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REVIEW

Development of neuroendocrine neurons in the mammalian hypothalamus

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Abstract

The neuroendocrine system consists of a heterogeneous collection of (mostly) neuropeptidergic neurons found in four hypothalamic nuclei and sharing the ability to secrete neurohormones (all of them neuropeptides except dopamine) into the bloodstream. There are, however, abundant hypothalamic non-neuroendocrine neuropeptidergic neurons developing in parallel with the neuroendocrine system, so that both cannot be entirely disentangled. This heterogeneity results from the workings of a network of transcription factors many of which are already known. *Olig2* and *Fezf2* expressed in the progenitors, acting through mantle-expressed *Otp* and *Sim1*, *Sim2* and *Pou3f2* (*Brn2*), regulate production of magnocellular and anterior parvocellular neurons. *Nkx2-1*, *Rax*, *Ascl1*, *Neurog3* and *Dbx1* expressed in the progenitors, acting through mantle-expressed *Isl1*, *Dlx1*, *Gsx1*, *Bsx*, *Hmx2/3*, *Ikzf1*, *Nr5a2* (*LH-1*) and *Nr5a1* (*SF-1*) are responsible for tuberal parvocellular (arcuate nucleus) and other neuropeptidergic neurons. The existence of multiple progenitor domains whose progeny undergoes intricate tangential migrations as one source of complexity in the neuropeptidergic hypothalamus is the focus of much attention. How neurosecretory cells target axons to the medial eminence and posterior hypophysis is gradually becoming clear and exciting progress has been made on the mechanisms underlying neurovascular interface formation. While rat neuroanatomy and targeted mutations in mice have yielded fundamental knowledge about the neuroendocrine system in mammals, experiments on chick and zebrafish are providing key information about cellular and molecular mechanisms. Looking forward, data from every source will be necessary to unravel the ways in which the environment affects neuroendocrine development with consequences for adult health and disease.

Keywords Arcuate nucleus · Genomic regulatory networks · Hypophysis · Magnocellular · Neurosecretory · Paraventricular · Parvocellular · Periventricular nucleus · Progenitor domain · Supraoptic nucleus

Introduction: neuropeptides and neurohormones

The neuroendocrine system is "the interface between the CNS [central nervous system] and the endocrine system. A remarkably diverse group of neurons in the hypothalamus secretes hormones into capillaries associated with the pituitary gland…" (Swanson 1986).

In order to understand the complexities of its development, it is useful to first review this heterogeneous neuronal group and the principles of its organization. Most hypothalamic neuroendocrine neurons are concentrated in the anterior and tuberal hypothalamic regions (Fig. 1a–c), where they form four neuronal nuclei (Fig. 1d–f): the paraventricular hypothalamic nucleus (PVH), the periventricular nucleus (PV), the supraoptic nucleus (SO) and the arcuate nucleus of the hypothalamus (ARH).

The neurohormones are oxytocin (*Oxt*), vasopressin (*Avp*), corticotropin-releasing hormone (*Crh*), thyrotropin-releasing hormone (*Trh*), somatostatin (*Sst*), growth hormone-releasing hormone (*Ghrh*), gonadotropin-releasing hormone (*Gnrh*) and dopamine. All of them are peptides except dopamine. Neurons producing dopamine are characterized by the expression of tyrosine hydroxylase (*Th*). *Gnrh*-expressing neuroendocrine neurons are born in the nasal placode and migrate into the CNS to settle mostly in the preoptic and anterior regions of the hypothalamus; their development is out of the scope of this review (Schwanzel-Fukuda and Pfaff 1989; Wray et al. 1989; Yoshida et al. 1995). This review focuses on the development of neurosecretory neurons expressing the seven (not counting

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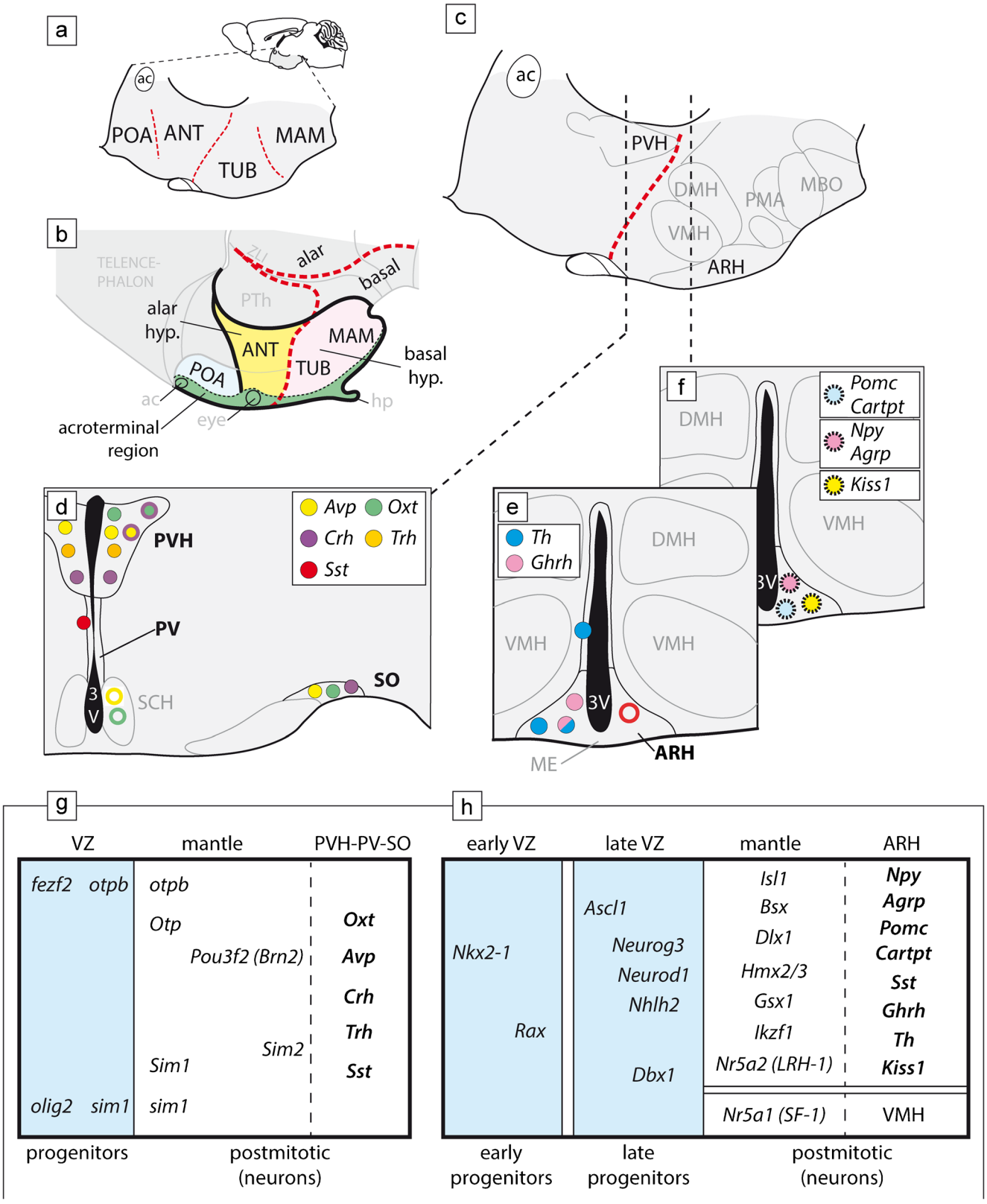

