Transcription factors in the development of medial hypothalamic structures

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The hypothalamus has historically been subdivided into nuclei, agglomerations of cell bodies that are visually distinct in histological sections. Regulatory functions of metabolism have been assigned to the various hypothalamic nuclei principally by analysis of animals with lesions of individual nuclei (39) but also via various means of stimulation, such as cooling or heating probes. Biochemical and molecular specificity of these studies became possible with the identification and synthesis of neurotransmitters (7, 38) as well as the means to manipulate the expression of endogenous neurotransmitters (35, 37) and their receptors by genetic means (6, 21). The arcuate nucleus (ARC) is likely to be the primary site for neurons that sense circulating fuels and energy reserves (POMC/CART neurons, NPY/AGRP neurons), whereas the paraventricular nucleus (PVN) receives input from the ARC and harbors many of the releasing factors (CRF, TRH, vasopressin, and oxytocin) that control pituitary hormone release. The ventromedial nucleus (VMN) receives input from the ARC and plays a critical role in energy balance in parallel with the ARC. The VMN and PVN also send descending projections to the autonomic nervous system and other pathways that control ingestive behavior and metabolism. Developmental analyses have revealed that the neurons that comprise the hypothalamic nuclei arise by differentiation and migration from stem cells within the ventricular zone. Based on recent work, it is becoming clear that coordination between numerous transcription factors that determine specification, survival, and migration is necessary for the formation of the hypothalamus, with each nucleus being determined by its own unique set of factors. In this minireview, we will provide a selective view of the roles that transcription factors play in the developing hypothalamus.

The VMN and SF-1

The VMN was regarded as a satiety center when early lesion studies described a syndrome of hyperphagic obesity in rats with bilateral electrolytic lesions (1). Unfortunately, the VMN has remained a difficult region to study due to the lack of identified neurotransmitters that would facilitate genetic manipulation of this hypothalamic nucleus. A major breakthrough was the discovery that mice with a genetic deletion of SF-1, an orphan nuclear hormone receptor, did not have a recognizable VMN and demonstrated a hyperphagic obesity syndrome as adults (after rescue with neonatal adrenal gland transplants) (29). Further work has shown that SF-1 neurons express MC4R as well as BDNF (42). These are significant associations because deficiency of MC4R or underexpression of tyrosine receptor kinase B, a receptor for BDNF, also causes hyperphagic obesity. Interestingly, a loss of SF-1 activity causes
a failure of the SF-1 neurons to coalesce into a recognizable nucleus rather than a reduction of the numbers of or the killing of SF-1 neurons (8). A transgene using the SF-1 promoter driving expression of a green fluorescent protein marker was used to show that the number of SF-1-expressing neurons is the same as wild-type cells, although these cells are widely dispersed in the mutant hypothalamus. These observations indicate that the defect is in the migration/coalescence of the neurons to form the ventromedial portion of the VMN. It remains to be determined whether the SF-1 deficiency causes a block in differentiation. In addition, it is not known whether the projections of these aberrantly located neurons are similarly affected.

Because BDNF is a proneural factor (18), promoting survival and differentiation of neurons, and its expression within the VMN is dependent upon SF-1, it is possible that a deficiency of BDNF (in SF-1-deficient states) could affect the growth or maintenance of non-SF-1 neurons within the VMN. This will require the identification of a marker for non-SF-1-expressing VMN neurons. BDNF also has actions in stimulating neuronal activity, indicative of a neuromodulator function (27). BDNF expression is regulated by MC4R, being stimulated by MSH but inhibited by AgRP (42). Therefore, developmental regulation could also involve activity-dependent release of BDNF from SF-1 neurons. Within the VMN, another population of SF-1-negative neurons that is located more ventrolaterally shows strong estrogen receptor-α expression and is critical for female lordosis (24). The location of the cell bodies of these ERα-positive neurons is shifted medially in SF-1-null mice. However, the projections of ERα neurons of the VMN are unaffected, suggesting that the functionality of these VMN neurons is likely to be fully functional (8).

