Editorial

Transcriptional Regulation of Sensed Energy Availability Within Hypothalamic Neurons

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INTRODUCTION

Obesity is a complex phenotype influenced by both genetics and the environment, the latter including learned behavioral effects, socioeconomic forces, cultural differences, and the ecosystem (weather, food choices, daylight etc). In this supplement, the authors of the five research articles will examine the link between gene regulation and environmental conditions—especially with respect to the individual's ecosystem and with a focus on transcriptional regulation of gene expression. In this introductory paper, an overview of the current state of knowledge about gene regulation in response to energy availability, and information on where further research is needed to augment deficiencies in our current knowledge will be provided.

THE OBESITY EPIDEMIC AND GENETIC CONTRIBUTIONS TO HUMAN OBESITY

The latest report from the Centers for Disease Control (CRC) indicates that obesity and the portion of overweight individuals in the US population have hit an all time high [1]. Six states now have an obesity prevalence of more than 30% (Alabama [31.4%], Mississippi [32/8%], Oklahoma [30.3%], South Carolina [30.1%], Tennessee [30.6%], and West Virginia [31.2%]). The World Health Organization (WHO) reports a similar trend worldwide with over 1 billion people overweight, including 300 million obese adults [2]. While some regions, such as China and Japan, have a relatively low rate of overweight and obesity, others have extremely high rates (e.g. 75% in urban areas of Samoa).

Concurrently with these increases in obese and overweight populations, the scientific community has identified new genes that contribute to obesity within populations (for a review—[3]). The latest edition of the Obesity Gene Map (through the end of October 2005, [4]) identifies 11 genes responsible for monogenetic obesity, and 64 other genes that are linked to some form of overweight phenotype, including complex syndromes. Another 127 genes, with some overlap to the previous two sets, are associated or linked to obesity-related phenotype [4]. Even more genes have been identified using mouse and other animal models; yet for some of these identified genes, the relevance in human obesity has not yet been established [5].

Of the over 150 genes that are involved in the etiology of obesity, the function of many of the protein products of these gene is known; however, there is much less information about how these genes are differentially-regulated in response to changing energy availability. In examining just the 11 genes responsible for human single gene obesity [4], most of the promoters of these genes have not been characterized with respect to gene regulation. For example, mutations in the melanocortin-4-receptor (MC4R) gene account for up to 5% of all forms of obesity. Studies have identified the role of the MC4R in binding to melanocortins and transmitting signals to the interior of the cell [6]. Mutations in the protein coding region of the gene can affect binding of melanocortins to this receptor, or transmission of the signal following binding [7]. However, there are also several mutations in the promoter region of MC4R [8], but little is known about the regulation of the MC4R gene. The last paper published on MC4R gene regulation was in 2005 and this work only identified the minimal promoter necessary for transgenic expression [9]. The specific transcriptional machinery governing basal and induced transcriptional regulation of MC4R is not yet known. A similar situation exists in our knowledge of leptin receptor gene regulation. Single nucleotide polymorphisms (SNPs) have been identified in the leptin receptor promoter, but the last paper examining the promoter and possible transcription factors conferring regulation was published in 2001 [10]. On the other hand, some genes responsible for human single gene obesities, including pro-opiomelanocortin (POMC) and prohormone convertase 1/3 (PC1/3) have been well characterized (for example [11, 12]. Thus, while more work needs to be done to fill in the gaps regarding hypothalamic gene regulation, we have some information to guide us in future studies.

HYPOTHALAMIC GENE REGULATION IN RESPONSE TO ENERGY AVAILABILITY

It is likely that complex molecular mechanisms with multiple gene targets and multiple transcription and post-transcriptional regulatory factors are activated and repressed following changes in energy availability as sensed by hypothalamic neurons. To date, researchers have only scratched the surface of these control pathways, identifying some important

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mechanisms for gene regulation and key transcriptional regulatory factors. Gene and protein expression can be regulated at multiple levels, including transcription initiation or repression, splicing, mRNA stability, mRNA editing, protein translation efficiency, proteolytic processing, and post-translational modification. The main focus of this supplement is in studying the role of transcription factors, transcription initiation, and transcriptional repression in hypothalamic gene regulation. Transcription factors bind to and interact with promoter motifs or other proteins binding to these motifs. The motifs can be located in the promoter region extending 5' of the transcription start site, within enhancer regions that can be located upstream, downstream, or even within the gene itself (i.e. within introns or other untranslated regions).

