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Role of homeobox genes in the hypothalamic development and energy balance

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1. ABSTRACT

Homeobox genes contribute to the regionalization, patterning and cell differentiation during embryogenesis and organ development. During mammalian embryonic development, homeobox genes, including orthopedia (Otp), a brain-specific homeobox transcription factor (Bsx) and a thyroid transcription factor-1 (TTF-1), are expressed in the hypothalamus. The genetic ablation of these genes indicated that Otp and TTF-1 are essential for the normal morphological development of the hypothalamus, including the arcuate nucleus (ARC), whereas Bsx is not required. In the adult stage, Bsx and TTF-1 continue to be expressed in the hypothalamus, including the ARC, and serve as transcription factors of neuropeptide Y and agouti-related protein. The expression of hypothalamic Bsx and TTF-1 can be altered by the feeding state and appetite regulatory hormones such as ghrelin and leptin. Although Bsx and TTF-1 are essential for normal feeding behavior in adult mice, they exert different effects on the expression of hypothalamic pro-opiomelanocortin (POMC) and body weight homeostasis. Thus, the hypothalamic homeobox genes may contribute to the dissociation of food intake and body weight via AgRP-POMC neurons.

2. INTRODUCTION

Homeobox genes are an evolutionarily conserved class of transcription factors that play important roles in regionalization, patterning, and cell differentiation during embryogenesis and organ development (1-4). They contain a homeobox, which is a segment of DNA of about 180 base pairs (5) that encodes a DNA-binding homeodomain of about 60 amino acids (Figure 1). The homeodomain includes a helix-turn-helix motif consisting of three α-helices and short loops that link them. All homeodomain transcription factors bind to DNA TAAT/ATTA motifs. Factors that bind to the motifs regulate the expression of downstream genes.

Homeobox genes were first identified in Drosophila by large scale forward genetic screens (6,7). Some Drosophila mutants showed homeotic transformation, which is the formation of body parts at inappropriate locations. Ectopic expression of the Antennapedia gene leads to the formation of middle legs in the location of antennae (8,9). Drosophila homeotic transformations are usually caused by the mutation of one gene in the cluster of homeotic genes, Drosophila homeotic complexes (HOM-C) (10). These genes are clustered and

arranged in a collinear manner on a single chromosome. Each of the HOM-C homeotic genes contains a homeobox. Drosophila Distal-less gene is another homeobox gene expressed in insect limbs during the larval stage (4,11). Drosophila Distal-less null mutants die as embryos; however, there are viable combinations of Distal-less alleles of some mutations that allow the embryos to live (12-14). The mutants show various severity of the phenotypes: the loss of the tibia and the tarsal segments or the size reduction of the distal tarsal segments of the legs in adult. Drosophila Distal-less mutants also have a loss of additional organs, including the antenna, larval sense organs, and vestigial larval legs, where Distal-less gene is expressed during development (4,11). These findings suggest that the Distal-less gene contributes to the normal development of organs.

The hypothalamus is the portion of brain which serves as the center of food intake regulation (15). The arcuate nucleus (ARC) is located at the base of the hypothalamus surrounding the third cerebroventricle. This nucleus contains two distinct neuronal populations: orexigenic neuropeptide Y/agouti-related protein (NPY/AgRP) neurons and anorexigenic pro-opiomelanocortin/cocaine- amphetamine-regulated transcript (POMC/CART) neurons. The contribution of homeobox genes to the development and function of the hypothalamus, including the ARC, however, has not yet been adequately reviewed. Here, we review the role of the homeobox genes expressed in the hypothalamus at the embryonic stage, including the ARC, in the regulation of hypothalamic development and energy homeostasis at the adult stage.

3. HOMEOBOX GENES EXPRESSED IN THE HYPOTHALAMUS AT EMBRYO STAGE

3.1. Otp

Otp (Orthopedia) is a homeobox gene encoding a homeodomain protein (16). The homology is shared by both *Drosophila* orthodenticle and Antennapedia homeodomains (16). The homeodomain of Otp is highly homologous to that of orthodenticle, but glycine substitutes lysine at residue 50. Likewise, residue 50 in the Antennapedia homeodomain is glycine; thus, the gene is named Orthopedia according to the mixed character of the otp homeodomain.

