Transcriptional and epigenetic regulation of POMC gene expression

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Abstract
Expression of the pro-opiomelanocortin (POMC) gene integrates numerous inputs that reflect the developmental history of POMC-expressing cells of the pituitary and hypothalamus, as well as their critical role in the endocrine system. These inputs are integrated at specific regulatory sequences within the promoter and pituitary or hypothalamic enhancers of the POMC locus. Investigations of developmental mechanisms and transcription factors (TFs) responsible for pituitary activation of POMC transcription led to the discovery of the Pitx factors that have critical roles in pituitary development and striking patterning functions in embryonic development. Terminal differentiation of the two pituitary POMC lineages, the corticotrophs and melanotrophs, is controlled by Tpit; mutations of the human TPIT gene cause isolated adrenocorticotropic hormone deficiency. Intermediate lobe and melanotroph identity is provided by the pioneer TF Pax7 that remodels chromatin to reveal a new repertoire of enhancers for Tpit action. Many signaling pathways regulate POMC transcription including activation by hypothalamic corticotrophin-releasing hormone acting through the orphan nuclear receptors of the Nur family and feedback repression by glucocorticoids and their glucocorticoid receptor. TFs of the basic helix-loop-helix, Smad, Stat, Etv, and nuclear factor-B families also mediate signals for control of POMC transcription. Whereas most of these regulatory processes are conserved in different species, there are also notable differences between specific targets for regulation of the human compared with mouse POMC genes.

Introduction
The POMC gene has proven to be incredibly informative to study various aspects of gene regulation. Indeed, its restricted expression in two pituitary cell lineages provided the system to identify cell-restricted transcription factors (TFs) such as Pitx1 and Tpit, and their interplay for setting up cell-specific gene expression. Further, the quest to understand cell-specific regulation of POMC in these two lineages, the corticotrophs of the anterior lobe (AL) and the melanotrophs of the intermediate lobe (IL), led to the discovery of the pioneer TF role of Pax7. The pioneering action of Pax7 on chromatin provides the selector function that establishes the difference between intermediate and anterior pituitary derivatives. The first part of this review thus deals with mechanisms for developmental control of POMC transcription in the two pituitary POMC lineages as well as with mechanisms for hypothalamic expression of the single copy POMC gene.

As central regulator of the hypothalamic–pituitary–adrenal axis, POMC transcriptional regulatory mechanisms integrate multiple inputs that ensure homeostasis of
this axis. Studies of CRH activation and of glucocorticoid repression (Gc) of the POMC gene have provided unique insight into the action of nuclear receptors and in particular, the interplay between activating and repressive actions. Further, investigation of mechanisms accounting for Gc resistance in the corticotroph pituitary adenomas that cause Cushing’s disease yielded new insight, not only on the mechanisms of hormone action, but also on the pathogenesis of the disease, including relationships with cell cycle control. Thus, the second part of this review surveys current knowledge on the hormonal control of POMC transcription.

Developmental control of POMC transcription

Mechanisms for pituitary expression of the POMC gene

The early studies of POMC 5′-flanking sequences indicated that these sequences exhibit cell-specific activity in cell culture experiments (Jeannotte et al. 1987) and most importantly that they are sufficient to recapitulate to a large extent the unique features of POMC transcriptional regulation in transgenic mice (Tremblay et al. 1988, Hammer et al. 1990). Further, the transgenic data indicate that 480 bp upstream of the rat gene transcription start site (TSS) contained most of the critical targets for POMC regulation, notwithstanding the possibility that other regulatory sequences may also contribute to control of POMC transcription. Initial studies thus focused on these 480 bp of the rat and human POMC promoters. These were shown to contain a proximal promoter and critical upstream regulatory sequences that behave as a corticotroph-specific enhancer. Molecular dissection of these enhancer sequences extending between −480 and −130 bp of the rat POMC promoter indicated that they contain multiple regulatory elements (Fig. 1A), and that all these elements are jointly required for activity (Therrien & Drouin 1991). Many regulatory elements are binding sites for TFs that appear to be widely distributed, if not ubiquitous. However, detailed analyses identified two elements that confer cell specificity to the enhancer and their identification led to the cloning of cognate TFs (Therrien & Drouin 1993, Lamonerie et al. 1996, Lamolet et al. 2001).

Thus, the corner stone for pituitary specificity of POMC expression lies within a composite regulatory element that is the binding site for cooperative binding of the bicoid-type homeodomain TF pituitary homeobox 1 (Pitx1, Ptx1) and for the Tbox TF Tpit (aka Tbx19). The roles and properties of these factors are discussed next. Another regulatory element provides critical cell-specific activity for corticotroph expression: this element is located about 60 bp upstream of the Pitx1 and Tpit binding sites and it is a target for heterodimers containing the basic helix-loop-helix (bHLH) factor neurogenic differentiation 1 (NeuroD1) (Poulin et al. 1997) as well as a binding site for orphan nuclear receptors (NR) of the Nur subfamily (Philips et al. 1997a). Thus, these two cell-specific regulatory elements, the Pitx/Tpit and NeuroD1 binding sites (Fig. 1A), synergistically control pituitary corticotroph transcription of the POMC gene (Poulin et al. 2000).