Gnrh) neurohormones with references to those non-neuroendocrine neuropeptidergic neurons whose development shares interesting genes and mechanisms.

Indeed many hypothalamic neurons express the same neurohormones as neuroendocrine neurons but they do not secrete them into the bloodstream. They release them locally instead,

◀ **Fig. 1** The four hypothalamic regions, the four neuroendocrine nuclei and their main neuronal types. **a** Hypothalamus region in the rodent brain showing the classical hypothalamic regions. **b** A different interpretation of the four regions based on the prosomeric model. The POA is here part of the telencephalon and the anterior and tuberal regions are alar and basal, respectively (from Haddad-Tóvolli et al. 2015). **c** The rodent hypothalamus showing some major nuclei and the plane of section of **d**–**f**. **d** Diagram of a transverse section through the anterior region showing the three neuroendocrine nuclei located in the anterior region, PVH, PV and SO, as well as the major neuroendocrine cell types. Part of the PV can also be seen in the tuberal region in **e**, **f**. Filled circles represent neuroendocrine cells and open circles represent their non-neuroendocrine versions (see inset for details). **e**, **f** Diagrams of a transverse section through the tuberal region showing the ARH and its major cell types expressing classical neuropeptides (**e**) as well as other (**f**). **g**, **h** Diagram of the hypothalamic VZ (blue) generating either the magnocellular and the anterior parvocellular neurons (**g**) or the tuberal parvocellular neurons (**h**) and the corresponding mantle layer (white), with indication of the known transcription expressed in the factors in that compartment. In **g**, the factors expressed by the VZ are known from zebrafish. On the rightmost side of each diagram, the PVH, PV and SO (**g**) and the ARH (**h**) with their characteristic neuropeptides (bold)

to act as modulators on the releasing neuron itself (autocrine function) or on nearby cells (paracrine function). Non-neuroendocrine neurons expressing the neurohormones can be found intermixed with their neuroendocrine counterparts as well as by themselves in many hypothalamic nuclei as well as the neuronal interstitium (neurons not organized as nuclei) between them (Markakis and Swanson 1997).

For instance, *Avp* neurons in the suprachiasmatic nucleus (SCH) are not neuroendocrine (reviewed in Kalsbeek et al. 2010); *Ghrh*-expressing cells in the arcuate nucleus of the hypothalamus (ARH) are neuroendocrine but in other locations (ventromedial nucleus of the hypothalamus (VMH)), they are mostly non-neuroendocrine (Sawchenko et al. 1985); the neuroendocrine *Sst*-expressing neurons are found in the PV and in the PVH; there are also *Sst*-expressing neurons in the ARH but they are not neuroendocrine (Kawano et al. 1982; Kawano and Daikoku 1988; Markakis and Swanson 1997).

Moreover, since there are many more neuropeptides than neurohormones (Fricker 2012), there are many neuropeptides that never work as hormones. Some of them are secreted as hormones by other organs (not the brain) or act as hormones in other, non-mammalian species.

Parvocellular and magnocellular

Neuroendocrine neurons of the magnocellular system project axons to the posterior hypophysis (neurohypophysis) where they secrete either Oxytocin (Oxt) or Vasopressin (Avp) into fenestrated capillaries devoid of blood-brain barrier and originating in the inferior hypophyseal artery. Neurons of the parvocellular system secrete one of the other neuropeptides (or dopamine) into the primary plexus of the hypophyseal portal system, found in the median eminence. This plexus is a fenestrated capillary bed from the superior hypophyseal that that flows in the hypophyseal portal veins. These in turn flow into a secondary plexus of fenestrated capillaries situated in the anterior hypophysis (adenohypophysis). There the parvocellular neurohormones exit the blood and exert their effects activating or inhibiting the secretion of other hormones by specific endocrine cell populations of the adenohypophysis. Magnocellular neurons reside in the PVH and SO and some more are scattered in an area between them, the paramagnocellular region. Of the parvocellular neurons, the ones expressing *Trh* or *Crh* are found in the PVH, the ones expressing *Sst* in the PVH and PV and those expressing *Th* and/or *Ghrh*, in the ARH and PV. A few neuroendocrine *Crh*-neurons can be found in the SO and there are some very few neuroendocrine *Ghrh*-neurons in the VMH and DMH (Markakis and Swanson 1997).