In the embryo of the mouse, the expression of Otp is seen in the hypothalamus, ventral diencephalon, ventral hindbrain, and spinal cord (16-18). In the embryonic hypothalamus, Otp is detectable in a restricted segment-like pattern in the anterior hypothalamus (AH), supraoptic/paraventricular area (spv), retrochiasmatic area (rch), and ventral tuberal area. The AH, spv, rch, and ventral tuberal areas become the anterior periventricular nucleus (aPV), paraventricular nucleus (PVN), supraoptic nucleus (SON), and ARC in the adult, respectively. Otp null mice die within 3 days of birth and their growth is severely retarded (17,18). Otp-deficient mouse embryos do not form the aPV, PVN, and SON, and have an impaired ARC (17,18) (Table 1). In addition, studies using Otp knockout mice in which the Otp gene is replaced with the lacZ reporter gene suggest that Otp contributes to the proper migration and differentiation of cells in the aPV, PVN, SON and ARC. Moreover, it has been recently demonstrated that Otp is involved in the development of diencephalic dopaminergic (DA) neurons in zebrafish and mouse (19-22). These findings suggest that the Otp is critical for the development of the hypothalamic neuroendocrine system of vertebrates (23).

3.2. TTF-1 (Nkx2.1)

Several members of the Nkx family, which are homeobox genes with an additional conserved motif, the Nk domain, are expressed in the embryonic ventral neural tube (24,25). Further, Nkx1.1, Nkx1.2, Nkx2.1, Nkx2.2, Nkx2.9, and Nkx6.1 are expressed in the embryonic central nervous system (26-29). During embryogenesis, misexpression of shh (sonic hedgehog), which is expressed in the ventral midline, the notochord and floor plate, and is known to be important in patterning in the embryonic ventral neural tube, induces ectopic expression of Nkx2.1, Nkx2.2, and Nkx6.1 within the embryonic central nervous system (CNS) (28,30,31). Developmental studies using Nkx-deficient mice suggest that some Nkx functions in the regionalization of the embryonic ventral central nervous system (32-34).

Thyroid transcription factor-1 (TTF-1; Nkx2.1) is a member of Nkx2 family of homeobox genes. TTF-1 was first found in thyroid cell lines (35). During the embryonic stage in mammals, the gene is expressed mainly in the thyroid gland and hypothalamus (36,37). Recently, genes homologous to mammalian TTF-1 were discovered, including in lamprey and amphioxus (38-41). The expression patterns were studied in these two animals, and it was found that the genes are expressed mainly in the thyroid gland or the endostyle, the organ homologous to the thyroid gland, and the ventral part of the anterior neural tube.

In TTF-1-deficient mice, the development of the hypothalamus is abnormal and the pituitary gland is absent (32) (Table 1). Anatomical studies of the hypothalamus of the mutant mice have also revealed that the ARC is absent. However, the VMH, DMH, and LHA are present. Knockdown experiments using morpholino antisense oligonucleotides of xNkx2.1 in the frog Xenopus laevis suggests that xNkx2.1 controls the relative size of major regions in the diencephalon, such as hypothalamus versus thalamus (42). In the xNkx2.1 morphants, the hypothalamus is smaller, and the position of the prethalamus is displaced rostrally and the thalamus is expanded. In Nkx2.1 knockout mouse embryos at embryonic day 11.5, changes in gene expression patterns are seen in the diencephalon; the expression of Pax6, a paired-homeobox gene, which marks the prethalamus is expanded ventrally into the hypothalamus, and the expression of Nkx2-4, a hypothalamus marker, is not detectable except in the most ventral region (43). The absence of ARC in TTF-1-deficient mice may be due to the consequence of patterning defects in the diencephalon in these mutant mice. These findings suggest that TTF-1 is essential for the formation of ARC at the embryonic stage.