The POMC locus includes another enhancer situated at about −7 kb from TSS that is conserved in different species (Langlais et al. 2011). Interestingly, this enhancer contains binding sites for many of the same TFs that act on the proximal −300 bp enhancer (Fig. 1B); the in vivo occupancy of these regulatory sequences by cognate TFs is revealed by chromatin immunoprecipitation (ChIP)-seq analyses (Fig. 2). In transgenic mice, the mouse −7 kb enhancer exhibited slightly more activity in corticotrophs compared with the −300 bp enhancer that showed slight preference for melanotrophs; together, they promoted similar levels of transcription in both corticotrophs and melanotrophs. A conserved feature of the −7 kb enhancer is the presence of a TpitREpal binding site for Tpit homodimers (Fig. 1B). The enhancer is also under regulation by signal/hormone-dependent TFs and the human and rat/mouse −7 kb enhancers appear to respond to different subsets of signals (Langlais et al. 2011).

Pitx1 and Pitx2, TFs required for pituitary development and gene expression

Detailed analyses of upstream POMC promoter regulatory sequences identified one element for its unique property to confer corticotroph-specific transcription in a reporter assay. This sequence was used as probe to obtain by expression cloning a cDNA for Pitx1 (Ptx1), a then novel homeodomain TF that defined the prototype of a subfamily of the paired/bicoid subclass (Lamonerie et al. 1996). The Pitx subfamily was later found to have two other members Pitx2 and Pitx3 (Gage et al. 1999a). The three members of this family differ only by one or two amino acids in their highly conserved 60 amino acids DNA-binding homeodomain and thus bind similar DNA sequences (Tremblay et al. 2000). Together with the Otx subfamily and goosecoid, they share DNA binding specificity recognizing the motif TAATC, where the C residue critically interacts with lysine 50 of the
The rat/mouse POMC −300 bp enhancer and promoter

Regulatory sequences of the POMC gene. In addition to the promoter, three enhancer sequences contribute to either pituitary or hypothalamic expression of POMC. The diagrams depict current knowledge on DNA binding transcription factors acting on these enhancers. (A) The rat −300 bp POMC enhancer has been the most used to dissect these regulatory sequences, whereas most in vivo data (i.e. ChIP-seq, Fig. 2) are for the mouse enhancer. These sequences are well-conserved, except for a 26 bp insertion in the rat compared with mouse sequences; positions shown on the diagram are for the rat sequence. Transcription factors known to bind the enhancer and to regulate POMC transcription are represented by pictograms on the enhancer. Coactivators that are recruited to these TFs are not shown here for clarity, but they are discussed in the text. (B) The mouse −7 kb enhancer is represented with known binding TFs together with predicted transcription factor binding sites (TFBS). (C) The mouse hypothalamic enhancer is constituted of two subenhancers named nPE1 and nPE2. The diagram represents transcription factors shown to bind the enhancer together with predicted transcription factor binding sites.

Figure 1

Regulatory sequences of the POMC gene. In addition to the promoter, three enhancer sequences contribute to either pituitary or hypothalamic expression of POMC. The diagrams depict current knowledge on DNA binding transcription factors acting on these enhancers. (A) The rat −300 bp POMC enhancer has been the most used to dissect these regulatory sequences, whereas most in vivo data (i.e. ChIP-seq, Fig. 2) are for the mouse enhancer. These sequences are well-conserved, except for a 26 bp insertion in the rat compared with mouse sequences; positions shown on the diagram are for the rat sequence. Transcription factors known to bind the enhancer and to regulate POMC transcription are represented by pictograms on the enhancer. Coactivators that are recruited to these TFs are not shown here for clarity, but they are discussed in the text. (B) The mouse −7 kb enhancer is represented with known binding TFs together with predicted transcription factor binding sites (TFBS). (C) The mouse hypothalamic enhancer is constituted of two subenhancers named nPE1 and nPE2. The diagram represents transcription factors shown to bind the enhancer together with predicted transcription factor binding sites.

homeodomain that typifies these subfamilies (Treisman et al. 1989). While we are guilty of giving the name Pitx1 to this subfamily when we cloned Pitx1 for its role in cell-specific transcription of the POMC gene, it must be recognized that this name does not do justice to the striking developmental roles of Pitx factors. Indeed, in addition to its role in development of oral ectoderm derivatives, Pitx1 is the master gene for specification of hindlimb identity.
POMC gene regulation

