworsky, G. L. Frehywot, C. A. Townsend, G. V. Ronnett, M. D. Lane, and F. P. Kuhajda. 2000. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. *Science* 288:2379–2381.
- Lovejoy, J. C., M. M. Windhauser, J. C. Rood, and J. A. de la Bretonne. 1998. Effect of a controlled high-fat versus low-fat diet on insulin sensitivity and leptin levels in African-American and Caucasian women. *Metabolism* 47:1520–1524.
- Lowell, B. B., S.-S. V., A. Hamann, J. A. Lawitts, J. Himms-Hagen, B. B. Boyer, L. P. Kozak, and J. S. Flier. 1993. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature (Lond.)* 366:740–742.
- Marsh, D. J., G. Hollopeter, D. Huszar, R. Laufer, K. A. Yagaloff, S. L. Fisher, P. Burn, and R. D. Palmiter. 1999. Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nat. Genet.* 21:119–122.
- Marsh, D. J., G. Hollopeter, K. E. Kafer, and R. D. Palmiter. 1998. Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nat. Med.* 4:718–721.
- Martinez, V., E. Barquist, J. Rivier, and Y. Tache. 1998. Central CRF inhibits gastric emptying of a nutrient solid meal in rats: the role of CRF2 receptors. *Am. J. Physiol.* 274:G965–970.
- Matthes, H. W., R. Maldonado, F. Simonin, O. Valverde, S. Slowe, I. Kitchen, K. Befort, A. Dierich, M. Le Meur, P. Dolle, E. Tzavara, J. Hanoune, B. P. Roques, and B. L. Kieffer. 1996. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature (Lond.)* 383:819–823.
- McKnight, G. S., D. E. Cummings, P. S. Amieux, M. A. Sikorski, E. P. Brandon, J. V. Planas, K. Motamed and R. L. Idzerda. 1998. Cyclic AMP, PKA, and the physiological regulation of adiposity. *Recent Prog. Horm. Res.* 53:139–159.
- McPherron, A. C., A. M. Lawler, and S. J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature (Lond.)* 387:83–90.
- Miner, J. L., M. A. Della-Fera, J. A. Paterson, and C. A. Baile. 1989. Lateral cerebroventricular injection of neuropeptide Y stimulates feeding in sheep. *Am. J. Physiol.* 257:R383–387.
- Moitra, J., M. M. Mason, M. Olive, D. Krylov, O. Gavrilova, B. Marcus-Samuels, L. Feigenbaum, E. Lee, T. Aoyama, M. Eckhaus, M. L. Reitman, and C. Vinson. 1998. Life without white fat: a transgenic mouse. *Genes Dev.* 12:3168–3181.
- Morley, J. E. 1987. Neuropeptide regulation of appetite and weight. *Endocr. Rev.* 8:256–287.
- Muglia, L. J., L. Jacobson, S. C. Weninger, C. E. Luedke, D. S. Bae, K. H. Jeong and J. A. Majzoub. 1997. Impaired diurnal adrenal rhythmicity restored by constant infusion of corticotropin-releasing hormone in corticotropin-releasing hormone-deficient mice. *J. Clin. Invest.* 99:2923–2929.
- Nottle, M. B., H. Nagashima, P. J. Verma, Z. T. Du, C. G. Grupen, S. M. McIlpatrick, R. J. Ashman, M. P. Harding, C. Giannakin, P. L. Wigley, I. G. Lyons, D. T. Harrison, B. G. Luxford, R. G. Campbell, R. J. Crawford, and A. J. Robins. 1999. Production and analysis of transgenic pigs containing a metallothionein porcine growth hormone gene construct. In: J. D. Murray, G. B.

- Anderson, A.M. Oberbaur, M. M. McGloughlin, (ed). Transgenic Animals in Agriculture. pp 145–156.
- Ollmann, M. M., B. D. Wilson, Y. K. Yang, J. A. Kerns, Y. Chen, I. Gantz, and G. S. Barsh. 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278:135–138.
- Palmiter, R. D., J. C. Erickson, G. Holoopeter, S. C. Baraban, and M. W. Schwartz. 1998. Life without neuropeptide Y. *Recent Prog. Horm. Res.* 53:163–199.
- Pedrazzini, T., J. Seydoux, P. Kunstner, J. F. Aubert, E. Grouzmann, F. Beermann, and H. R. Brunner. 1998. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. *Nat. Med.* 4:722–726.
- Rossi, M., S. J. Choi, D. O'Shea, T. Miyoshi, M. A. Ghatei, and S. R. Bloom. 1997. Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight. *Endocrinology* 138:351–355.
