60 YEARS OF POMC

Transcriptional and epigenetic regulation of *POMC* gene expression

Jacques Drouin

Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal (IRCM), Montréal, Québec, Canada

Correspondence should be addressed to J Drouin
Email
jacques.drouin@ircm.qc.ca

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 adrenocorticotrophic 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.

Key Words
- gene regulation
- transcription
- pituitary
- hypothalamus
- ACTH
- MSH

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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

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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*. 1997*a*). 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*. 1999*a*). 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

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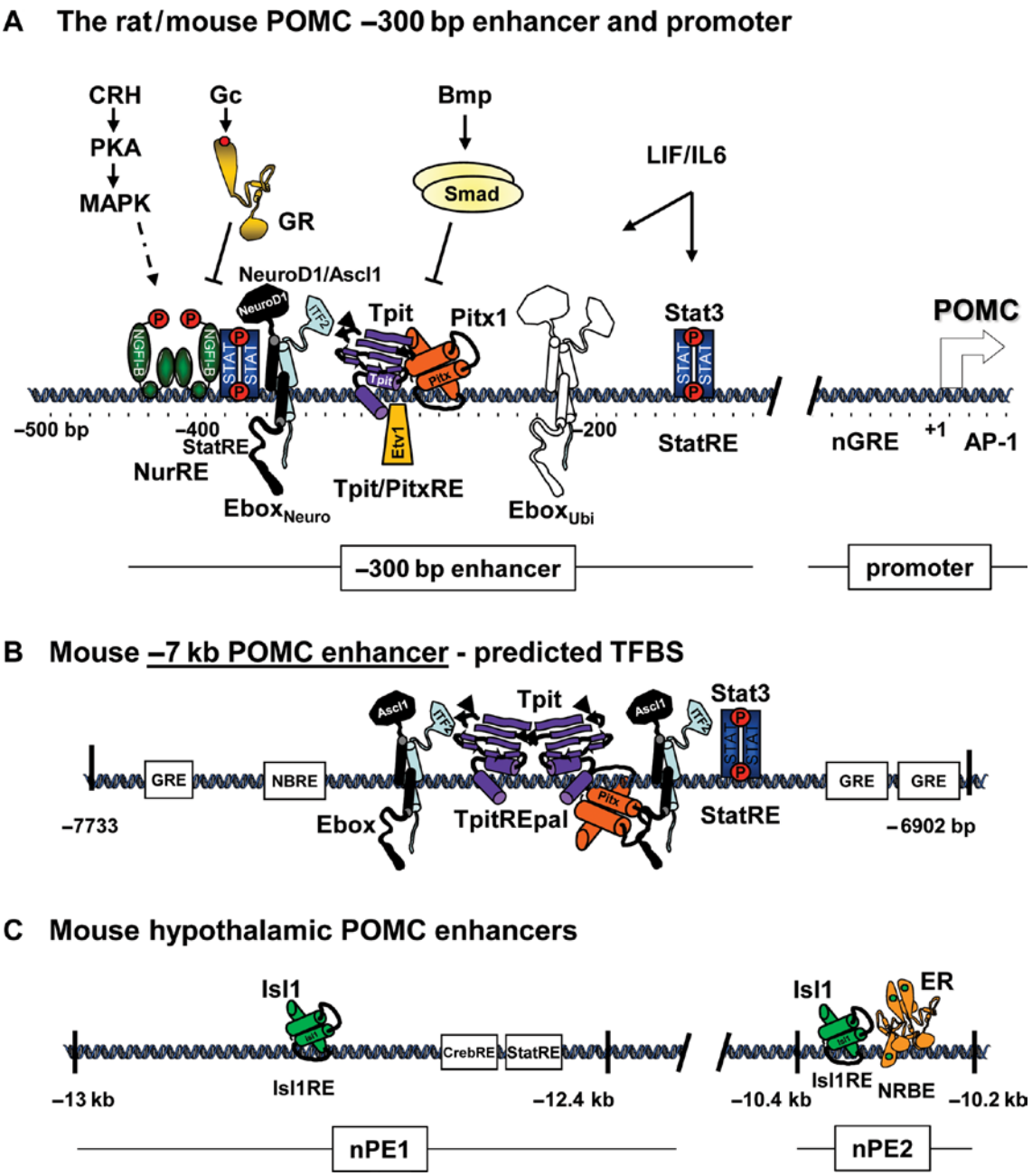