Intriguingly, there are in the rat PVH magnocellular *Oxt*-expressing neurons as well as parvocellular (!) *Avp*-expressing neurons coexpresing *Crh* (Sawchenko et al. 1984a, b). There is also evidence of neurons expressing *Avp* or *Oxt* but belonging to the parvocellular system, which is, sending axons to the hypophysial portal system and secreting their neuropeptides onto the anterior hypophysis (Horn et al. 1985; Johnston and Negro-Vilar 1988). Moreover, some PVH neurons express *Avp* or *Oxt* but send descending axons to autonomic nuclei of the hindbrain and spinal cord (Sawchenko and Swanson 1982). The actual functional importance of these "exceptions" is starting to be unveiled (Eliava et al. 2016; Wircer et al. 2017).

In some occassions, the terms magnocellular and parvocellular have been used as synonymous, respectively, for neuroendocrine (i.e., neurosecretory) and non-neuroendocrine (i.e., non-neurosecretory) (see for instance Eliava et al. 2016; Luther et al. 2000, 2002). This use is not standard and might cause confusion.

The ability to extend axons to fenestrated capillaries of the median eminence (parvocellular system) or the posterior hypophysis (magnocellular system) and secrete neurohormones into the bloodstream is the essential phenotypical trait of the neuroendocrine system. To classify a neuron as neuroendocrine or non-neuroendocrine, it is necessary to inject in the bloodstream of the experimental animal a marker (usually Fast Blue or Fluorogold) that can be acquired by the axons of neuroendocrine cells and retrogradely label them specifically. Any study of the developing neuroendocrine system on normal or genetically altered brains must include this analysis; otherwise, we are missing key information. The function of neurons able to influence the secretion of corticoids (*Crh*-expressing neuroendocrine neurons) is quite different from that of neurons secreting Crh as a local modulator (*Crh*-expressing neurons). How the neuroendocrine trait is acquired and if the development of these neurons is differently regulated as that of non-neuroendocrine neurons are important questions.

Non-neuroendocrine neuropeptides that act as hormones elsewhere

Finally, there are neuropeptides acting as hormones, only not in mammals or in the hypothalamus.

α-Melanocyte-stimulating hormone (α-MSH), a derivative of pro-opiomelanocortine (*Pomc*), is a neuropeptide delivered to the bloodstream from the intermediate pituitary: it is a pituitary hormone. However, the α-MSH-expressing neurons of the hypothalamus, which find themselves in the arcuate nucleus, do not send axons to the median eminence or to the posterior hypophysis. Instead, they secrete their α-MSH into their local environment, where it acts as a neuromodulator.

Melanin-concentrating hormone (MCH) is a neuropeptide secreted by certain neurons to the bloodstream (i.e., it is a neurohormone) in Osteichthyes (bony fishes) but not in reptiles, amphibians, or mammals, where it is not a neurohormone (Croizier et al. 2013). Therefore, the MCH-expressing neurons of the lateral hypothalamic area (LHA) do not belong to the neuroendocrine system in rodents, for instance, or in humans.

Kisspeptin (Kiss1) is a peptide expressed by neurons in the PV, where it locally regulates the secretion of Gnrh (Clarkson and Herbison 2006) as well as, in pregnant animals, that of Oxt (Seymour et al. 2017). Kisspeptin is secreted as a hormone by the placenta (Horikoshi et al. 2003) but does not belong to the neuroendocrine system, since it is not secreted into the bloodstream from the hypothalamus.

Progenitor domains and neuronal migration routes

Regional determination of the hypothalamus

The mechanisms by which a certain area of the neuroepithelium (ventricular zone (VZ)) is instructed to become hypothalamus are very complex; a detailed comment of them is beyond the scope of this review. Suffice to say that the work on zebrafish, chicken and mouse embryos has shown that Shh from the axial mesoderm has a key role (Chiang et al. 1996; Szabó et al. 2009a) i.e., that morphogenetic movements of the neural plate and axial mesoderm are important because they sequentially expose the presumptive hypothalamic floor plate to the influence of various secreted proteins from the underlying mesoderm (Manning et al. 2006; Placzek and Briscoe 2005) and that, later on, Wnt (Kapsimali et al. 2004; Lee et al. 2006) and Shh (Haddad-Tóvolli et al. 2015; Szabó et al. 2009a) and other signaling cascades interact to specify the progenitors that will generate the four classical hypothalamic regions (preoptic, anterior, tuberal and mamillary; Swanson 1987) (Fig. 1a). These general mechanisms apply also to a recent reinterpretation of hypothalamic organization based on phylogenetic hypotheses; accordingly, the preoptic region would be part of the telencephalon rather than the hypothalamus, the anterior region would be part of the alar plate of the neural tube and the other two part of the basal plate (Puelles et al. 2012) (Fig. 1b).

Progenitor domains and neuronal migration routes

The regionalization of the neural plate translates into a mosaic of gene expression in the presumptive hypothalamic neuroepithelium (see for instance Alvarez-Bolado et al. 1995; Shimogori et al. 2010 and many others). Restricted areas of presumptive hypothalamic neuroepithelium characterized by the expression of specific combinations of transcription factors constitute "progenitor domains." These will generate specific neuronal subpopulations each of them characterized by common phenotypic traits like neurotransmitter or connections. The microorganization of the PVH suggests a very high degree of precision in the specification of the neuroepithelium giving rise to neurosecretory neurons. The five neuroendocrine neuronal types of the PVH distribute forming five restricted areas (or "hot spots") where one single neuronal type is predominant and almost no other type is found. Between any two of these restricted areas, both neuronal types are found intermixed (Simmons and Swanson 2009). This contrasts with the large progenitor domain of *Pmch* neurons, for instance, which generates a large, relatively disperse group of MCH-expressing neurons to the LHA, itself an anatomically poorly defined area (Brischoux et al. 2001).