- Rubinstein, M., J. S. Mogil, M. Japon, E. C. Chan, R. G. Allen, and M. J. Low. 1996. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. *Proc. Natl. Acad. Sci. U S A* 93:3995–4000.
- Sahu, A. 1998a. Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. *Endocrinology* 139:795–798.
- Sahu, A. 1998b. Leptin decreases food intake induced by melanin-concentrating hormone (MCH), galanin (GAL) and neuropeptide Y (NPY) in the rat. *Endocrinology* 139:4739–4742.
- Sahu, A., J. D. White, P. S. Kalra, and S. P. Kalra. 1992. Hypothalamic neuropeptide Y gene expression in rats on scheduled feeding regimen. *Brain Res. Mol. Brain Res.* 15:15–18.
- Sanacora, G., M. Kershaw, J. A. Finkelstein, and J. D. White. 1990. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. *Endocrinology* 127:730–737.
- Schwartz, M. W., M. F. Dallman, and S. C. Woods. 1995. Hypothalamic response to starvation: implications for the study of wasting disorders. *Am. J. Physiol.* 269:R949–957.
- Shimada, M., N. A. Tritos, B. B. Lowell, J. S. Flier, and E. Maratos-Flier. 1998. Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature (Lond.)* 396:670–674.
- Smith, G. W., J. M. Aubry, F. Dellu, A. Contarino, L. M. Bilezikjian, L. H. Gold, R. Chen, Y. Marchuk, C. Hauser, C. A. Bentley, P. E. Sawchenko, G. F. Koob, W. Vale, and K. F. Lee. 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* 20:1093–1102.
- Smith, T. P., N. L. Lopez-Corrales, S. M. Kappes, and T. S. Sonstegard. 1997. Myostatin maps to the interval containing the bovine mh locus. *Mamm. Genome* 8:742–744.
- Soloveva, V., R. A. Graves, M. M. Rasenick, B. M. Spiegelman, and S. R. Ross. 1997. Transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue are resistant to obesity. *Mol. Endocrinol.* 11:27–38.
- Stanley, B. G., A. S. Chin, and S. F. Leibowitz. 1985. Feeding and drinking elicited by central injection of neuropeptide Y: evidence for a hypothalamic site(s) of action. *Brain Res. Bull.* 14:521–524.
- Stenzel-Poore, M. P., V. A. Cameron, J. Vaughan, P. E. Sawchenko, and W. Vale. 1992. Development of Cushing's syndrome in corticotropin-releasing factor transgenic mice. *Endocrinology* 130:3378–3386.
- Stenzel-Poore, M. P., S. C. Heinrichs, S. Rivest, G. F. Koob, and W. W. Vale. 1994. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J. Neurosci.* 14:2579–2584.
- Strand, F. 1999. *Neuropeptides: Regulators of Physiological Processes*. MIT Press, Cambridge, MA.
- Susulic, V. S., R. C. Frederich, J. Lawitts, E. Tozzo, B. B. Kahn, M. E. Harper, J. Himms-Hagen, J. S. Flier, and B. B. Lowell. 1995. Targeted disruption of the beta 3-adrenergic receptor gene. *J. Biol. Chem.* 270:29483–29492.
- Turnbull, A. V., G. W. Smith, S. Lee, W. W. Vale, K. F. Lee, and C. Rivier. 1999. CRF type I receptor-deficient mice exhibit a pronounced pituitary-adrenal response to local inflammation. *Endocrinology* 140:1013–1017.
- Wilding, J. P., S. G. Gilbey, M. Mannan, N. Aslam, M. A. Ghatei, and S. R. Bloom. 1992. Increased neuropeptide Y content in individual hypothalamic nuclei, but not neuropeptide Y mRNA, in diet-induced obesity in rats. *J. Endocrinol.* 132:299–304.
- Wilmot, I., A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. Campbell. 1997. Viable offspring derived from fetal and adult mammalian cells. *Nature (Lond.)* 385:810–813.
- Wilson, B. D., D. Bagnol, C. B. Kaelin, M. M. Ollmann, I. Gantz, S. J. Watson, and G. S. Barsh. 1999. Physiological and anatomical circuitry between Agouti-related protein and leptin signaling. *Endocrinology* 140:2387–2397.
- Yamashita, T., T. Murakami, M. Iida, M. Kuwajima, and K. Shima. 1997. Leptin receptor of Zucker fatty rat performs reduced signal transduction. *Diabetes* 46:1077–1080.
- Yang, X. W., P. Model, and N. Heintz. 1997. Homologous recombination based modification in *Escherichia coli* and germline transmission in transgenic mice of a bacterial artificial chromosome. *Nat. Biotechnol.* 15:859–865.
- Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature (Lond.)* 372:425–432.