Figure 1

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

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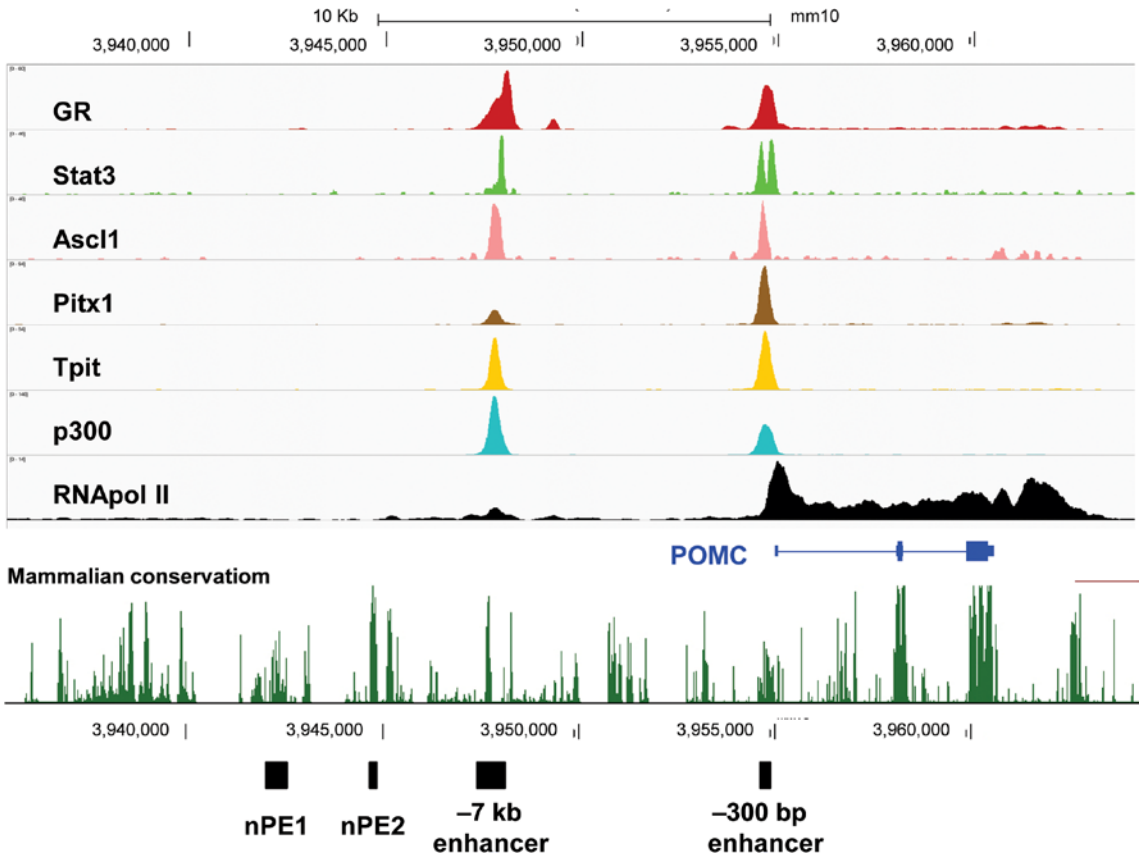

Figure 2
The mouse *POMC* locus. *In vivo* occupancy of the mouse *POMC* locus by different transcription factors, the coactivator p300 and RNA polymerase II (RNApol II) is revealed by ChIP-seq. ChIP-seq profiles for the indicated factors are shown above diagrams showing mouse chromosomal positions (mm10) on chromosome 12, the *POMC* locus together with a plot of mammalian conservation for this locus. ChIP-seq data are from the author's laboratory except Ascl1 data recomputed from Zhang *et al.* (2015).

(Lanctôt *et al.* 1997, 1999*b*, 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.* 1999*b*, 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

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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 (Lanctôt *et al*. 1997, 1999*a*). 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 (Lanctôt *et al*. 1999*a*). *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*. 1999*b*, 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*. 2003*a*). 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*. 2003*b*). 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*. 2003*b*). 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, p57^{Kip1} and p27^{Kip2}, 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 adrenocorticotrophic 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*. 2003*a*) and this is a hallmark of neonatal, but not juvenile, IAD

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(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 spicing, 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 $Ebox_{neuro}$, 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 $Ebox_{neuro}$ (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 ITF2, 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*. 2005*b*), whereas the activity of the Nur factors that binds the neighboring NurRE is coactivated by Rb, p107, or p130 (Batsche *et al*. 2005*a*) 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 $Ebox_{neuro}$ sequence. Ascl1 may thus be the major bHLH factor acting on the $Ebox_{neuro}$ 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

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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*$^{-/-}$ IL cells is significantly (but not completely) switched to 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, nPEI 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

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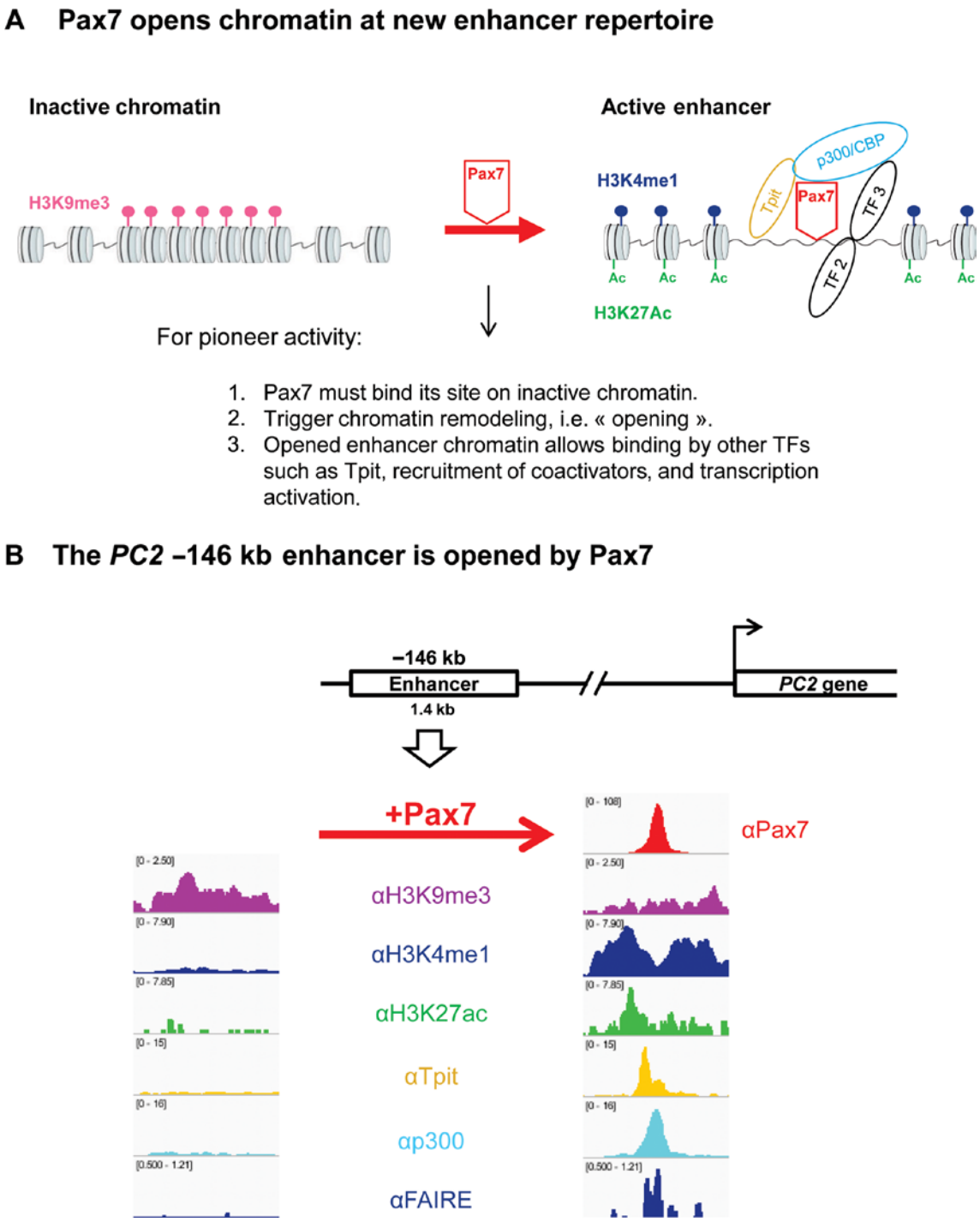