The complexity of hypothalamic-specific gene regulation is evident even if just the arcuate nucleus of the hypothalamus is examined. Within the arcuate nucleus of the hypothalamus, multiple neuron subtypes exist, including POMC, neuropeptide Y (NPY)/Agouti-related protein (AgRP), kisspeptin, and dopamine-expression cells [13, 14]. If regulation by energy availability also is considered, the complexity of gene regulation increases, as neurons such as POMC and NPY respond in opposing fashion to signals such as leptin, glucose, insulin, or fasting [14]. The promoter or enhancer elements conferring neuron-specific expression, as well as those elements (or other mechanisms) that confer energy availability-dependent expression are only in the beginning stages of elucidation. In addition, it appears that hypothalamic promoter and enhancer regions may be quite large compared to those in other neuroendocrine tissues such as the pituitary. The POMC gene serves as a good example of this. POMC is expressed within hypothalamic arcuate nucleus neurons, hindbrain neurons, and within the corticotroph cells of the pituitary. For the pituitary, tissue- and signal-specific POMC gene expression requires just 400 base pairs (bp) of proximal promoter [15, 16]. However, the transgenic mouse created for these studies did not express the transgene in its hypothalamus, suggesting that the hypothalamic-specific elements were more distal. A 27 kb region containing the entire POMC coding region, 13 kb of upstream, and 8 kb of downstream regions was shown to confer neuron-specific expression in both the hypothalamic and hindbrain of transgenic mice carrying the 27kb tagged DNA fragment [17]. Using additional transgenes and phylogenetic analysis of conserved regions, the same group was able to identify two enhancers located at -11kb and -8kb in the POMC proximal region [18]. In a similar example using AgRP, an enhancer region conferring fasting-induced upregulation to AgRP is located approximately 3 kb downstream from the transcription initiation site of AgRP [19]. However, the enhancer does not confer *in vivo* hypothalamic-specific expression, although expression was found for N38 hypothalamic cells. Thus, the region conferring hypothalamic-specific expression has yet to be identified for the AgRP gene even though 9 kb of surrounding DNA was examined [19]. These two examples suggest that hypothalamic-specific gene regulation may require more distal promoter elements for cell-specific expression, but could rely on proximal elements for signal-specific expression. This supplement focuses on signal-specific expression of hypothalamic genes in response to changes in energy availability.

How many transcription factors might be involved in hypothalamus-specific gene expression? A search of the Mouse Genome Informatics (MGI) site using the search terms [expression detected in; anatomical structure equals] "hypothalamus", [gene ontogeny contains] "transcription" and [developmental stage] "any", reveals 588 matching records. When this search is limited to post-natal hypothalamic expression only, the number drops to 192 genes (Fig. **1**) [20, 21]. A search of the PubMed [22] using the search terms hypothalamus and [gene name] for each of the genes in this list indicates that less than 50% of these have yet to be studied in the hypothalamus. Interestingly, several of the genes indicated in Fig. **1** by a "#" symbol will be discussed by several of the authors in this supplement, but were not identified during the search of the MGI database. This suggests that there is a need for more updates to the MGI resource. Indeed, the MGI gene expression database relies on just a few select large-scale expression studies for its data [20, 21].

Overall, the current knowledge in the area of hypothalamic gene expression suggests that hypothalamic promoters may be more complex and larger in size (kilobases) than promoters that specify tissue- and signal-specific regulation. In most cases where the expressions of hypothalamic body-weight regulatory genes are being studied, we do not yet know the promoter elements responsible for basal, induced, or repressed expression. Furthermore, analysis of the MGI database suggests there are still a large number of transcription factors that are expressed but not yet characterized in the hypothalamus. Lastly, while this supplement mainly focuses on transcriptional mechanisms of gene regulation, there are several other possible levels at which the level of protein or neuropeptide can be modulated, and this area of study is even less characterized in the area of energy availability signals in the hypothalamus.