(Lanctôt et al. 1997, 1999b, Szeto et al. 1999), whereas Pitx2 is the major effector for left–right asymmetric development of internal organs such as heart, lungs, and stomach (Gage et al. 1999b, Kitamura et al. 1999, Lin et al. 1999, Lu et al. 1999). Pitx2 has partly redundant roles with Pitx1 in pituitary development (Charles et al. 2005). The third member of the subfamily Pitx3 is not expressed in pituitary or oral ectoderm, but plays critical roles in eye and midbrain development (Semina et al. 1997, 1998, Smidt et al. 1997) and in skeletal myogenesis where it is partly redundant with Pitx2 (Coulon et al. 2007, L’Honoré et al. 2007, 2010, 2014). Pitx3 is a critical survival gene for midbrain dopaminergic neurons of the ventral substantia nigra, the subset of midbrain dopaminergic neurons that preferentially degenerate in Parkinson’s disease (Hwang et al. 2003, Nunes et al. 2003, van den Munckhof et al. 2003, 2006). The discovery of Pitx1 for its role in the pituitary thus provided new impetus to unforeseen areas of biology.

Within the POMC promoter, the Pitx1 binding site is essential for activity and it is present within a composite regulatory element that contains a DNA binding sequence for another TF, Tpit, discussed below. In fact, the presence of a half-site for Tpit binding within the composite Pitx/Tpit regulatory element (Tpit/PitxRE) creates an absolute requirement for prior binding of Pitx1 in order to cooperatively bind Tpit; nevertheless, both factors are required for transcriptional activity (Lamolet et al. 2001). Thus, Pitx1 not only constitutes the cornerstone of the Tpit/Pitx composite regulatory element, but also of the entire −300 bp enhancer that is centered around. It is noteworthy that the Pitx1 binding site is conserved across very divergent species (Bumaschny et al. 2007). Pitx1 plays a similar central role for transcription of other pituitary hormone-coding genes such as those for the gonadotrophins, prolactin, and growth hormone (Tremblay et al. 1998). Similar to its role in POMC transcription, Pitx1 interacts with the gonadotroph-restricted SF1 and...
with the signal-dependent factor Egr1 for control of LHβ transcription (Tremblay & Drouin 1999, Tremblay et al. 1999), and with the somatolactotrope-restricted Pit1 for transcription of the prolactin and growth hormone genes (Tremblay et al. 1998).

This role as cornerstone for cell-specific transcription in each pituitary lineage is consistent with the pan-pituitary expression of Pitx1 and Pitx2, and with their very early onset of expression in the developing oral ectoderm from which the pituitary gland derives (Lancot et al. 1997, 1999a). This is exemplified by the pituitary phenotypes of knockout mice for Pitx1, Pitx2, and their double knockouts (Charles et al. 2005). Pitx1 gene inactivation has the least severe phenotype with underrepresentation of the gonadotroph and POMC lineages, those lineages that express the highest levels of Pitx1 protein in the adult pituitary (Lancot et al. 1999a). Pitx2 knockout leads to arrested development of the pituitary at the primitive Rathke’s pouch stage with differentiation of only one lineage, the AL corticotrophs (Gage et al. 1999b, Kitamura et al. 1999, Lin et al. 1999). However, the Pitx1, Pitx2 double knockout showed redundancy between these two factors and pituitary development aborted at the earliest Rathke’s pouch stage. Thus, Pitx1 and Pitx2 are essential for early pituitary development and their maintained expression in all adult pituitary lineages is critical for the cell-specific transcription program of each lineage.

**Tpit, a Tbox TF restricted to POMC lineages**

As discussed previously, the Tpit/PitxRE contains two DNA binding sites of different DNA sequence. Recognition that the sequence motif flanking the Pitx1 binding site is similar to the consensus binding site for Tbox factors led us to clone Tpit (also known as Tbx19), which has highly restricted expression in two pituitary lineages, the corticotrophs and melanotrophs (Lamolet et al. 2001). On its own, Tpit has relatively low affinity for the Tpit/PitxRE DNA sequence and its binding is highly cooperative with Pitx1 already bound to its site on the Tpit/Pitx/RE. Together, the two factors form a critically inter-dependent combination required for activity of the POMC ~300 bp enhancer. This composite element and the dependence on Pitx1 for binding is, however, not the most common mode of Tpit action genome-wide as revealed by ChIP-seq studies (Budry et al. 2012). Indeed, genome-wide ChIP-seq identified a palindrome regulatory element (TpitREpal) that contains two half-sites for recognition by Tpit and at the POMC locus (Figs 1B and 2), this palindrome is present and critical for activity of the ~7 kb enhancer (Langlais et al. 2011).