Figure 3
Pioneer factor action of Pax7 opens new enhancer repertoire for implementation of the melanotroph gene expression program. (A) Schematic representation of chromatin remodeling exerted by Pax7 at melanotroph-specific enhancers. (B) The *PC2* gene –146 kb enhancer is remodeled by Pax7 to allow 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.* 2003*a*), 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

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associated with activation of AP1 TFs constituted of heterodimers between *jun* and *fos* and putative targets of AP1 were identified on the *POMC* promoter (Boutillier *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*. 1997*a*). 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*. 2003*b*). 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*. 2003*b*). 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*. 2003*b*). 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*. 2005*a*).

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*. 1997*a*,*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*. 1997*b*, 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.

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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 that overexpress the chaperone HSP90 (Riebold *et al*. 2015). Rather, it is often the Gc-dependent corticotroph response mechanisms that are altered. The identification of critical roles of BRG1 and HDAC2 for GR transrepression of POMC led to assessment of the expression of these two essential components for Gc feedback in Cushing's disease adenomas. The loss of nuclear expression of either of these proteins, BRG1 or HDAC2, may account for Gc resistance in over 50% of adenomas, both in dogs and humans (Bilodeau *et al*. 2006).

The loss of BRG1 expression in corticotroph adenomas may also lead to upregulation of cyclin E. Accordingly, upregulation of cyclin E is correlated with the loss of BRG1 in these adenomas together with loss of $p27^{Kip1}$ (Roussel-Gervais *et al*. 2010). It had already been observed that cyclin E is overexpressed in about 75% of corticotroph adenomas (Jordan *et al*. 2000) and that these adenomas also often exhibit the loss of $p27^{Kip1}$ expression (Lidhar *et al*. 1999). Direct assessment of the relative contributions of cyclin E overexpression and loss of $p27^{Kip1}$ showed a synergistic effect in a mouse model leading to bigger, faster, and more aggressive adenomas (Roussel-Gervais *et al*. 2010).

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 EGFR 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 (Takayasu *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

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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 *NFK2B* 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.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

Akita S, Webster J, Ren SG, Takino H, Said J, Zand O & Melmed S 1995 Human and murine pituitary expression of leukemia inhibitory factor. Novel intrapituitary regulation of adrenocorticotropin hormone synthesis and secretion. *Journal of Clinical Investigation* **95** 1288–1298. (doi:10.1172/JCI117779)

Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, *et al*. 2003 STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature* **421** 856–859. (doi:10.1038/nature01388)

Batsche E, Desroches J, Bilodeau S, Gauthier Y & Drouin J 2005*a* Rb enhances p160/SRC coactivator-dependent activity of nuclear receptors and hormone responsiveness. *Journal of Biological Chemistry* **280** 19746–19756. (doi:10.1074/jbc.M413428200)

Batsche E, Moschopoulos P, Desroches J, Bilodeau S & Drouin J 2005*b* Retinoblastoma and the related pocket protein p107 act as coactivators of NeuroD1 to enhance gene transcription. *Journal of Biological Chemistry* **280** 16088–16095. (doi:10.1074/jbc.M413427200)

Bilodeau S, Vallette-Kasic S, Gauthier Y, Figarella-Branger D, Brue T, Berthelet F, Lacroix A, Batista D, Stratakis S, Hanson J, *et al*. 2006 Role of Brg1 and HDAC2 in GR *trans*-repression of pituitary POMC gene and misexpression in Cushing disease. *Genes & Development* **20** 2871–2886. (doi:10.1101/gad.1444606)

Bilodeau S, Roussel-Gervais A & Drouin J 2009 Distinct developmental roles of cell cycle inhibitors p57Kip2 and p27Kip1 distinguish pituitary progenitor cell cycle exit from cell cycle re-entry of differentiated cells. *Molecular and Cellular Biology* **29** 1895–1908. (doi:10.1128/MCB.01885-08)

Boutillier AL, Sassone-Corsi P & Loeffler JP 1991 The protooncogene c-fos is induced by corticotropin-releasing factor and stimulates proopiomelanocortin gene transcription in pituitary cels. *Molecular Endocrinology* **5** 1301–1310. (doi:10.1210/mend-5-9-1301)