Recently, the importance of tangential migrations from multiple progenitor domains for the development of the hypothalamic neuropeptidergic neurons has been recognized. The migration of young hypothalamic neurons was for many years described as mostly radial and following "three waves" of neurogenesis, each of them settling in a more medial position than the previous (outside-in pattern), in this way defining an outer "shell" of neurons, generated by an early wave of neurogenesis, an intermediate layer generated by the next wave of neurogenesis and lastly a periventricular layer generated (Altman and Bayer 1986). Work on neuropeptide-expressing neurons started to throw some doubt on this simple scheme. For these cells, it has been shown that they are born relatively early but occupy a much more medial position than would correspond to them by the three waves model. They behave rather like "pioneer neurons" of their respective hypothalamic nuclei, because they appear to be born and settle earlier than other, non-neuroendocrine neurons fated to the same hypothalamic nuclei (Markakis and Swanson 1997). Later work following Shh lineages showed a more complex pattern than the three waves for most hypothalamic cells (Alvarez-Bolado et al. 2012). Finally, other analyses have shown an important degree of tangential migration for neuropeptidergic hypothalamic cells from their progenitor domains molecularly defined by expression of combinations of transcription factors, originating specific neuronal

subpopulations each sharing expression of the same neuropeptide, tangential migration pathways and settling places in the hypothalamic mantle (Daikoku et al. 1983; Diaz et al. 2014; Morales-Delgado et al. 2011, 2014). An example of how tangential migration from diverse progenitor domains can work out in the formation of hypothalamic nuclei can be found in the *Th*-expressing neurons of the ARH (the A12 dopaminergic group). In the mouse ARH, this subpopulation of neuroendocrine cells is heterogeneous, since 30% of them also express dopamine transporter (DAT, *Slc6a3*) (Yip et al. 2017) and 15% of them coexpress *Ghrh* (representing most *Ghrh*-expressing ARH neurons (Meister et al. 1986; Phelps et al. 2003). Accordingly, some *Th*-expressing cells of the ARH originate in one single restricted region of the hypothalamic VZ (the anterobasal domain), rostral to the presumptive ARH (Diaz et al. 2014), while the *Ghrh*-expressing neurons originate in a progenitor domain immediately contiguous to the presumptive ARH (Morales-Delgado et al. 2014).

At this point, a succint comparison with thalamic developmental mechanisms can be enlightening. The thalamus is another complex brain region formed by a large number of nuclei and subnuclei. The mechanisms by which an initially undifferentiated thalamic cell mass becomes gradually subdivided by differential gene expression are becoming clear (Ebisu et al. 2017; Hashimoto-Torii et al. 2003; Kataoka and Shimogori 2008; Szabó et al. 2009b). However, thalamic nuclei are tightly "embedded" into each other in way reminiscent of Russian dolls. Complex migration routes and reticular areas not organized as nuclei are not present in this region. The hypothalamus, with its many "loose" nuclei and large interstitial areas including the very large LHA, presents a different kind of problem, still awaiting full clarification.

Introduction to genomic regulatory networks and neuropeptidergic cells in the developing hypothalamus

Much research has focused on the identification of the transcription factors regulating the development of the neuroendocrine system. The phenotypes of mice carrying targeted mutations of genes expressed in the embryonic hypothalamus have provided a great deal of information. Work on zebrafish has focused on this system more recently but it is rapidly gaining predominance. Many of the major regulators of neuroendocrine development are known. One task ahead is to recognize their relations with each other as well as with effector genes and to understand them as a network.

The first insights into transcriptional control of differentiation were published half a century ago (Britten and Davidson 1969; Davidson and Britten 1974). The first answers to the question of how an embryo goes from undifferentiated to differentiated were based on *Drosophila* work; two key novel concepts from this work were the partition of an originally undifferentiated epithelium by morphogen gradients and the importance of different "combinations" of regulators to confer differential properties to the newly appeared compartments (reviewed in Kauffman 1981). Combinations of transcription factors hierarchically organized were then shown to underlie differentiation in yeast (Herskowitz 1989) and "combinatorial codes" were rapidly adapted to explain differentiation in the developing nervous system (He and Rosenfeld 1991). Soon, combinations of transcription factors were suggested, on the basis of differential expression, to underlie hypothalamic differentiation (Alvarez-Bolado et al. 1995). At the same time, the concept of rostrocaudal and transversal gradients setting up regions of differential expression of combinations of transcriptional regulators was also co-opted from *Drosophila* work to explain the regionalization of the neural plate and neural tube (Rubenstein et al. 1998). In 2002, work by Davidson and colleagues summarizing decades of mutant phenotype analysis on the sea urchin *Strongylocentrotus purpuratus* (re-)introduced their concept of genomic regulatory networks (GRNs) (Davidson et al. 2002) as a more advanced way to look at combinatorial codes. The vague concept of "developmental program" has acquired finally a more stringent definition and this can be applied systematically to the unraveling of the genetic basis of patterning processes (Vokes et al. 2007). The evolution of thinking from "combinatorial code" to GRNs as applied to neural tube regionalization has been recognized and reviewed (Beccari et al. 2013).