To summarize, SF-1 is required for the migration and coalescence of neurons to form the ventromedial nucleus. A green fluorescent protein reporter tag driven by the SF-1 promoter indicates that the neurons in SF-1-deficient mice retain their differentiated state and are of normal abundance.

The PVN and PAS Domain Transcription Factors

The PVN is considered a satiety center. Lesions of the PVN produce hyperphagic obesity (4, 17). The PVN is the host of numerous neuroendocrine cell types. One transcription factor, orthopedia (OTP), contains a homeodomain and is necessary for the genesis of the PVN, supraoptic nucleus (SON), and anterior periventricular nucleus (aPV) (2, 40). Mouse embryos with a complete deficiency of OTP do not form the PVN, SON, or aPV. The OTP-null mice do not express oxytocin (OXT), vasopressin (AVP), corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), or somatostatin (SST) within the hypothalamus, and they fail to generate a recognizable PVN, SON, or aPV. As a result of the deficiency in the generation of the magnocellular PVN neurons, the posterior pituitary does not develop in OTP-null mice. Complete OTP deficiency results in late embryonic lethality, although individual deficiencies of any of the neuropeptides affected by OTP are not associated with embryonic lethality. Additionally, OTP is expressed in portions of the arcuate nucleus and the premammillary area. OTP has been reported to be necessary for the generation of dopaminergic arcuate hypothalamic neurons in zebrafish (36), and it is likely that this feature is evolutionarily conserved. Because the knockout allele was generated by replacing the coding sequence with a reporter gene, it was possible to follow the numbers and fates of the OTP-expressing cells. There is a gradual reduction in the numbers of lacZ-expressing cells in the OTP-null mice during embryonic development. There is a reduction in the numbers of proliferating cells in the ventricular zone without an effect on rates of cell death, although OTP is not expressed in this area (2). This suggests that OTP is necessary for the production of factors for the proliferation and/or maintenance of the precursor cells that give rise to the PVN, SON, and aPV.

Phenocopies of the OTP-null embryos have been reported in SIM1-null (33) and ARNT2-null mice (23). SIM1 (homolog of the Drosophila simple-minded) and ARNT2 (arylhydrocarbon receptor nuclear translocator) are obligate dimerizing partners from a family of proteins that share the feature of bearing a homeobox DNA-binding domain and a PAS (per-αh-sim)-dimerizing domain (32). SIM1- and ARNT2-null embryos fail to produce a recognizable PVN, SON, and aPV. They also fail to generate a posterior pituitary. The mutant mice can survive for several days, although the pups inevitably die within the first week of life. Tracking of the SIM1- and ARNT2-expressing neurons was possible with the replacement of the coding sequence with a reporter gene, as was done with the Otp knockout alleles. Surprisingly, the SIM1- and ARNT2-expressing neurons are found in equal numbers between null and heterozygous embryos but are scattered in atypical regions of the hypothalamus, suggestive of a failure of migration and coalescence of the neurons rather than any primary effects on rates of proliferation or death (32, 33). Furthermore, the lack of the posterior pituitary points to a defect in the development of efferent projections. Thus, the combined failures of neuropeptide gene expression and functional efferent projections are major features of the null phenotype in SIM1 and ARNT2 deficiencies. It is interesting to note that SIM1 can bind to multiple partners in vitro, although expression of ARNT and BMAL1 is very low within the hypothalamus.

SIM1 is semidominant in that hemizygosity of SIM1 causes early-onset obesity and hyperphagia in rodents (26, 31) and humans (19). This feature of the gene was initially reported for a patient with a balanced 1p;6q chromosome translocation. Analysis of the breakpoint indicated a disruption of the SIM1 gene on 6q without any functional consequence to genes on 1p. Subsequent literature reviews have suggested up to five cases to have been reported (13). The mouse Sim1 hemizygote similarly shows an early-onset obesity and hyperphagia along with partial deficiencies in the numbers of at least five neuroendocrine cells in the PVN and SON (31). The phenotypes of Otp and Arnt2 hemizygotes are reported to be normal.