Boutillier AL, Monnier D, Lorang D, Lundblad JR, Roberts JL & Loeffler JP 1995 Corticotropin-releasing hormone stimulates proopiomelanocortin transcription by cFos-dependent and -independent pathways: characterization of an AP1 site in exon 1. *Molecular Endocrinology* **9** 745–755. (doi:10.1210/mend.9.6.8592520)

Brue T, Quentien M, Khetchoumian K, Bensa M, Capo-Chichi J, Delemer B, Balsalobre A, Nassif C, Papadimitriou DT, Pagnier A, *et al*. 2014 Mutations in NFKB2 and potential genetic heterogeneity in patients with DAVID syndrome, having variable endocrine and

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-15-0289

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immune deficiencies. *BMC Medical Genetics* **15** 139. (doi:10.1186/s12881-014-0139-9)

Budry L, Couture C, Balsalobre A & Drouin J 2011 The Ets factor Etv1 interacts with Tpit protein for pituitary pro-opiomelanocortin (POMC) gene transcription. *Journal of Biological Chemistry* **286** 25387–25396. (doi:10.1074/jbc.M110.202788)

Budry L, Balsalobre A, Gauthier Y, Khetchoumian K, L'Honore A, Vallette S, Brue T, Figarella-Branger D, Meij B & Drouin J 2012 The selector gene Pax7 dictates alternate pituitary cell fates through its pioneer action on chromatin remodeling. *Genes & Development* **26** 2299–2310. (doi:10.1101/gad.200436.112)

Bumaschny VF, de Souza FS, Lopez Leal RA, Santangelo AM, Baetscher M, Levi DH, Low MJ & Rubinstein M 2007 Transcriptional regulation of pituitary POMC is conserved at the vertebrate extremes despite great promoter sequence divergence. *Molecular Endocrinology* **21** 2738–2749. (doi:10.1210/me.2006-0557)

Campos-Melo D, Galleguillos D, Sanchez N, Gysling K & Andres ME 2013 Nur transcription factors in stress and addiction. *Frontiers in Molecular Neuroscience* **6** 44. (doi:10.3389/fnmol.2013.00044)

Charles MA, Suh H, Drouin J, Camper SA & Gage PJ 2005 PITX genes are required for cell survival and *Lhx3* activation. *Molecular Endocrinology* **19** 1893–1903. (doi:10.1210/me.2005-0052)

Chen K, Coonrod EM, Kumanovics A, Franks ZF, Durtschi JD, Margraf RL, Wu W, Heikal NM, Augustine NH, Ridge PG, *et al*. 2013 Germline mutations in NFKB2 implicate the noncanonical NF-kappaB pathway in the pathogenesis of common variable immunodeficiency. *American Journal of Human Genetics* **93** 812–824. (doi:10.1016/j.ajhg.2013.09.009)

Coulon V, L'Honoré A, Ouimette JF, Dumontier É, van den Munckhof P & Drouin J 2007 A muscle-specific promoter directs *Pitx3* gene expression in early muscle cells. *Journal of Biological Chemistry* **282** 33192–33200. (doi:10.1074/jbc.M706119200)

Coupe B & Bouret SG 2013 Development of the hypothalamic melanocortin system. *Frontiers in Endocrinology (Lausanne)* **4** 38. (doi:10.3389/fendo.2013.00038)

Couture C, Saveanu A, Barlier A, Baechler A, Wherett D, Houang Y, Lebouc Y, Fassnacht M, Maes D, Fluck C, *et al*. 2012 Phenotypic homogeneity and genotypic variability in a large series of congenital isolated ACTH deficiency patients with TPIT gene mutations. *Journal of Clinical Endocrinology & Metabolism* **97** 486–495. (doi:10.1210/jc.2011-1659)

de Souza FS, Santangelo AM, Bumaschny V, Avale ME, Smart JL, Low MJ & Rubinstein M 2005 Identification of neuronal enhancers of the proopiomelanocortin gene by transgenic mouse analysis and phylogenetic footprinting. *Molecular and Cellular Biology* **25** 3076–3086. (doi:10.1128/MCB.25.8.3076-3086.2005)

de Souza FS, Nasif S, Lopez-Leal R, Levi DH, Low MJ & Rubinsten M 2011 The estrogen receptor alpha colocalizes with proopiomelanocortin in hypothalamic neurons and binds to a conserved motif present in the neuron-specific enhancer nPE2. *European Journal of Pharmacology* **660** 181–187. (doi:10.1016/j.ejphar.2010.10.114)

Drouin J, Trifiro MA, Plante RK, Nemer M, Eriksson P & Wrange Ö 1989 Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of pro-opiomelanocortin gene transcription. *Molecular and Cellular Biology* **9** 5305–5314. (doi:10.1128/MCB.9.12.5305)

Drouin J, Sun YL, Chamberland M, Gauthier Y, De Léan A, Nemer M & Schmidt TJ 1993 Novel glucocorticoid receptor complex with DNA element of the hormone-repressed POMC gene. *EMBO Journal* **12** 145–156.

Drouin J, Bilodeau S & Vallette-Kasic S 2007 Of old and new diseases: genetics of pituitary ACTH excess (Cushing) and deficiency. *Clinical Genetics* **72** 175–182. (doi:10.1111/j.1399-0004.2007.00877.x)

Elkabes S, Loh YP, Nieburgs A & Wray S 1989 Prenatal ontogenesis of pro-opiomelanocortin in the mouse central nervous system and pituitary gland: an in situ hybridization and immunocytochemical study. *Developmental Brain Research* **46** 85–95. (doi:10.1016/0165-3806(89)90145-4)

Gage PJ, Suh H & Camper SA 1999*a* The bicoid-related Pitx gene family in development. *Mammalian Genome* **10** 197–200. (doi:10.1007/s003359900970)

Gage PJ, Suh HY & Camper SA 1999*b* Dosage requirement of Pitx2 for development of multiple organs. *Development* **126** 4643–4651.