ARTICLES IN THIS SUPPLEMENT

The objective of this supplement is to provide several articles that relate to the topic of hypothalamic gene regulation in response to energy availability. As discussed above, this is an area that is in need of more studies. The authors were chosen by the guest editor because each is conducting research that is likely to yield interesting new information on hypothalamic gene regulation within the next few years.

In the first article, Dr. Denise Belsham and her colleagues describe creation, characterization, and use of hypothalamic cell lines. Dr. Belsham has created 38 different lines of hypothalamic neurons from mouse embryos using retroviral transfer of SV-40 T-antigen to immortalize the cells. These cell lines have now been used by many researchers, including the guest editor [11] to study hypothalamic gene regulation and signaling. Each cell line has been characterized and shown to express a unique set of markers, representing distinct neuron types within the hypothalamus. Dr. Belsham illustrates how these lines can be used to study gene regulation by providing new data on the regulation of neurotensin by leptin using mHypoE-46 lines and the regulation of NPY/AgRP using mHypoE-39 and mHypoE-36/1 lines.

Adar
Arid3b
Arntl
Arnt2
Ascc1
Ascl1
Atxn7
Barhl1
Barx1
Bmal*#
Boll
Bsx
Btg2
Carm1
CBP*#
Celsr2
Clock*#
Cited2
Cjun#
Cnbp
Cnot4
Cops5
Cpeb1
Creb1*#
Creb3
Csrnp3
Ctcf
Cux1
Cux2
Cxxc1
Dazl

Dlx1
Dlx2
Dlx5
Dpf3
Egr4
Eif2c2
Eif2c3
Eif2c4
Esr1
Esr2
Esrra
Esrrg
Etv1
Ewsr1
Ferd3l
Fos*
Foxa1
Foxa2
Foxb1
Foxk1
Foxl1
FoxO1*#
Foxp2
Foxq1
Fubp1
Fus
Fusip1
Fzd1
Gabpa
Gata2

Gbx2
Gsx1
Gtf2ird1
Hdac1*#
Hdac2
Hes5
Hmg20a
Hmgb1
Hmgb2
Hmx3
Hnrnpab
Hnrpdl
Hoxa7
Hoxa9
Hoxb6
Hr
Id2
Id3
Igf2bp1
Ilf3
Impact
Irx1
Isl1
Kcnh5
Kcnip3
Khdrbs1
Khdrbs2
Khdrbs3
Klf6

Ldb1
Lhx1
Lhx2
Lhx5
Lhx6
Lhx8
Lhx9
Lmo4
Lmx1b
Mapkapk
Mecp2
Mesp1
Mta2
Mycl1
Myef2
Ncoa3
NcoR*#
Neurod1
Neurod6
Nfe2l1
Nfib
Nfkbie
Nhlh2*
Nkx2-1
Nkx2-2
Noc2l
Nr0b1
Nr1d1

Nr2f1
Nr2f2
Nr4a1
Nr4a2
Olig1
Olig2
Otp
Pbx1
Pbx3
Pelp1
Per1*#
Per2*#
Per3*#
Phox2a
Pitx2
Piwil2
Polr1b
Pou3f4
Pou4f1
Ppargc1a
Ppargc1b
Pprc1
Preb
Prmt6
Prpf6
Pspc1
Pum1
Qk
Rbm14

Rbm15b
Rbm3
Rbm39
Rere
Rfx1
Rfx3
Rfx4
Rnf4
Rora
Rxra
Rxrb
Sall2
Sap18
Sf1
Sfpq
Sfrs5
Sim1#
Six2
Six3
Sltm
Snai2
Snd1
Sox2
Sox4
Sox5
Sox9
Sox10
Sox14
Sox21
Sp7
Spen

Srebf1
Stat3*
Sub1
Supt5h
Supt6h
Supt7l
Tarbp2
Tardbp
Tbx4
Tcea1
Tcf12
Tcf7l2
Tgs1
Thra
Tia1
Tlx3
Tnrc6a
Trnau1ap
Usf1
Vax1
Vps72
Zcchc12
Zeb2
Zfhx3
Zfp238
Zfp57

Fig. (1). List of hypothalamic transcription factors as identified using the Mouse Genome Informatics Database. The search terms used were as follows: [expression detected in; anatomical structure equals] "hypothalamus", [gene ontogeny contains] "transcription" and [developmental stage] "TS28, postnatal." Genes where there is at least one study on them in hypothalamus, as identified by a PubMed search with the search terms hypothalamus and [gene name], indicating that at least one are in underlined and bold font. Genes that are not identified by their proper names in articles would have been missed by this type of search. *Indicates a transcription factor discussed in the supplement. #Indicates a transcription factor that is known to be expressed in the hypothalamus but was not identified in the database search. These are discussed by authors of this supplement.