Table 1. A summary of expression domains and functions of the three homeobox genes, TTF-1, Bsx, and Otp, at embryonic stages, postnatal, adult stages

Gene	Organ	E xpression Region	Function		
		Embryonic Stage	Postnatal and Adult	Embryonic Stage	Postnatal and Adult
TTF-1	Brain	Hypothalamus, Medial ganglionic eminence	ARC, VMH, DMH, LHA, PVN (In postnatal, VMH)	ARC development (brain patterning), Pituitary gland development	Feeding behavior (food intake)
	Other region	Lung, Thyroid	Subfornical organ, Ependymal/subepen-dymal cell of third ventricle in postnatal	Thyroid development, Branching morphogenesis in lung	Control of body fluid homeostasis
Bsx	Brain	ARC, Epiphysis, Telencephalic septum, Mammillary body	ARC, DMH (In postnatal, ARC, LHA)	None	Feeding behavior (food intake), Locomotor activity
	Other region	None	None	None	Proper growth of the whole body, Nursing, Mammary gland development
Otp	Brain	Hypothalamus, Hindbrain, Spinal cord	ARC, PVN (In postnatal, ARC, PVN, LHA, LA)	ARC development, aPV, PVN, SON development (cell migration, differentiation)	Unknown
	Other region	None	None	None	Unknown

ARC, arcuate nucleus; DMH, dorsomedial hypothalamic nucleus; LA, lateroanterior hypothalamic nucleus; LHA, lateral hypothalamic area; aPV, anterior periventricular nucleus; PVN, paraventricular nucleus; SON, supraoptic nucleus; VMH, ventromedial hypothalamic nucleus.

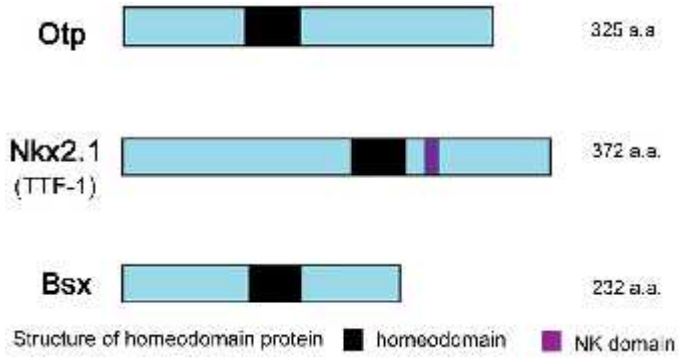

Figure 1. Structures of three homeodomain proteins that are expressed in the hypothalamus of adults. Note that the positions of the homeodomains in the proteins are similar to each other. The sizes of the homeodomain proteins are represented by those of mouse.

In the mammalian postnatal and adult stages, TTF-1 is widely expressed in the hypothalamus, including the ARC, VMH, DMH, paraventricular nucleus (PVN), and LHA (37,44) (Table 1 and Figure 2). Some cells which coexpress TTF-1 and AgRP or alpha-MSH in the ARC have recently been identified (45). Intracerebroventricular (Icv) injection of TTF-1 antisense oligonucleotides into rats decreases hypothalamic TTF-1 expression associated with a decrease in food intake (44) (Table 2). In addition, icv injection of the TTF-1 antisense oligonucleotides decreases hypothalamic NPY and AgRP expression and increases hypothalamic POMC expression, leading to anorexia (44,45) (Table 2). The anorexia induced by TTF-1 antisense oligonucleotides is attenuated by the administration of MC3/4R antagonists (45). Moreover, icv injection of leptin decreases hypothalamic TTF-1 expression (45). These findings suggest that TTF-1 is essential for normal feeding behavior and body weight homeostasis via the central leptin-melanocortin pathway in adult mice (Table 2).