Gagner J-P & Drouin J 1985 Opposite regulation of pro-opiomelanocortin gene transcription by glucocorticoids and CRH. *Molecular and Cellular Endocrinology* **40** 25–32. (doi:10.1016/0303-7207(85)90154-6)

Gagner J-P & Drouin J 1987 Tissue-specific regulation of pituitary proopiomelanocortin gene transcription by corticotropin-releasing hormone, 3′, 5′-cyclic adenosine monophosphate, and glucocorticoids. *Molecular Endocrinology* **1** 677–682. (doi:10.1210/mend-1-10-677)

Gali Ramamoorthy T, Begum G, Harno E & White A 2015 Developmental programming of hypothalamic neuronal circuits: impact on energy balance control. *Frontiers in Neuroscience* **9** 126. (doi:10.3389/fnins.2015.00126)

Ge C, Che L, Ren J, Pandita RK, Lu J, Li K, Pandita TK & Du C 2015 BRUCE regulates DNA double-strand break response by promoting USP8 deubiquitination of BRIT1. *PNAS* **112** E1210–E1219. (doi:10.1073/pnas.1418335112)

Gee CE, Chen CLC, Roberts JL, Thompson R & Watson SJ 1983 Identification of pro-opiomelanocortin neurones in rat hypothalamus by in situ cDNA-mRNA hybridization. *Nature* **306** 374–376. (doi:10.1038/306374a0)

Hammer GD, Fairchild-Huntress V & Low MJ 1990 Pituitary-specific and hormonally regulated gene expression directed by the rat proopiomelanocortin promoter in transgenic mice. *Molecular Endocrinology* **4** 1689–1697. (doi:10.1210/mend-4-11-1689)

Hwang DY, Ardayfio P, Kang UJ, Semina EV & Kim KS 2003 Selective loss of dopaminergic neurons in the substantia nigra of Pitx3-deficient aphakia mice. *Molecular Brain Research* **114** 123–131. (doi:10.1016/S0169-328X(03)00162-1)

Jeannotte L, Trifiro MA, Plante RK, Chamberland M & Drouin J 1987 Tissue-specific activity of the pro-opioomelanocortin gene promoter. *Molecular and Cellular Biology* **7** 4058–4064. (doi:10.1128/MCB.7.11.4058)

Jordan S, Lidhar K, Korbonits M, Lowe DG & Grossman AB 2000 Cyclin D and cyclin E expression in normal and adenomatous pituitary. *European Journal of Endocrinology* **143** R1–R6. (doi:10.1530/eje.0.143R001)

Kitamura K, Miura H, Miyagawa-Tomita S, Yanazawa M, Katoh-Fukui Y, Suzuki R, Ohuchi H, Suehiro A, Motegi Y, Nakahara Y, *et al*. 1999 Mouse Pitx2 deficiency leads to anomalies of the ventral body wall, heart, extra- and periocular mesoderm and right pulmonary isomerism. *Development* **126** 5749–5758.

Kovalovsky D, Refojo D, Liberman AC, Hochbaum D, Pereda MP, Coso OA, Stalla GK, Holsboer F & Arzt E 2002 Activation and induction of NUR77/NURR1 in corticotrophs by CRH/cAMP: involvement of calcium, protein kinase A, and MAPK pathways. *Molecular Endocrinology* **16** 1638–1651. (doi:10.1210/mend.16.7.0863)

L'Honoré A, Coulon V, Marcil A, Lebel M, Lafrance-Vanasse J, Gage PJ, Camper SA & Drouin J 2007 Sequential expression and redundancy of *Pitx2* and *Pitx3* genes during muscle development. *Developmental Biology* **307** 421–433. (doi:10.1016/j.ydbio.2007.04.034)

L'Honoré A, Ouimette JF, Lavertu Jolin M & Drouin J 2010 Pitx2 defines alternate pathways acting through MyoD during limb and somitic myogenesis. *Development* **137** 3847–3856. (doi:10.1242/dev.053421)

L'Honore A, Commere PH, Ouimette JF, Montarras D, Drouin J & Buckingham M 2014 Redox regulation by Pitx2 and Pitx3 is critical for fetal myogenesis. *Developmental Cell* **29** 392–405. (doi:10.1016/j.devcel.2014.04.006)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-15-0289

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Lam DD, de Souza FS, Nasif S, Yamashita M, Lopez-Leal R, Otero-Corchon V, Meece K, Sampath H, Mercer AJ, Wardlaw SL, *et al*. 2015 Partially redundant enhancers cooperatively maintain Mammalian pomc expression above a critical functional threshold. *PLoS Genetics* **11** e1004935. (doi:10.1371/journal.pgen.1004935)

Lamberts SWJ 2002 Glucocorticoid receptors and Cushing's disease. *Molecular and Cellular Endocrinology* **197** 69–72. (doi:10.1016/S0303-7207(02)00280-0)

Lamolet B, Pulichino AM, Lamonerie T, Gauthier Y, Brue T, Enjalbert A & Drouin J 2001 A pituitary cell-restricted T-box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell* **104** 849–859. (doi:10.1016/S0092-8674(01)00282-3)

Lamolet B, Poulin G, Chu K, Guillemot F, Tsai MJ & Drouin J 2004 Tpit-independent function of NeuroD1(BETA2) in pituitary corticotroph differentiation. *Molecular Endocrinology* **18** 995–1003. (doi:10.1210/me.2003-0127)

Lamonerie T, Tremblay JJ, Lanctôt C, Therrien M, Gauthier Y & Drouin J 1996 PTX1, a *bicoid*-related homeobox transcription factor involved in transcription of pro-opiomelanocortin (POMC) gene. *Genes & Development* **10** 1284–1295. (doi:10.1101/gad.10.10.1284)

Lanctôt C, Lamolet B & Drouin J 1997 The *bicoid*-related homeoprotein Ptx1 defines the most anterior domain of the embryo and differentiates posterior from anterior lateral mesoderm. *Development* **124** 2807–2817.