Within the POMC promoter, Tpit binding to the Tpit/PitxRE is essential and provides transcriptional activation function. This activation function is in part mediated by transcriptional coactivators of the SRC family that also contribute to CRH-dependent activation of POMC transcription (Maira et al. 2003a). Tpit-dependent transcription is enhanced by association with the Ets family TF Etv1 that binds a sequence between Pitx and Tpit binding sites (Budry et al. 2011). During pituitary development, Tpit is expressed about 12 h before POMC and the first Tpit-positive corticotrophs are detected in the developing anterior lobe around day e12 of mouse development and the first Tpit-positive melanotrophs around e14.5 (Lamolet et al. 2001, Pulichino et al. 2003b). Tpit is thus an excellent marker of the pituitary POMC lineages including in humans, where it has proven useful to characterize pituitary adenomas (Vallette-Kasic et al. 2003).

In view of this highly cell-restricted expression, it was not surprising to find that inactivation of the Tpit gene in mice prevents differentiation of corticotrophs and melanotrophs: it is thus a positive regulator for terminal differentiation of these lineages (Pulichino et al. 2003b). Unexpectedly, the Tpit knockout also revealed that Tpit is a negative regulator for differentiation toward the gonadotroph lineage: indeed, the Tpit−/− IL not only fails to differentiate melanotrophs, but also presents with 10–15% of cells with a cell fate change to gonadotroph. A similar cell fate switch is observed in the AL and the cells that have switched are marked by expression of the gonadotroph-specific factor SF1. Further, cells that fail to complete that cell fate switch remain in somewhat of a limbo between progenitor and differentiated status as revealed by the coexpression of two cell cycle inhibitors, p57Kip1 and p27Kip2, the former normally marking only pituitary progenitors that recently exited the cell cycle and progenitor state, and the latter marking differentiated cells (Bilodeau et al. 2009).

**TPIT mutations in isolated ACTH deficiency**

The highly cell-restricted expression of Tpit and its critical role for POMC lineage differentiation led us to postulate that its inactivation in humans would produce isolated adrenocorticotropic hormone (ACTH) deficiency (IAD), a condition that had only been described in a few children. And indeed, we found that 2/3 IAD neonates have TPIT gene mutations (Lamolet et al. 2001, Pulichino et al. 2003a) and this is a hallmark of neonatal, but not juvenile, IAD.
(Vallette-Kasic et al. 2005). IAD causing TPIT mutations produce either complete or severe loss of function for DNA binding and/or transactivation. Many TPIT mutations are replacement mutations within the DNA binding Tbox domain that are incompatible with DNA binding or protein–protein interactions (Vallette-Kasic et al. 2007). Other mutations cause premature stop, aberrant splicing, or have chromosomal deletions (Couture et al. 2012).

IAD is a lethal condition in children as the resulting hypocortisolism leads to unstable glycemia, severe hypoglycemia, convulsions, and rapid death; however when recognized, the condition is effectively treated with glucocorticoid replacement therapy (Vallette-Kasic et al. 2005). Molecular diagnosis of TPIT mutations is thus extremely useful for patient follow-up.