Some targets of SIM1 have been putatively found: Brain-2 (BRN2) and SIM2. Knockout alleles of the two genes have been generated, and the mutant phenotypes have been described. Indeed, the BRN2 mutants were reported prior to the descriptions of the Otp, Sim1, and Arnt2 mutants. BRN2 is a POU domain (PIT1-OCT-UNC86) transcription factor that is required for the differentiation and survival of three neuroendocrine cell types within the PVN and SON: AVP, OXT, and CRH (34). The PVN and SON of mutant mice are hypocellular, although the numbers and expression of TRH and SST cells are unaffected. BRN2 deficiency is a perinatal lethal phenotype, with the pups rarely surviving beyond the first week of post-
natal life. BRN2 expression is severely compromised in the absence of OTP, SIM1, or ARNT2, indicating that the BRN2 is likely to be involved in mediating the effects of these transcription factors. SIM2 (15) is highly homologous to SIM1, although its dimerizing partner remains to be discovered. SIM2 has a repressor function, and its expression is also dependent on OTP, SIM1, and ARNT2. SIM2 mutant mice show a hypocellular aPV with deficiencies in numbers of TRH and SST cells.

To summarize, OTP is likely to drive expression of factors necessary for the proliferation of progenitor cells destined to become PVN neurons, although it is not expressed in the progenitor cells themselves. SIM1 and ARNT2 are heterodimeric binding factors that are necessary for the migration of the progenitor cells into the PVN as well as the formation of their projection fields. Downstream target genes for SIM1 and ARNT2, such as SIM2 and BRN2, have also been shown to be important for the development of subsets of PVN neurons.

The Arcuate Nucleus and Achaete-Scute Complex 1

The arcuate nucleus is the home of POMC and NPY/AgRP neurons, two of the major neuronal subtypes involved in leptin signaling, deriving a great deal of attention due to their effects on feeding behavior, autonomic nervous system control, and substrate metabolism (11, 41). The arcuate nucleus also harbors growth hormone-releasing hormone (GHRH) neurons (5, 16), dopaminergic neurons (3), and kisspeptin neurons (10). Most of these neurons are dependent upon the activity of a basic helix-loop-helix transcription factor called achaete-scute complex 1 (ASCL1), also called MASH1 (30). ASCL1 is another proneural factor that is important for the generation of neural progenitor cells (20). Complete loss of ASCL1 causes a failure of the ARC and VMN to develop as distinct anatomical structures, with increased rates of cell death. All lineages of the ARC and VMN are affected, although not all lineages are equally affected by the loss of ASCL1 (30). There is a dramatic reduction (<90%) in SF-1, POMC, NPY/AGRP, GHRH, and catecholaminergic neurons, although only the GHRH lineage is completely absent in the ASCL1-null mutant. Analysis of the progenitors clearly shows that there is a cell type that is SF-1/POMC double positive. Subsequent development is impaired but not completely lost, because SF-1 neurons appear within the presumptive VMN and POMC neurons appear within the presumptive ARC, albeit at greatly reduced numbers. This could be explained by the observation that ARC-specific POMC gene expression is controlled by two separate enhancers within the POMC upstream regulatory region (9). Thus, alternative pathways that can partially compensate for defects in development and differentiation exist. The GHRH lineage is completely dependent upon ASCL1 activity because Ascl1-null embryos have very low expression of genomic screen homeobox 1 (GSH1), a homeobox domain transcription factor necessary for expression of Ghrh (28). A zinc finger containing the transcription factor Ikaros has been found to be necessary for Ghrh expression (12). Interestingly, both GSH1 and Ikaros are similarly expressed in pituitary somatotropes, and loss of either protein results in extreme dwarfism and loss of somatotropes.