3.3. Bsx

Bsx shows similarities to Barx, Barh1, and the Nkx family (46). This gene is the mammalian homologue of the Drosophila brain specific homeobox gene, bsh (47). In Drosophila, bsh is strongly expressed in the optic lobes of the larval brain. The gene expression pattern, which is expressed in about 30 cells in each brain hemisphere, suggests that it plays a role in determining cell type in the insect brain. However, in the Drosophila bsh mutant, no obvious phenotypes are observed in the embryonic brain (47). Mammalian Bsx is expressed in the ARC, pineal gland, septum, and mammillary body in the embryo and after birth (46). In the adult, expression of Bsx is seen in the ARC and the DMH (48,49).

NPY/AgRP neurons express Bsx in the adult ARC (48). Expression of the gene is not seen in POMC/CART neurons labeled using anti- β-endorphin antibody or anti-α-MSH antibodies. Despite normal POMC and CART expression in the ARC, Bsx-deficient mice display decreases in food intake and locomotor activity associated with decreases in the NPY and AgRP expression in the ARC (48) (Tables 1 and 2). These findings suggest that NPY/AgRP and POMC/CART neurons are not always linked by their opposing functions.

Table 2. Effects of icv injection of TTF-1 antisense oligonucleotides and genetic ablation of Bsx on the expression of hypothalamic neuropeptides and energy balance (44,45,48)

	***TTF-1* (knockdown)**	***Bsx* (-/-)**
Npy/Agrp gene expression	Decrease	Decrease
Pomc/Cart gene expression	Increase	No change
Food Intake	Decrease	Decrease
Locomotor Activity	?	Decrease
Body Weight	Decrease	No change

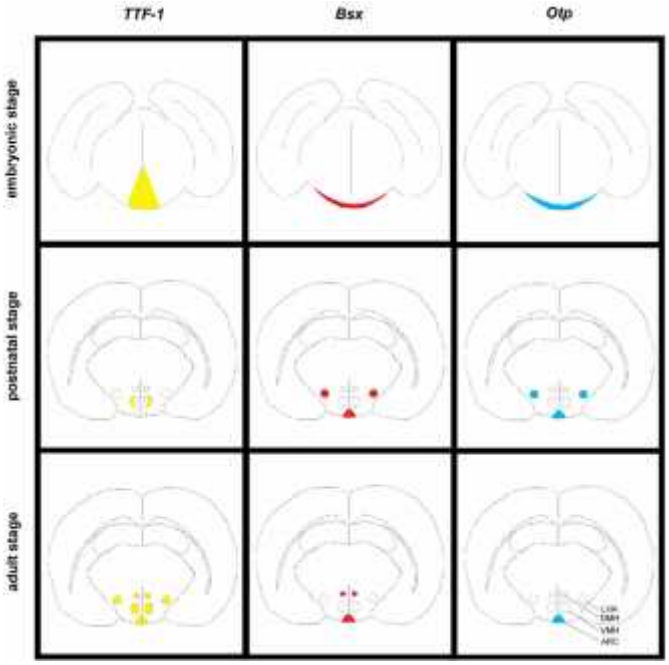

Figure 2. The brain regions where the homeobox genes are expressed in the mouse brain in embryos, postnatal animals, and adults. All drawings represent frontal sections of brain. The gene expression domains in the brain are represented by the different colors. ARC, arcuate nucleus; LHA, lateral hypothalamic area; DMH, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus.

Fasting and feeding can alter the expression of Bsx in the ARC (49). The level of Bsx expression becomes high during fasting. Refeeding returns expression to the normal low level, comparable to that during feeding. In addition, ghrelin, a hormone secreted by stomach, and leptin, a hormone secreted by white adipose tissue, alter the expression of Bsx in the ARC (49). Icv injection of ghrelin increases the expression of Bsx in the ARC (49). The level of expression becomes high, almost comparable to the level observed during fasting. On the other hand, icv injection of leptin lowers the fasting-induced increase in Bsx in the ARC (49). The expression level remains a little higher than that during feeding.

These findings suggest that Bsx is essential for normal feeding behavior but not body weight homeostasis, and that ghrelin upregulates while leptin donwregulates the expression of Bsx in the ARC. Factors other than ghrelin and leptin may also contribute to the expression of Bsx in the ARC during fasting and feeding.