**Corticotroph specificity of POMC expression**

Whereas broad pituitary specificity is conferred by Pitx1 and POMC lineage identity by Tpit, corticotroph-specific expression of POMC also relies on the neurogenic bHLH factor NeuroD1 (Poulin et al. 1997). Indeed, early transcriptional studies of the pituitary POMC promoter had identified in the distal region of the −300 bp enhancer a complex regulatory element that synergistically activates transcription when associated with the Tpit/PitxRE (Therrien & Drouin 1991). This regulatory element is also bipartite with one part corresponding to a binding site for NeuroD1-containing bHLH heterodimers, the Ebox_n熬 and the other part constituted of the Nur response element (NurRE) that provides signal-dependent inputs for activation of POMC transcription in response to CRH (discussed next). The Ebox_n熬 (originally called DE2C) is critical for synergism with the Tpit/PitxRE and for the full activity of the −300 bp enhancer (Therrien & Drouin 1993). This synergism relies on direct protein interactions between the NeuroD1 dimerization partner, such as IFT2, with the homeodomain of Pitx1 (Poulin et al. 2000). Further, NeuroD1 itself interacts directly with Tpit to support their synergistic interaction. Transcriptional activation by NeuroD1 heterodimers is enhanced by recruitment of Rb and the related coactivator p107 (Batsche et al. 2005b), whereas the activity of the Nur factors that binds the neighboring NurRE is coactivated by Rb, p107, or p130 (Batsche et al. 2005a) as discussed later. It is possible that the combination of Pitx1, Tpit, and NeuroD1 might suffice to specify the corticotroph genetic program compared with other anterior pituitary lineages; in contrast, the IL melanotrophs do not express NeuroD1 at any time during development or in adults. In early development, NeuroD1 is strongly expressed in developing corticotrophs, but its expression diminishes at mid-development to remain low during adult life when NeuroD1 is also expressed in other anterior lineages, in particular gonadotrophs (Lamolet et al. 2004). The exclusion of NeuroD1 from the IL and the decreasing NeuroD1 expression in corticotrophs might indicate a transient role of NeuroD1 in activation of POMC expression in fetal corticotrophs as supported by the transient phenotype observed in NeuroD1 knockout pituitaries (Lamolet et al. 2004). However, mutagenesis of the NeuroD1 binding site within the POMC promoter resulted in severe reduction of POMC promoter activity both during development and in the adult pituitary (Lavoie et al. 2008): these in vivo analyses clearly support a role for the neurogenic bHLH target sequence in the adult when NeuroD1 levels are low. It is not clear whether these low levels of NeuroD1 are sufficient to maintain this activity or whether other neurogenic bHLH might take over in adult corticotrophs. Indeed, another bHLH factor contributes to POMC expression in corticotrophs and also in melanotrophs. Indeed, Ascl1 (Mash1) is expressed in the early Rathke’s pouch and remains expressed in all adult lineages with particularly high levels in melanotrophs, corticotrophs, and gonadotrophs (Zhang et al. 2015) (L. Budry and JD, unpublished observations). Ascl1 contributes to POMC transcription and its genomic binding profile overlaps significantly with Tpit (Zhang et al. 2015). It is present at both −7 kb enhancer and POMC promoter (Fig. 2), where it binds the Ebox_n熬 sequence. Ascl1 may thus be the major bHLH factor acting on the Ebox_n熬 in adult POMC cells.

**Melanotroph identity and Pax7**

The single copy POMC gene is transcribed from the same TSS in corticotrophs and melanotrophs and differential promoter usage does not explain how very different signaling pathways modulate POMC transcription in each lineage. Similarly, both Pitx1 and Tpit are as critical for expression of POMC in corticotrophs and melanotrophs and hence, they do not explain melanotroph-specific POMC transcription. Candidates for this specificity were identified using developmental expression profiling of micro-dissected mouse pituitaries. This approach yielded one striking candidate, Pax7, which turns out to be a major determinant of IL identity and melanotroph-specific transcription (Budry et al. 2012). Indeed, Pax7 is only expressed in the IL of the pituitary and no other oral ectoderm derivatives.

Whereas Tpit gene inactivation results in abrogation of POMC expression, Pax7 knockout does not prevent POMC
expression in the IL (although reduced), but the Pax7−/− IL shows a striking switch in expression profile from melanotroph to corticotroph. Indeed, melanotroph markers such as the protein convertase PC2 and dopamine DRD2 receptors are severely reduced in the Pax7−/− IL, whereas corticotroph-specific markers such as the CRH and vasopressin V1b receptors are upregulated; in addition, the glucocorticoid receptor (GR), which is usually not expressed in IL but is expressed in all AL cells, is expressed at a similar level in the Pax7−/− IL. Global analysis of gene expression supports the interpretation that the genetic program of Pax7 is expressed in all AL cells, is expressed at a similar level in the corticotroph-specific markers such as the CRH and vasopressin V1b receptors are upregulated; in addition, the glucocorticoid receptor (GR), which is usually not expressed in IL but is expressed in all AL cells, is expressed at a similar level in the Pax7−/− IL. Global analysis of gene expression supports the hypothesis that the genetic program of Pax7 is expressed in all AL cells, is expressed at a similar level in the corticotroph program supporting a major role of Pax7 in determination of IL identity. Gain-of-function transgenic experiments corroborated this interpretation and further suggested that Pax7 may have the ability to drive pituitary progenitors out of the progenitor state and engage them into the differentiated status.

Most striking, however, is the mechanism by which Pax7 implements this identity switch: indeed, the contribution of Pax7 for implementation of melanotroph-specific gene expression goes beyond the simple combinatorial action of TFs because many melanotroph-specific Tpit target genes are inaccessible to Tpit in the corticotroph AtT-20 cells. However, following Pax7 action, these melanotroph-specific targets (enhancers) become accessible to Tpit; hence, Pax7 acts as a pioneer TF that remodels chromatin and changes enhancer accessibility to other TFs such as Tpit (Fig. 3A). This was indeed shown to be the case by genome-wide analyses of chromatin marks for active enhancers (Budry et al. 2012). It was found that a few thousand enhancers change their chromatin structure upon Pax7 binding, making them accessible to other TFs such as Tpit: a good example of this is the melanotroph-specific enhancer of the PC2 gene that has no epigenetic mark of activity in normal corticotroph AtT-20 cells, but acquires marks of active enhancers (H3K4me1, H3K27Ac) after Pax7-dependent chromatin remodeling (Fig. 3B).