Transcription factors expressed in the progenitors that form the VZ (neuroepithelium) are very high in the GRN hierarchy. They specify the fate of the neurons produced by the progenitor cells as well as the number of them, i.e., how abundant a certain neuronal type is going to be. Later on, transcription factors down the GRN will be expressed by the postmitotic neurons as they abandon the VZ and enter the mantle layer. These mantle-expressed regulators will control the migration of the young neurons, their settling in the appropriate place of the hypothalamus, their axonal outgrowth and navigation and the expression of appropriate phenotypal traits, among them the ability to secrete neuropeptides into the bloodstream: the neuroendocrine phenotype. Finally, at the end of the GRN, some transcription factors will bind the regulatory sequences of the genes encoding the neuropeptides and other recognizable proteins, in this way revealing the fate that had been specified in the VZ days before. Developmental GRNs use time and again a few transcription factor proteins of "proven efficiency," so to speak, which sometimes leads to multiple unrelated effects of the mutation of one given gene.

Transcriptional control of the development of PVH, SO and PV

From the point of view of developmental regulation, we can define three neuroendocrine systems: the magnocellular (neurosecretory neurons expressing *Avp* or *Oxt*) and the anterior parvocellular (neurosecretory neurons expressing *Crh*, *Trh* or *Sst*) reside in the anterior region of the hypothalamus and the tuberal parvocellular (neurosecretory neurons expressing *Ghrh* or *Th*) resides in the ARH of the tuberal region.

A great deal of the original research on this subject is reduced to a dozen publications, most of them analyzing mouse mutant phenotypes and most of them 10 years old at least (Acampora et al. 1999; Blechman et al. 2007; Borodovsky et al. 2009; Goshu et al. 2002, 2004; Michaud et al. 2001, 1998, 2000; Nakai et al. 1995; Schonemann et al. 1995; Wang and Lufkin 2000; Xu and Fan 2007).

The development of the magnocellular and anterior parvocellular systems is regulated to a great extent by transcription factor genes *orthopedia homeobox* (*Otp*) and *single-minded 1* (*Sim1*) working in parallel (i.e., both are necessary and do not regulate each other's expression). In zebrafish, the expression of *otpb* (zebrafish paralog of *Otp*) and *sim1* is under the control of *fezf2* and *olig2*, respectively, which are expressed by hypothalamic progenitors in the VZ (Blechman et al. 2007; Borodovsky et al. 2009). Additionally, a mechanism based on adenylate cyclase activating polypeptide 1 (*adcyap1*) and its receptor *adcyap1r1* increases the translation of *otpb* mRNA (Blechman et al. 2007).

Both *Otp* and *Sim1* work at least in part by maintaining expression of transcription factor gene *Pou3f2* (*Brn2*), which is necessary for the development of the magnocellular lineage as well as the *Crh*-expressing parvocellular neurons. Both *Otp* and *Sim1* are required for expression of *Sim2*, which is necessary for the development of the *Sst* and *Trh* parvocellular neurons.

Most parvocellular and magnocellular neurons of the PVH and SO are generated between embryonic day (E) 10.5 and E12.5 in the mouse (Karim and Sloper 1980) and from E12.5 to E14.5 in the rat (Markakis and Swanson 1997). Many of these cells do not migrate very far away but remain very close to the VZ (i.e., to the midline) and build the PVH: those fated for the SO migrate away. Their terminal differentiation becomes obvious as soon as their characteristic neuropeptides are detectable around two days after they are born (Acampora et al. 1999). *Otp* is expressed by postmitotic cells fated to become magnocellular or anterior parvocellular immediately before they leave the VZ, as they still find themselves in a sublayer of the VZ contiguous to the mantle zone. In the mouse, *Otp* is apparently not expressed by dividing progenitors and therefore is probably not involved in the specification of fate; however, it positively influences progenitor proliferation, very likely in a non-cell-autonomous way (by regulating the secretion of mitogenic factors, for instance, Wang and Lufkin 2000). Intriguingly, in zebrafish, *otpb* is expressed by progenitors as well as by the postmitotic neurons they generate (Blechman et al. 2007). As they exit the VZ and start migrating, *Otp*-expressing neurons start expressing *Sim1* also as they will do until they settle and differentiate; again, in zebrafish, *sim1* is expressed also by the corresponding progenitors (Borodovsky et al. 2009). *Otp* and *Sim1* are essential for the proper migration of these neurons, which without either of them would "lose their way" and settle in inappropriate hypothalamic localizations. *Otp* and *Sim1* regulate migration by acting on different pathways, i.e., the migration defects found in *Otp*-deficient mouse brains are different from those found in *Sim1*-deficient brains. Two important protein families involved in neuronal migration as well as axonal navigation are the semaphorins (secreted ligands) and the plexins (signaling subunits of the semaphorin receptors) (Fiore and Püschel 2003; Püschel 2002). *Sim1* is required for activation of *PlexinC1* (and possibly downregulation of *PlexinA1*) in the developing hypothalamus. *PlexinC1* is very likely involved in the migration of magnocellular neurons.

Otp directs migration of postmitotic cells to the appropriate settling places where they form three of the four neuroendocrine nuclei: PVH, SO and PV. Then, *Otp* is required for these neurons to differentiate into their adult phenotypes. This includes particularly two properties: first of all, the expression of specific neuropeptides within those nuclei (*Avp*, *Oxt*, *Crh*, *Trh* and *Sst*). *Otp* is also necessary for the ability of the neuroendocrine neurons of PVH, SO and PV to send axons to the fenestrated capillaries of either the median eminence (parvocellular) or the posterior hypophysis (magnocellular): the "neuroendocrine phenotype." Additionally, both *Otp* and *Sim1* are required for the expression of the appropriate neuropeptides, that is, for terminal differentiation.