4. GENETIC ABLATION OF NPY/AGRP AND POMC

Within the hypothalamus, the NPY/AgRP and POMC/CART neurons originating in the ARC send projections to other nuclei, heavily so in the case of the PVN, where melanocortin 4 receptors (MC4R) are densely expressed, and also to the DMH, VMH and lateral hypothalamic area (LHA) (50). AgRP inhibits the melanocortin pathway (50-52). In addition, the activation of AgRP neurons releases the inhibitory neurotransmitter GABA (γ-aminobutyric acid) onto neighboring POMC neurons (53). This GABA reportedly decreases the release of α-MSH from POMC neurons, leading to an increase in food intake.

However, mice with a genetic ablation of NPY, AgRP or both exhibit no feeding or body weight phenotypes and maintain a normal response to starvation (54-57). All of these mutants displayed a normal expression of hypothalamic POMC. On the other hand, mice with a genetic ablation of POMC exhibit hyperphagia and obesity on a normal chow-fed diet (58). The selective ablation of AgRP expressing neurons in adult mice, however, results in decreases in food intake and body weight. Because the ablation of AgRP in adult mice is associated with increased expression of hypothalamic POMC (55), the changes in food intake and body weight between the congenital and acquired ablation of AgRP may in fact be due to a

difference in the expression of hypothalamic POMC. These findings suggest that the hypothalamic POMC neurons rather than NPY/AgRP neurons are critical for body weight homeostasis.

5. SUMMARY AND PERSPECTIVE

During mammalian embryonic development, Otp, Bsx, and TTF-1 are expressed in the hypothalamus. Otp and TTF-1 are essential for the normal morphological development of the hypothalamus, including the ARC, whereas Bsx is not. In the adult stage, Bsx and TTF-1 continue to be expressed in the hypothalamus, including the ARC. Bsx and TTF-1 are transcriptional factors of NPY and AgRP in the ARC of adult mice and are essential for normal feeding behavior.

Interestingly, TTF-1 is essential for body weight homeostasis, whereas Bsx is not. Because Bsx-deficient mice display a normal expression of hypothalamic POMC and TTF-1 knockdown in the brain of adult mice resulted in the decreased expression of hypothalamic POMC, the differences in body weight changes may be due to the differences in the expression of hypothalamic POMC. TTF-1 and Bsx both are transcriptional factors of NPY and AgRP, and it remains unknown why Bsx does not affect the expression of hypothalamic POMC or body weight.

A dissociation of food intake and body weight has previously been reported in serotonin 5-HT2C receptor mutant mice (59,60). Despite hyperphagia, 5-HT2C receptor mutants display normal body weight by 6 month of age, and then develop “middle age-onset” obesity. The homeobox genes expressed in the adult brain may contribute to the central dissociation of food intake and body weight. To further study the role of sustained expression of homeobox genes in the adult brain may be useful in the determination of novel brain functions in the regulation of energy homeostasis.

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Abbreviations: AH: anterior hypothalamus; AgRP: agouti-related protein; aPV: anterior periventricular nucleus; ARC: arcuate nucleus; BMP: bone morphogenetic protein; Bsx: brain-specific homeobox transcription factor; CART: cocaine- and amphetamine-regulated transcript; DMH: dorsomedial hypothalamic nucleus; GABA: γ-aminobutyric acid; LA: lateroanterior hypothalamic nucleus; LHA: lateral hypothalamic area; MC4R: melanocortin 4 receptor; NPY: neuropeptide Y; OB-R: leptin receptor; Otp: Orthopedia; POMC: pro-opiomelanocortin; PVN: paraventricular nucleus; rch: retrochiasmatic area; SHH (Shh): sonic hedgehog; SON: supraoptic nucleus; spv: supraoptic/paraventricular area; TTF-1: thyroid transcription factor-1;
VMH: ventromedial hypothalamic nucleus.

Key Words: homeobox gene, TTF-1, Bsx, Otp, hypothalamus, food intake, body weight, NPY, AgRP, POMC, Review

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