Pax7 expression appears slightly before Tpit in the IL and hence this is consistent with epigenome remodeling by Pax7 to implement the Tpit-dependent melanotroph program of gene expression. Such role is consistent with the selector gene model that acts early in development to condition later cell differentiation. Selection of IL identity during early pituitary development is consistent with the developmental importance of this tissue for proper pituitary development and with the maintenance of this tissue even in species such as humans, where the IL regresses during mid-gestation and where the pituitary melanotroph function is lost and transferred to skin.

Hypothalamic expression of POMC

The POMC gene is expressed in a few thousand neurons of the hypothalamus; in particular, the POMCergic system plays a key role in energy homeostasis (Mountjoy 2010, Gali Ramamoorthy et al. 2015). During fetal development, there is strong POMC expression in a cluster of ventrally located neurons in the developing diencephalon (Gee et al. 1983, Elkabes et al. 1989). This early cluster of POMC-positive neurons contains the progenitors of a fraction of the adult hypothalamic POMC network (Padilla et al. 2010, Coupe & Bouret 2013). Analyses of POMC regulatory sequences have clearly identified separate sequences for hypothalamic expression of POMC compared with the pituitary regulatory sequences. Indeed, the 5′ proximal −480 bp upstream of the POMC TSS do not drive hypothalamic expression in a transgenic assay, whereas an enhancer present at about −12 kb from the POMC TSS is primarily responsible for hypothalamic expression (Young et al. 1998, Lam et al. 2015). Conversely, the −12 kb enhancer does not have any pituitary activity (Rubinstein et al. 1993). The hypothalamic −12 kb enhancer (Fig. 1C) was subdivided into two subregions, NPE1 and NPE2 (de Souza et al. 2005), and recent studies indicated that the TF Isl1 is a major determinant for hypothalamic expression of POMC; further, Isl1 acts on target sequences within the NPE1 and NPE2 subdomains of the hypothalamic enhancer (Nasif et al. 2015). The NPE2 element is also a target of estrogen receptor (de Souza et al. 2011). Hypothalamic POMC expression is stimulated by leptin; this action is mediated by the Jak2/Stat3 pathway (Bates et al. 2003), and is counteracted by the FoxO1 TF that blocks Stat3 by direct interaction (Yang et al. 2009).

In agreement with the clear separation of regulatory sequences for pituitary and hypothalamic expression, chromatin marks associated with transcriptional activity in pituitary cells are restricted to the 5′-proximal sequences and the −7 kb pituitary enhancer (figs 1C and 2), but are completely absent from the hypothalamic −12 kb enhancer (Langlais et al. 2011).

Hormonal control of POMC transcription

Activation of pituitary transcription function by CRH signaling

The hypothalamic hormone CRH stimulates the release of POMC-derived peptides from pituitary corticotrophs and from the model cells AtT-20. In parallel, CRH signaling activates POMC transcription (Gagner & Drouin 1985, 1987) and may act through different signaling
A  Pax7 opens chromatin at new enhancer repertoire

1. Pax7 must bind its site on inactive chromatin.
2. Trigger chromatin remodeling, i.e. « opening ».
3. Opened enhancer chromatin allows binding by other TFs such as Tpit, recruitment of coactivators, and transcription activation.

B  The PC2 −146 kb enhancer is opened by Pax7

Figure 3
Pioneer factor action of Pax7 opens new enhancer repertoire for implementation of the melanotroph gene expression program. (A) Schematic representation of chromatin remodeling exerted by Pax7 at melanotroph-specific enhancers. (B) The PC2 gene −146 kb enhancer is remodeled by Pax7 to allow Tpit binding and enhancer activity. ChIP-seq profiles illustrate the changes in transcription factor occupancy and chromatin state at the −146 kb PC2 enhancer before and after Pax7.

Pathways. Indeed, CRH action on its receptor leads to activation of the cAMP/PKA pathway and of the MAPK pathway (Kovalovsky et al. 2002, Maira et al. 2003a), the pituitary corticotrophs being one of the unusual tissues where these two pathways are positively linked rather than antagonizing each other. CRH signaling has been
associated with activation of AP1 TFs constituted of heterodimers between jun and fos and putative targets of AP1 were identified on the POMC promoter (Boutilier et al. 1991, 1995). Growth and serum stimulation also activates these TFs and their role in hormonal control of POMC transcription by CRH remains ill defined.