Of interest to understand the evolution of the neuropeptidergic system is that, in the mouse, *Otp* is expressed by the dopaminergic neurons of group A11 (in the posterior hypothalamus, around the mamillothalamic tract) but not for those of groups A8–10 and A12–15 (group A12 corresponds to the ARH). In zebrafish, however, all dopaminergic neurons of the hypothalamus are under *Otp* control; during evolution, this requirement has been greatly restricted in mammals (Ryu et al. 2007).

In the embryonic hypothalamic mantle, *Sim1* is expressed by all neurons fated to form the PVH and SO and coexpresses with *Pou3f2* (*Brn2*) in a subset of them and with *Sim2* in another, complementary, subset. *Pou3f2* (*Brn2*) and *Sim2* do not coexpress in this region and *Sim1* is required to activate expression of both of them. The subset of migrating neurons expressing *Sim1* and *Pou3f2* (*Brn2*) is fated to become all magnocellular cells as well as the *Crh*-expressing parvocellular cells. *Sim1* is essential for the correct migration and neuropeptide expression of those neurons and exerts this function through activation of *Pou3f2 (Brn2)* expression. In this way, the magnocellular lineage is born in a very restricted progenitor domain encoding not only the properties of *Avp* expression or *Oxt* expression but also the

crucial ability to send axons to fenestrated capillaries of the posterior hypophysis.

The subset of migrating neurons expressing *Sim1* and *Sim2* will become *Sst*- or *Trh*-expressing neurons of the PV and PVH. Also here, *Sim1* is essential for the correct migration and neuropeptide expression of those neurons and exerts its function on neuropeptide expression through activation of *Sim2* expression (*Sim2* is not required for correct migration).

Therefore, the magnocellular and the anterior parvocellular neurons are born from a circumscribed neuroepithelial region harboring two very restricted and contiguous progenitor domains. One of them will generate *Crh*-, *Avp*- and *Oxt*-expressing neurons migrating and differentiating under the control of *Sim1* mostly through *Pou3f2* (*Brn2*) activation and the other will generate *Trh*- and *Sst*-expressing neurons migrating and differentiating under the control of *Sim1*, which activates specific neuropeptide expression through *Sim2*.

Since SIM1 and SIM2 are able to bind the same DNA regulatory sequence (Probst et al. 1997; Swanson et al. 1995), *Sim1* can compensate for *Sim2* in *Sim2* null mutant mice; accordingly, these mutants show some *Trh* and *Sst* expression in reduced numbers of neurons. Moreover, *Sim2* has a direct, cell-autonomous influence on *Trh* expression as well as a non-cell-autonomous one, still mysterious (non-*Sim2*-expressing *Trh* cells lose *Trh* expression in the *Sim2* mutant).

The proteins SIM1 and ARNT2 are probably heterodimeric partners, since it is known that SIM1 and SIM2 can work forming heterodimers with ARNT proteins (Swanson et al. 1995; Michaud et al. 2000; Moffett and Pelletier 2000) and the hypothalamic defects in *Sim1* and *Arnt2* mutant mice are similar. SIM2 and ARNT2 are probably also partners in the activation of *Trh* and *Sst* expression (Hosoya et al. 2001; Keith et al. 2001; Michaud et al. 1998, 2000).

Finally, the differential functions of transcription factors on hypothalamic neurons expressing the same neuropeptide in different regions or in neuroendocrine and non-neuroendocrine neurons showcase the complexity of heterogeneous neuropeptide populations caused by different progenitor domains and tangential migrations (Morales-Delgado et al. 2011; Diaz et al. 2014; Morales-Delgado et al. 2014). Some examples refer to the neurons expressing either *Sst*, *Avp*, *Crh*, or *Trh*.

The case of *Sst* *Sst*-expressing cells in the ARH, which are not neuroendocrine in the rat (Daikoku et al. 1983; Kawano et al. 1982; Kawano and Daikoku 1988), require *Otp* for normal development. They are born in a different—but also *Otp*-expressing—progenitor domain as the *Sst*-expressing cells of the PV (Morales-Delgado et al. 2011), which are neuroendocrine. But *Otp* and *Sim1* do not coexpress in the area where the *Sst* cells of the ARH come from, which could be the reason why loss of those cells has not been reported in the *Sim1* mutant.

The case of *Avp* This cell type disappears as neuroendocrine in PVH and SO (in the *Otp* mutant as well as the *Sim1* mutant), remains in the non-neuroendocrine cells in SCH (in agreement with the differential progenitor domains of endocrine and non-endocrine *Oxt* and *Avp*; Diaz et al. 2014).

Conversely, *Crh*- and *Trh*-expressing cells are absent in the *Sim1* and in the *Otp* mutants not only from neuroendocrine cells in PVH but also from non-neuroendocrine cells from LHA.

Transcriptional control of the development of the neuroendocrine ARH (as well as some notes on its non-neuroendocrine portion)

The fourth and last hypothalamic neuroendocrine nucleus, the ARH, is generated by a portion of neuroepithelium that produces most cells fated for the tuberal region. The ARH is different from the PVH, PV and SO in that it includes not only neuropeptidergic neuroendocrine neurons (expressing*Th* and/or *Ghrh*) but also a variety of non-neuroendocrine neurons expressing neuropeptides with important functions. In particular, the ARH harbors orexigenic and anti-orexigenic neurons, benefiting from the current wave of obesity-related research. The regulation of its development has been elucidated to a great extent in the last ten years.

In the tuberal VZ, *Nkx2-1* determines the main general traits of the tuberal region and *Rax* specifies ARH and VMH in general. Downstream these genes, *Ascl1*, *Neurog3* and *Dbx1* specify neuroendocrine (*Ghrh*- or *Th*-expressing) and non-neuroendocrine cell types as well as the number of cells appropriate to each of them. Later, genes like *Dlx1*, *Gsx1*, *Isl1*, *Lrh1* and *SF1* expressed by postmitotic neurons of the mantle layer regulate their final differentiation.