Lanctôt C, Gauthier Y & Drouin J 1999*a* Pituitary homeobox 1 (Ptx1) is differentially expressed during pituitary development. *Endocrinology* **140** 1416–1422. (doi:10.1210/endo.140.3.6549)

Lanctôt C, Moreau A, Chamberland M, Tremblay ML & Drouin J 1999*b* Hindlimb patterning and mandible development require the *Ptx1* gene. *Development* **126** 1805–1810.

Langlais D, Couture C, Sylvain-Drolet G & Drouin J 2011 A pituitary-specific enhancer of the POMC gene with preferential activity in corticotrope cells. *Molecular Endocrinology* **25** 348–359. (doi:10.1210/me.2010-0422)

Langlais D, Couture C & Drouin J 2012 The Stat3/GR interaction code: predictive value of direct/indirect DNA recruitment for transcription outcome. *Molecular Cell* **47** 38–49. (doi:10.1016/j.molcel.2012.04.021)

Lavoie PL, Budry L, Balsalobre A & Drouin J 2008 Developmental dependence on NurRE and EboxNeuro for expression of pituitary POMC. *Molecular Endocrinology* **22** 1647–1657. (doi:10.1210/me.2007-0567)

Liddle GW 1960 Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. *Journal of Clinical Endocrinology & Metabolism* **20** 1539–1560. (doi:10.1210/jcem-20-12-1539)

Lidhar K, Korbonits M, Jordan S, Khalimova Z, Kaltsas G, Lu X, Clayton RN, Jenkins PJ, Monson JP, Besser GM, *et al*. 1999 Low expression of the cell cycle inhibitor p27Kip1 in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors. *Journal of Clinical Endocrinology & Metabolism* **84** 3823–3830. (doi:10.1210/jcem.84.10.6066)

Lin CR, Kioussi C, O'Connell S, Briata P, Szeto D, Liu R, Izpisua Belmonte JC & Rosenfeld MG 1999 Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphegenesis. *Nature* **401** 279–282. (doi:10.1038/45803)

Lu MF, Pressman C, Dyer R, Johnson RL & Martin JF 1999 Function of Rieger syndrome gene in left-right asymmetry and craniofacial development. *Nature* **401** 276–278. (doi:10.1038/45797)

Maira MH, Martens C, Philips A & Drouin J 1999 Heterodimerization between members of the Nur subfamily of orphan nuclear receptors as a novel mechanism for gene activation. *Molecular and Cellular Biology* **19** 7549–7557. (doi:10.1128/MCB.19.11.7549)

Maira MH, Couture C, Le Martelot G, Pulichino AM, Bilodeau S & Drouin J 2003*a* The T-box factor Tpit recruits SRC/p160 coactivators and mediates hormone action. *Journal of Biological Chemistry* **278** 46523–46532. (doi:10.1074/jbc.M305626200)

Maira MH, Martens C, Batsche E, Gauthier Y & Drouin J 2003*b* Dimer-specific potentiation of NGFI-B (Nur77) transcriptional activity by the protein kinase A pathway and AF-1-dependent coactivator recruitment. *Molecular and Cellular Biology* **23** 763–776. (doi:10.1128/MCB.23.3.763-776.2003)

Martens C, Bilodeau S, Maira M, Gauthier Y & Drouin J 2005 Protein: protein interactions and transcriptional antagonism between the subfamily of NGFI-B/Nur77 orphan nuclear receptors and glucocorticoid receptor. *Molecular Endocrinology* **19** 885–897. (doi:10.1210/me.2004-0333)

Mountjoy KG 2010 Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. *Biochemical Journal* **428** 305–324. (doi:10.1042/BJ20091957)

Nasif S, de Souza FS, Gonzalez LE, Yamashita M, Orquera DP, Low MJ & Rubinstein M 2015 Islet 1 specifies the identity of hypothalamic melanocortin neurons and is critical for normal food intake and adiposity in adulthood. *PNAS* **112** E1861–E1870. (doi:10.1073/pnas.1500672112)

Nudi M, Ouimette JF & Drouin J 2005 Bone morphogenic protein (Smad)-mediated repression of proopiomelanocortin transcription by interference with Pitx/Tpit activity. *Molecular Endocrinology* **19** 1329–1342. (doi:10.1210/me.2004-0425)

Nunes I, Tovmasian LT, Silva RM, Burke RE & Goff SP 2003 Pitx3 is required for development of substantia nigra dopaminergic neurons. *PNAS* **100** 4245–4250. (doi:10.1073/pnas.0230529100)

Padilla SL, Carmody JS & Zeltser LM 2010 Pomc-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits. *Nature Medicine* **16** 403–405. (doi:10.1038/nm.2126)

Perez-Rivas LG, Theodoropoulou M, Ferrau F, Nusser C, Kawaguchi K, Stratakis CA, Rueda Faucz F, Wildemberg LE, Assie G, Beschorner R, *et al*. 2015 The gene of the ubiquitin-specific protease 8 is frequently mutated in adenomas causing Cushing's disease. *Journal of Clinical Endocrinology & Metabolism* **100** E997–E1004. (doi:10.1210/jc.2015-1453)

Philips A, Lesage S, Gingras R, Maira MH, Gauthier Y, Hugo P & Drouin J 1997*a* Novel dimeric Nur77 signaling mechanisms in endocrine and lymphoid cells. *Molecular and Cellular Biology* **17** 5946–5951. (doi:10.1128/MCB.17.10.5946)