A major target of CRH action on POMC transcription was mapped to a target regulatory element located at \(-404\) bp in the rat promoter: this regulatory element defined a palindromic target of action for orphan NR related to nerve growth factor I-B (NGFI-B) (Nur77) that are activated through the MAPK pathway (Philips et al. 1997a). Indeed, the POMC gene NurRE defined this dimer mechanism of action for the Nur subfamily of orphan NRs by comparison to the previously held view that these orphan NRs acted primarily as monomers on a target sequence that is similar to one half-site of the NurRE. This monomer target of action, the NBRE, is a far less potent target for NGFI-B activation, in particular in response to activation of the MAPK pathway (Maira et al. 2003b). The POMC gene NurRE exhibits a preference for Nur factor dimers that include NGFI-B compared with the activity of a consensus NurRE that is equally activated by the three members of this NR subfamily (Maira et al. 1999). The Nur subfamily of orphan NRs includes in addition to NGFI-B (Nur77), the factors Nur-related factor 1 (Nurr1) and NOR1 (Campos-Melo et al. 2013).

CRH and Nur factor activation of POMC are negatively regulated by a miRNA, miR-375, which targets the 3′-UTR of the MAPK 8 mRNA (Zhang et al. 2013). Downregulation of MAPK 8 decreases Erk1/2 and Nur factor activity, hence POMC transcription.

**Coactivators of CRH signaling**

Transcriptional activation of the POMC gene through the Nur and NeuroD1 dimers is enhanced by coactivators of the SRC family, including SRC1, SRC2, and SRC3. Indeed, these coactivators interact with many NRs including the Nur factors, but their action on this latter group is far greater (by almost two orders of magnitude) on Nur dimers than monomers (Maira et al. 2003b). In contrast to many ligand-dependent NRs where SRC coactivators are recruited to the ligand-dependent C-terminal AF2 activation domain, their recruitment to Nur factors is through the N-terminus. The recruitment is dependent on MAPK signals and activates the NGFI-B N-terminal AF1 domain. For NGFI-B, the AF1 domain was dissected into two subdomains, one being constitutively active and the other being dependent on MAPK signaling (Maira et al. 2003b). This AF1 domain is a site of intense phosphorylation following activation by MAPK.

Direct identification by mass spectrometry of proteins associated with NGFI-B following CRH signaling identified another unexpected coactivator. Indeed, CRH-dependent proteins recruited to NGFI-B complexes included the coregulator Tif1β (also known as KAP1), which was primarily known as a corepressor (Rambaud et al. 2009). However, in the context of CRH signaling and NurRE-dependent transcription, Tif1β exhibits coactivator activity and this coactivation is synergistic with that of SRC2. Nur factor action on the NurRE is also enhanced by Rb and its related proteins p107 and p130 through direct protein–protein interactions (Batsche et al. 2005a).

**Glucocorticoid repression of POMC transcription**

In counterbalance to CRH activation, transcription of the POMC gene is subject to feedback repression by Gc and their receptor, GR (Gagner & Drouin 1985). The first transgenic analysis of the 5′ regulatory sequences of the POMC gene indicated that these sequences were sensitive to both CRH activation and Gc repression (Tremblay et al. 1988). Further dissection of these sequences led first to identification of a negative glucocorticoid response element (nGRE) centered at \(-63\) bp of the rat POMC promoter (Drouin et al. 1989). The unique property of this nGRE is to bind three moieties of GR in the form of a homodimer followed by monomer binding (Drouin et al. 1993). While the nGRE regulatory sequences confer Gc repression in some contexts, they may not be primarily responsible for Gc repression in other contexts and it is still unclear which in vivo context would favor this relative to another mechanism. Indeed, mapping of Gc responsive sequences using transient transfection in AtT-20 cells with luciferase reporters mapped responsive sequences to the NurRE (Philips et al. 1997a,b). Further investigation indicated that GR does not bind these sequences directly, but that it interacts with Nur factors through protein–protein interactions and thus may repress transcription by a transrepression mechanism (Philips et al. 1997b, Martens et al. 2005). This GR recruitment requires BRG1, the ATPase component of the SWI/SNF chromatin remodeling complex (Bilodeau et al. 2006). The GR transrepression complex also recruits the histone deacetylase 2 (HDAC2) and this is also dependent on BRG1. BRG1 appears to be constitutively present at the POMC promoter before Gc/GR activation.
GR recruitment results in decreased histone acetylation at the POMC promoter and gene body. Discovery of the hormone-regulated −7 kb POMC gene enhancer further supported a role for GR-dependent repression through transrepression, but comparison of human and mouse −7 kb enhancer and 5′ regulatory sequences suggested that their relative importance for hormonal control of transcription may have species-specific features (Langlais et al. 2011). Indeed, whereas the rodent 5′ proximal regulatory sequences (rather than −7 kb enhancer) appear primarily responsible for both CRH activation and Gc repression, the human −7 kb human enhancer appeared more sensitive to CRH activation and Gc/GR repression (Langlais et al. 2011). Thus, the relative importance of the −7 kb enhancer for hormonal control of POMC may differ between species despite the fact that the overall sequence conservation is high when comparing different genomes.