The regulatory processes of neuroendocrine and non-neuroendocrine peptidergic neurons intersect very often and are difficult to disentangle.

Expression of *Nkx2-1* and *Rax* by the VZ specifies the general fate of the tuberal region and its nuclei

Nkx2-1 is essential for the specification of the tuberal region, including the ARH, the VMH and DMH, the median eminence and the entire hypophysis (Kimura et al. 1996; Marin et al. 2002). *Nkx2-1* is also required for the development of the preoptic and mamillary regions, a discussion of which is out of the scope of this paper (Sussel et al. 1999; Takuma et al. 1998).

The anterior hypothalamic region, including the SCH and the major magnocellular nuclei PVH and SO, does not depend on *Nkx2-1* except for the fact that all neuroendocrine neurons send their axons to either the median eminence or the posterior hypophysis, whose development is *Nkx2-1* dependent (Kimura et al. 1996).

The ARH contains many *Th*-expressing and *Ghrh*-expressing neuroendocrine neurons (Markakis and Swanson 1997) as well as many non-neuroendocrine neuropeptidergic neurons with important functions: neurons (expressing *Npy* and *Agrp*), anorexigenic neurons (expressing *Pomc* and *Cartpt*) and *Kiss1*-expressing neurons.

Rax specifies the fate of ARH and VMH progenitors

Rax is a homeobox transcription factor gene expressed by the progenitors of the ARH and the VMH (Lu et al. 2013). Conditional inactivation of *Rax* at different times during early embryonic development (Lu et al. 2013; Miranda-Angulo et al. 2014; Orquera et al. 2016; Salvatierra et al. 2014) shows sequential functions for this regulator, as has been pointed out (Orquera et al. 2016). Starting at E8.5, *Rax* is required for the proliferation and fate specification (including ventralization) of the progenitors of the infundibulum (presumptive posterior hypophysis), the ARH and the VMH. These functions are mediated at least in part by the secreted morphogen protein Shh and the proneural genes *Ascl1* and *Neurog3*, whose expression by the same progenitors is not initiated but strongly promoted by *Rax*. Finally, there is some evidence that membrane proteins involved in the migration or specific adhesion of young neurons fated for the ARH could be downstream *Rax* (Orquera et al. 2016). After promoting progenitor proliferation, *Rax* is necessary for the fate determination of the progenitors that will generate the ARH and VMH (Lu et al. 2013). Finally, Rax expression is also required for proper differentiation and function of hypothalamic tanycytes (Salvatierra et al. 2014), ependymal cells with radial glial characteristics that can generate neurons in the adult.

Expression of *Ascl1*, *Neurog3* and *Dbx1* by VZ progenitors specifies fate and cell number of ARH neuronal subpopulations

Next in the ARH-generating hierarchy are *Ascl1*, *Neurog3* and *Dbx1*, expressed in progenitor cells of the tuberal hypothalamic region (Orquera et al. 2016; Lu et al. 1992; Sokolowski et al. 2016).

Ascl1 and *Neurog3* are proneural genes, that is, bHLH transcription factors responsible for the fate specification of progenitor cells in the VZ (Bertrand et al. 2002). *Ascl1* has the most general function of securing the generation of the right number of ARH cells of all kinds. Both *Ascl1* and *Neurog3* have fate-specification functions at different levels, some broader and other more restricted, on certain subpopulations of neuroendocrine and non-neuroendocrine ARH neurons. Both genes are also required to reach the appropriate number of *SF-1 (Nr5a1)*-expressing neurons in the VMH (non-neuroendocrine) (McNay et al. 2006). Finally, *Dbx1* is a homeobox transcription factor essential for the progenitors to generate the appropriate number of orexigenic neurons (Sokolowski et al. 2015).

Ascl1 regulates the fate of certain endocrine ARH subpopulations as well as general ARH proliferation

Ascl1 acts upstream three other proneural genes: *Neurog3*, *Neurod1* and *Nhlh2*. It is also essential for expression of *Dll1* and *Hes5* and therefore for *Notch*-mediated lateral inhibition (Artavanis-Tsakonas et al. 1999; Aujla et al. 2013) and contributes to the survival of progenitors. One major function of *Ascl1* is to promote proliferation of ARH progenitors, so that the ARH reaches the appropriate number of neurons of all kinds in the right proportions: not all neuronal fates are promoted equally. A prime example is the role of *Ascl1* inhibiting the *Th*-expressing fate, ensuring an appropriate number of *Th*-neurons in the ARH. The regulation of *Th*-expressing neuron development is shared with that of orexigenic and antiorexigenic neurons in ways difficult to unravel.

In the other neuroendocrine ARH subpopulation, the *Ghrh*-expressing cells, *Ascl1* is essential (but not sufficient) for expression of *Gsx1*, which in turn is required for expression of *Ghrh* (see as follows for more about the *Ghrh* regulation).

Ascl1 also regulates some important traits of non-neuroendocrine ARH neurons, like the number of *SF1*-expressing cells of the VMH, the number of orexigenic (*Npy*- and *Cartpt*-expressing) neurons and the expression of *Pomc* in neurons fated to be anorexigenic; see as follows.

Ascl1 and *Neurog3* regulate the fate of orexigenic and antiorexigenic neurons in the ARH

Neurog3 is expressed in a more restricted set of progenitors than *Ascl1*. *Ascl1* is required for the appropriate onset of *Neurog3* expression.