In the second article, Dr. Leona Plum describes her work on the FoxO1 transcription factor.This transcription factor was not identified in the MGI database search. A search of the literature identifies only 12 papers describing FoxO1's role in the hypothalamus with the earliest from three years ago in 2006. Thus, there is still much to know about the role of FoxO1 in hypothalamic gene regulation. In Dr. Plum's article, she describes the opposing activity of FoxO1 on the POMC and AgRP promoters through its ability to recruit co-repressors and co-activators to the respective promoters. While FoxO1 acts to suppress POMC gene expression, it further affects downstream processing of POMC to neuropeptides by suppression of the Carboxypepsidase gene (Cpe) (Plum *et al*., in press). Dr. Plum describes how FoxO1 is regulated by phosphorylation, ubiquitination, and acetylation, and how these secondary modifications may result in the nuclear localization of the protein in fasting conditions. Overall, translocation of FoxO1 to the nucleus of POMC and AgRP neurons with energy deficit results in reduced production of anorexigenic neuropeptides and increased production of orexigenic peptides in the hypothalamus.

The analysis of gene regulation within POMC neurons continues with the third article by Dr. Jennifer Hill. POMC neurons also express cocaine- and amphetamine-regulated transcript (CART), and the two anorexigenic genes are both induced by leptin and increased energy availability. Current knowledge shows that regulation of these two genes appears to occur via two different mechanisms—POMC is regulated directly through leptin receptor activation of the Jak/Stat3 pathway (for example [23]), while CART expression is controlled through complexes involving the CRE-binding protein (CREB) and other transcription factors (for example [24]. However, the presence of a putative Stat3 site on the CART promoter suggests that it too may be directly regulated through leptin receptor signaling.

In the fourth article, Dr. Oren Froy describes the effect of light and darks signals on gene expression in the hypothalamus and peripheral metabolism. Circadian rhythms can be perturbed by environmental changes, such as exposure to complete darkness for a defined period of time, or in animals carrying mutations in specific "clock" family transcription factors that control the 24-hour period. The transcription factors, many of which are PER, ARNT, SIM and basic helix-loop-helix (PAS-bHLH)

domain proteins, are subject to transcriptional and post-transcriptional regulation, leading to an approximately 24-hour cycling signal. Dr. Froy identifies several gene products and hormones that show diurnal rhythms, such as the POMC. However, more research is needed to determine which of these are directly regulated by Clock transcription factors.

The final article was co-written by the guest editor and Dr. Kristen Vella. We have previously shown that gene expression of the bHLH transcription factor Nhlh2 is negatively regulated by food deprivation and induced by food intake or leptin [25]. In this article, we describe a new environmental condition that affects hypothalamic Nhlh2 mRNA levels: temperature. Overall, the effect seems to be caused by perceived energy availability; in low energy conditions such as food deprivation or cold exposure, Nhlh2 expression is reduced while in high energy conditions simulated by leptin injection or food intake following deprivation, Nhlh2 expression is induced. The downsteam effect of this differential regulation of Nhlh2 is to induce anorexigenic peptide production and increase energy expenditure in situations of high energy availability. While this work characterizes an environmental signal that modulates expression level of a hypothalamic transcription factor, and presumably its downstream gene targets, transcription factors that act on the Nhlh2 promoter are still awaiting identification.

CONCLUSIONS

We have only just begun to understand the role of transcriptional regulators in hypothalamic neurons, especially with respect to transcriptional mechanisms that are activated or repressed in response to changes in energy availability or environmental conditions. The articles in this supplement review some of our knowledge in this area, and seek to engage the scientific community in further study of the signaling pathways leading to differential gene expression in hypothalamic neurons.

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