Philips A, Maira MH, Mullick A, Chamberland M, Lesage S, Hugo P & Drouin J 1997*b* Antagonism between Nur77 and glucocorticoid receptor for control of transcription. *Molecular and Cellular Biology* **17** 5952–5959. (doi:10.1128/MCB.17.10.5952)

Poulin G, Turgeon B & Drouin J 1997 NeuroD1/BETA2 contributes to cell-specific transcription of the POMC gene. *Molecular and Cellular Biology* **17** 6673–6682. (doi:10.1128/MCB.17.11.6673)

Poulin G, Lebel M, Chamberland M, Paradis FW & Drouin J 2000 Specific protein: protein interaction between basic Helix-Loop-Helix transcription factors and homeoproteins of the Pitx family. *Molecular and Cellular Biology* **20** 4826–4837. (doi:10.1128/MCB.20.13.4826-4837.2000)

Pulichino AM, Vallette-Kasic S, Couture C, Gauthier Y, Brue T, David M, Malpuech G, Deal C, Van Vliet G, De Vroede M, *et al*. 2003*a* Human and mouse Tpit gene mutations cause early onset pituitary ACTH deficiency. *Genes & Development* **17** 711–716. (doi:10.1101/gad.1065603)

Pulichino AM, Vallette-Kasic S, Tsai JP, Couture C, Gauthier Y & Drouin J 2003*b* Tpit determines alternate fates during pituitary cell differentiation. *Genes & Development* **17** 738–747. (doi:10.1101/gad.1065703)

Quentien MH, Delemer B, Papadimitriou DT, Souchon PF, Jaussaud R, Pagnier A, Munzer M, Jullien N, Reynaud R, Galon-Faure N, *et al*. 2012 Deficit in anterior pituitary function and variable immune deficiency (DAVID) in children presenting with adrenocorticotropin deficiency and severe infections. *Journal of Clinical Endocrinology & Metabolism* **97** E121–E128. (doi:10.1210/jc.2011-0407)

http://jme.endocrinology-journals.org
DOI: 10.1530/JME-15-0289

Published by Bioscientifica Ltd.

Rambaud J, Desroches J, Balsalobre A & Drouin J 2009 TIF1beta/KAP-1 is a coactivator of the orphan nuclear receptor NGFI-B/Nur77. *Journal of Biological Chemistry* **284** 14147–14156. (doi:10.1074/jbc.M809023200)

Ray DW, Ren SG & Melmed S 1996 Leukemia inhibitory factor (LIF) stimulates proopiomelanocortin (POMC) expression in a corticotroph cell line. Role of STAT pathway. *Journal of Clinical Investigation* **97** 1852–1859. (doi:10.1172/JCI118615)

Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, Meitinger T, Mizuno-Yamasaki E, Kawaguchi K, Saeki Y, *et al*. 2015 Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nature Genetics* **47** 31–38. (doi:10.1038/ng.3166)

Riebold M, Kozany C, Freiburger L, Sattler M, Buchfelder M, Hausch F, Stalla GK & Paez-Pereda M 2015 A C-terminal HSP90 inhibitor restores glucocorticoid sensitivity and relieves a mouse allograft model of Cushing disease. *Nature Medicine* **21** 276–280. (doi:10.1038/nm.3776)

Roussel-Gervais A, Bilodeau S, Vallette S, Berthelet F, Lacroix A, Figarella-Branger D, Brue T & Drouin J 2010 Cooperation between cyclin E and p27Kip1 in pituitary tumorigenesis. *Molecular Endocrinology* **24** 1835–1845. (doi:10.1210/me.2010-0091)

Roussel-Gervais A, Couture C, Langlais D, Takayasu S, Balsalobre A, Rueda BR, Zukerberg LR, Figarella-Branger D, Brue T & Drouin J 2016 The Cables1 gene in glucocorticoid regulation of pituitary corticotrope growth and Cushing disease. *Journal of Clinical Endocrinology & Metabolism* **101** 513–522. (doi:10.1210/jc.2015-3324)

Rubinstein M, Mortrud M, Liu B & Low MJ 1993 Rat and mouse proopiomelanocortin gene sequences target tissue-specific expression to the pituitary gland but not to the hypothalamus of transgenic mice. *Neuroendocrinology* **58** 373–380. (doi:10.1159/000126566)

Semina EV, Reiter RS & Murray J 1997 Isolation of a new homeobox gene belonging to the Pitx/Rieg family: expression during lens development and mapping to the aphakia region on mouse chromosome 19. *Human Molecular Genetics* **6** 2109–2116. (doi:10.1093/hmg/6.12.2109)

Semina EV, Ferrell RE, Mintz-Hittner HA, Bitoun P, Alward WLM, Reiter RS, Funkhauser C, Daack-Hirsch S & Murray JC 1998 A novel homeobox gene *PITX3* is mutated in families with autosomal-dominant cataracts and ASMD. *Nature Genetics* **19** 167–170. (doi:10.1038/527)

Smidt MP, van Schaick HS, Lanctôt C, Tremblay JJ, Cox JJ, van der Kleij AA, Wolterink G, Drouin J & Burbach JP 1997 A homeodomain gene Ptx3 has highly restricted brain expression in mesencephalic dopaminergic neurons. *PNAS* **94** 13305–13310. (doi:10.1073/pnas.94.24.13305)

Szeto DP, Rodriguez-Esteban C, Ryan AK, O'Connell SM, Liu F, Kioussi C, Gleiberman AS, Izpisua-Belmonte JC & Rosenfeld MG 1999 Role of the Bicoid-related homeodomain factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. *Genes & Development* **13** 484–494. (doi:10.1101/gad.13.4.484)