**Glucocorticoid repression of POMC and Cushing's disease**

Cushing’s disease is characterized by the loss of Gc feedback repression on pituitary POMC (Drouin et al. 2007). The relative insensitivity of pituitary POMC to Gc is indeed used as a discriminating clinical test to diagnose Cushing’s disease (Liddle 1960). The cause of Gc resistance in corticotroph adenomas of patients with Cushing’s disease was very rarely ascribed to GR mutations (Lamberts 2002), but processing of GR may be altered in a subset of adenomas may be involved in linking these mutations to the hallmark of corticotroph adenomas, namely their resistance to Gc feedback.

Another regulator of p27^Kip1, Cables1, was recently found to be Gc-dependent and lost in ~55% of Cushing’s adenomas. Indeed, the Cables1 negative regulator of cell cycle progression was identified in a genome-wide screen of Gc-dependent cell cycle regulators in AtT-20 cells (Roussel-Gervais et al. 2016).

**Defective signaling in Cushing's disease**

A new and significant inroad into the pathogenesis of Cushing’s disease was provided through transcriptome analysis. Indeed, this revealed recurrent mutations in the USP8 gene that encodes a deubiquitinase (Perez-Rivas et al. 2015, Reincke et al. 2015). Although USP8 could have many different substrates (Ge et al. 2015), current analyses indicate that corticotroph adenomas with USP8 mutations have persistent EGF signaling. The sustained EGF signaling was ascribed to increased cell surface EGF receptor due to enhanced deubiquitination activity caused by the USP8 mutations. The upregulation of EGF signaling likely increases POMC gene expression (Reincke et al. 2015) and this may contribute to upregulation of ACTH secretion. However in itself, this action does not account for Gc resistance and hence, it is likely that other targets of USP8 may be involved in linking these mutations to the hallmark of corticotroph adenomas, namely their resistance to Gc feedback.

**Other signaling pathways affecting POMC gene expression**

Signals related to transforming growth factor-β modulate POMC transcription through their action on the Smad family of TFs. The Smad factors acting as heterodimers containing a signal responsive Smad together with the general partner Smad4 act on the POMC promoter through recruitment to the Tpit/PitxRE (Nudi et al. 2005). While exogenous ligands repress POMC transcription, it appears from studies in AtT-20 cells that the inhibitory Smad 6 and Smad 7 may also operate as an autocrine regulatory loop on the system.

The inflammatory response TF nuclear factor-B (NFkB) also stimulates POMC transcription (Takahasu et al. 2010), but details of this action remain limited. However, it is noteworthy that a form of human ACTH deficiency is ascribed to activating mutations in
the related NFKB2 gene (Chen et al. 2013, Brue et al. 2014). These NFKB2 mutations also cause variable immunodeficiency and the association of IAD with immunodeficiency constitutes the David syndrome (Quentien et al. 2012). While these NFKB2 mutations have been well documented in human patients, the loss of Nfkb2 function in mouse does not affect pituitary development and in all likelihood, function. Once again, human and mouse appear to have differences in rate-limiting processes, such as the relative importance of NFKB2 or the role of −7 kb POMC enhancer for hormonal regulation.

The cytokine and STAT signaling on POMC transcription

Cytokines related to leukemia inhibitory factor (LIF) and interleukin (IL)-6 stimulate POMC transcription and ACTH release and this action may be synergistic with CRH (Ray et al. 1996). Both IL-6 and LIF are released from the hypothalamus and systemically in response to inflammation and thus may serve to coordinate inflammatory response with pituitary function; hence, this may provide an important immune-endocrine connection for coordination of inflammatory responses. In addition, LIF is produced within the pituitary gland and there, it may provide paracrine and autocrine regulation (Akita et al. 1995). These cytokines act through activation of the Stat3 TF, which has two binding sites on the POMC promoter, one at −380 bp and the other at −150 bp (Fig. 2). As for CRH-dependent activation, LIF activation of POMC transcription is antagonized by Gc and GR, and this effect is exerted through transrepression (Langlais et al. 2012).

Conclusion

This review has outlined knowledge gained over the last three decades on POMC gene regulation from studies mostly performed in mouse models, cells, and in vivo. These studies have been very informative of the mechanisms and crosstalk between various developmental and signaling pathways and they provide the conceptual backdrop to understand their regulatory interplays. However, it is noteworthy that the emerging picture for transposition of the knowledge to the human POMC gene, as for many other similar comparisons, reveals that the regulatory processes are well conserved, but that the specific targets may have evolved differently, even for relatively close species such as human and mouse.
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