![Fig. 1. Transcription factors determine proliferation of progenitor cells and migration into medial hypothalamic nuclei. Two transcription factors, orthopedia (OTP) and achaete-scute complex 1 (ASCL1), are necessary for the proliferation of progenitor cells near the subventricular zone. OTP drives the proliferation of all cell types for the paraventricular nucleus (PVN) as well as dopaminergic neurons of the arcuate nucleus (ARC). ASCL1 drives proliferation of neuropeptide Y (NPY)/Agouti-related protein (AgRP), growth hormone-releasing hormone (GHRH), and catecholaminergic neurons of the ARC. Migration of neurons is driven by steroidogenic factor-1 (SF-1) for brain-derived neurotropic factor (BDNF) neurons of the ventromedial nucleus (VMN), whereas SIM1 (homolog of the Drosophila simple-minded) and ARNT2 (arylhydrocarbon receptor nuclear translocator) are necessary for migration of all PVN neurons. BRN2, necessary for the development of vasopressin (AVP), corticotropin-releasing hormone (CRH), and oxytocin (OXT) neurons, and SIM2, necessary for somatostatin (SST) and thryotropin-releasing hormone (TRH) neuronal development, are transcription factors that are dependent upon the SIM1/ARNT2 complex, suggesting that neuropeptide gene expression would also be dependent upon continued SIM1/ARNT2 function. Nescient helix-loop-helix (NHLH2) is important for proopiomelanocortin (POMC) expression because it modulates the synthesis of prohormone convertase 1. Please note that, although there is a progenitor cell that expresses both SF-1 and POMC, the mechanisms that produce the 2 types of neurons remain unknown. SON, supraoptic nucleus; aPV, anterior periventricular nucleus; GLUT, glucose transporter.](http://ajpendo.physiology.org/)

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**Review**

**E565 TRANSCRIPTION FACTORS AND HYPOTHALAMIC DEVELOPMENT**

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Expression of POMC peptides within the ARC has been shown to be dependent partially upon expression of nescent helix-loop-helix (NHLH2) (22), another basic helix-loop-helix transcription factor. Mice without NHLH2 activity exhibit hyperphagia and obesity as well as infertility (14). Expression of POMC mRNA is not altered by loss of NHLH2, although the amount of α-MSH is reduced. This is potentially explained by a reduction in the expression and activity of prohormone convertase 1 (22). Thus, reduced processing of MSH would result in diminished melanocortinergic tone and hyperphagic obesity. Additionally, the infertility of the mutant mice is revealed by the observation that migration of GnRH neurons to their final hypothalamic sites is significantly reduced in double-null NHLH1 and NHLH2 mutants (25). The numbers of GnRH efferents to the median eminence were also greatly reduced. Further work will be necessary to determine whether a defect in neuronal migration affects synaptic inputs to POMC neurons.

To summarize, ASCL1 is important in stimulating the proliferation of ARC progenitor cells but is necessary only for the GHRH neuron. Complex effects on NPY/AGRP neuron numbers are observed Ascl1 heterozygous and homozygous knockout mice. NHLH2 is important for the expression of prohormone convertases, indirectly affecting the expression of Pomc and MSH synthesis.

We have summarized the literature and attempted to synthesize the roles of transcription factors in the development of the midline structures of the hypothalamus (Fig. 1). We have described the role of proneural factors ASCL1 and OTP in stimulating the proliferation of progenitor cells. We have provided examples of factors (SF-1, SIM1, ARNT2) that are necessary for cell migration and the formation of discrete nuclei. We have examples of transcription factors that are critical to the expression of specific neuropeptide genes; BRN2 is necessary for expression of AVP, OXT, and CRH, whereas ASCL1 is necessary for expression of GHRH. Thus, the major themes of developmental biology figure strongly in the formation of hypothalamic nuclei: specification of progenitor cells, cell migration, and maintenance of a differentiated phenotype. Genetic manipulations that permit analysis of the fates of these cells are also crucially important for obtaining insights into the developmental biology of the hypothalamus. An important feature that has not been addressed adequately is the requirement for the development of the projection fields of neurons. It is to be hoped that future work will identify the signaling cascades and sequential activation of differentiation factors that determine the fates of the component neurons of each hypothalamic structure as well as an overarching view of the origins of hypothalamic neurons.

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**REFERENCES**