Takayasu S, Iwasaki Y, Nigawara T, Asai M, Yoshida M, Kageyama K & Suda T 2010 Involvement of nuclear factor-kB and Nurr-1 in cytokine-induced transcription of proopiomelanocortin gene in AtT20 corticotroph cells. *Neuroimmunomodulation* **17** 88–96. (doi:10.1159/000258691)

Therrien M & Drouin J 1991 Pituitary pro-opiomelanocortin gene expression requires synergistic interactions of several regulatory elements. *Molecular and Cellular Biology* **11** 3492–3503. (doi:10.1128/MCB.11.7.3492)

Therrien M & Drouin J 1993 Cell-specific helix-loop-helix factor required for pituitary expression of the pro-opiomelanocortin gene. *Molecular and Cellular Biology* **13** 2342–2353. (doi:10.1128/MCB.13.4.2342)

Treisman J, Gonczy P, Vashishtha M, Harris E & Desplan C 1989 A single amino acid can determine the DNA binding specificity of homeodomain proteins. *Cell* **59** 553–562. (doi:10.1016/0092-8674(89)90038-X)

Tremblay JJ & Drouin J 1999 Egr-1 is a downstream effector of GnRH and synergizes by direct interaction with Ptx1 and SF-1 to enhance luteinizing hormone ? gene transcription. *Molecular and Cellular Biology* **19** 2567–2576. (doi:10.1128/MCB.19.4.2567)

Tremblay Y, Tretjakoff I, Peterson A, Antakly T, Zhang CX & Drouin J 1988 Pituitary-specific expression and glucocorticoid regulation of proopiomelanocortin fusion gene in transgenic mice. *PNAS* **85** 8890–8894. (doi:10.1073/pnas.85.23.8890)

Tremblay JJ, Lanctôt C & Drouin J 1998 The pan-pituitary activator of transcription, Ptx-1 (pituitary homeobox1), acts in synergy with SF-1 and Pit1 and is an upstream regulator of the Lim-homeodomain gene Lim3/Lhx3. *Molecular Endocrinology* **12** 428–441. (doi:10.1210/mend.12.3.0073)

Tremblay JJ, Marcil A, Gauthier Y & Drouin J 1999 Ptx1 regulates SF-1 activity by an interaction that mimics the role of the ligand-binding domain. *EMBO Journal* **18** 3431–3441. (doi:10.1093/emboj/18.12.3431)

Tremblay JJ, Goodyer CG & Drouin J 2000 Transcriptional properties of Ptx1 and Ptx2 isoforms. *Neuroendocrinology* **71** 277–286. (doi:10.1159/000054547)

Vallette-Kasic S, Figarella-Branger D, Grino M, Pulichino AM, Dufour H, Grisoli F, Enjalbert A, Drouin J & Brue T 2003 Differential regulation of proopiomelanocortin and pituitary-restricted transcription factor (TPIT), a new marker of normal and adenomatous human corticotrophs. *Journal of Clinical Endocrinology & Metabolism* **88** 3050–3056. (doi:10.1210/jc.2002-021934)

Vallette-Kasic S, Brue T, Pulichino AM, Gueydan M, Barlier A, David M, Nicolino M, Malpuech G, Déchelotte P, Deal C, *et al*. 2005 Congenital isolated adrenocorticotropin deficiency: an underestimated cause of neonatal death, explained by TPIT gene mutations. *Journal of Clinical Endocrinology & Metabolism* **90** 1323–1331. (doi:10.1210/jc.2004-1300)

Vallette-Kasic S, Couture C, Balsalobre A, Gauthier Y, Metherell LA, Dattani M & Drouin J 2007 The TPIT gene mutation M86R associated with isolated ACTH deficiency interferes in protein: protein interactions. *Journal of Clinical Endocrinology & Metabolism* **92** 3991–3999. (doi:10.1210/jc.2007-0284)

van den Munckhof P, Luk KC, Sainte-Marie L, Montgomery J, Blanchet PJ, Sadikot AF & Drouin J 2003 Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. *Development* **130** 2535–2542. (doi:10.1242/dev.00464)

van den Munckhof P, Gilbert F, Chamberland M, Lévesque D & Drouin J 2006 Striatal neuroadaptation and rescue of locomotor deficit by L-dopa in *aphakia* mice, a model of Parkinson's disease. *Journal of Neurochemistry* **96** 160–170. (doi:10.1111/j.1471-4159.2005.03522.x)

Yang G, Lim CY, Li C, Xiao X, Radda GK, Li C, Cao X & Han W 2009 FoxO1 inhibits leptin regulation of pro-opiomelanocortin promoter activity by blocking STAT3 interaction with specificity protein 1. *Journal of Biological Chemistry* **284** 3719–3727. (doi:10.1074/jbc.M804965200)

Young JI, Otero V, Cerdan MG, Falzone TL, Chan EC, Low MJ & Rubinstein M 1998 Authentic cell-specific and developmentally regulated expression of pro-opiomelanocortin genomic fragments in hypothalamic and hindbrain neurons of transgenic mice. *Journal of Neuroscience* **18** 6631–6640.

Zhang N, Lin JK, Chen J, Liu XF, Liu JL, Luo HS, Li YQ & Cui S 2013 MicroRNA 375 mediates the signaling pathway of corticotropin-releasing factor (CRF) regulating pro-opiomelanocortin (POMC) expression by targeting mitogen-activated protein kinase 8. *Journal of Biological Chemistry* **288** 10361–10373. (doi:10.1074/jbc.M112.425504)

Zhang F, Tanasa B, Merkurjev D, Lin C, Song X, Li W, Tan Y, Liu Z, Zhang J, Ohgi KA, *et al*. 2015 Enhancer-bound LDB1 regulates a corticotrope promoter-pausing repression program. *PNAS* **112** 1380–1385. (doi:10.1210/jc.2008-1102)

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