Loss of *Ascl1* results in a severe reduction of *Npy*-expressing neurons. Whereas *Ascl1* is required for neurogenesis of ARH neurons, including the *Npy*-expressing, *Ascl1* (and *Ngn2*) suppresses *Npy* expression. *Ascl1* is required for the initial expression of *Pomc* but not for the specification of *Pomc*-expressing neurons, in agreement with the presence of two independently functioning *Pomc* neural enhancers (de Souza et al. 2005). These data indicate that *Ascl1* is required for neurogenesis of ARH neurons, as well as for early *Pomc* expression but not for the specification of *Pomc* neurons.

Neurog3 works at least in part through activation of the expression of proneural genes *Nhlh2* and *Neurod1* (Pelling et al. 2011). One main function of *Neurog3* is to bias the progenitors towards the production of anorexigenic neurons (those expressing *Pomc* and *Cartpt*) at the expense of *Th*-expressing neuroendocrine neurons as well as orexigenic neurons (those expressing *Npy* and *Agrp*). In addition, *Neurog3* is necessary for the anorexigenic neurons to express *Pomc* but not for them to express *Cartpt*. *Neurog3* has no effect on *Ghrh* neurons of the ARH (Pelling et al. 2011; Anthwal et al. 2013).

Finally, *Ascl1* positively regulates the number of cells expressing *Isl1*, which is essential for the expression of Ghrh as well as Pomc. *Neurog3* is not upstream *Isl1* but works in parallel to maintain *Pomc* expression (Nasif et al. 2015).

Dbx1 specifies the number of orexigenic neurons as well as their terminal differentiation

Dbx1 ensures that the appropriate number of ARH orexigenic neurons (i.e., *Npy*-and-*Agrp*-expressing neurons) is generated (Sokolowski et al. 2015). In addition, it activates *Bsx*, a transcription factor necessary for the expression of *Agrp* and *Npy* (in the ARH) through direct interaction with their regulatory sequences (Lee et al. 2013; Sakkou et al. 2007). The fate of orexigenic neurons is specified in the VZ by *Ascl1* and *Neurog3* but the production of the right number of orexigenic neurons as well as the specific expression of *Npy* and *Agrp* by those neurons (i.e., a trait of their terminal differentiation) depend on *Dbx1*.

Dbx1 is also necessary for *Pmch*, *Hcrt* and *Lhx9* in the LHA, (Sokolowski et al. 2015).

Expression of *Dlx1*, *Gsx1*, *Isl1*, *Lrh1* and *SF1* by postmitotic neurons regulates terminal differentiation of neuronal subpopulations

After *Nkx2-1*, *Rax*, *Ascl1*, *Neurog3* and *Dbx1* have specified the ARH and the fate, number and proportions of its neurons, a number of transcription factors regulate expression of the genes characteristic of the adult phenotype of ARH neurons. The ones we know are those regulating properties easy to detect, like neuropeptide expression.

Dlx1 regulates the number of *Th*-expressing neurons in the ARH

We have seen previously that both proneural genes *Ascl1* and *Neurog3* have a role in the regulation of the number of *Th*-expressing neurons. There is one more known transcription factor with an important role in the number of *Th*-expressing neurons in the ARH and that is the mantle-expressed transcription factor *Dlx1* (Yee et al. 2009).

Gsx1 and *Ikzf1* directly activate *Ghrh* expression in the ARH

Growth hormone releasing hormone (Ghrh) is a parvocellular hypothalamic hormone expressed by a subset of neurons in the ARH. Its function is to activate the secretion of growth hormone (Gh) by specialized cells of the anterior hypophysis called somatotropes. At least five transcriptional regulators have been found that are crucial for the specification of *Ghrh* neurons in the ARH: *Ascl1*, *Hmx2*, *Hmx3*, *Gsx1* and *Ikzf1*.

Gsx1 is a transcription factor expressed in the hypothalamic mantle at E13.5. (Valerius et al. 1995). *Gsx1* positively regulates expression of *Ghrh* (Li et al. 1996) by binding directly to the *Ghrh* promoter (Mutsuga et al. 2001).

Hmx2 and *Hmx3* are homeobox transcription factors expressed in restricted regions of the mantle layer (postmitotic neurons). Their expression pattern is identical in the brain and fully redundant, so that the single mutants do not have an abnormal phenotype in the hypothalamus (Wang et al. 2004). *Hmx2* and *Hmx3* are required to regulate *Ghrh* (and Galanin (*Gal*)) expression in the ARH through activation of *Gsx1* expression (Wang et al. 2004). For this function, *Hmx2* and *Hmx3* act in parallel (not downstream) to *Ascl1* (McNay et al. 2006). Although the majority of *Ghrh* neurons also express *Th*, *Gsx1* is not upstream *Th* (Wang et al. 2004). This means that the expression of both neurohormones is regulated independently during development as well as during the postnatal period (Phelps et al. 2003).

Finally, the transcription factor *Ikzf1* positively regulates expression of *Ghrh* in the ARH by binding *Ghrh* regulatory sequences (Ezzat et al. 2006).

Isl1 specifies the expression of several non-endocrine peptides as well as *Ghrh* in the ARH

Isl1 is expressed in the mantle and is required for the terminal differentiation of four neuronal types in the ARH by activating the genes for the neuropeptides *Agrp*, *Npy*, *Pomc*, *Sst* and *Ghrh*. This represents four neuronal types, of which only one (*Ghrh*) is neuroendocrine. *Sst*-expressing ARH neurons do not send axons to the median eminence (Kawano et al. 1982; Kawano and Daikoku 1988; Markakis and Swanson 1997). A role of *Isl1* in the survival of the early postmitotic neurons is also possible. *Isl1*, however, is not required for the development of *Th*-expressing ARH cells. It is also interesting that, although *Isl1* is required for *Ghrh* expression in the ARH, it does not regulate the expression of *Gsx1* and *Hmx2/3*, which are themselves required for *Ghrh* activation.

Isl1 activates directly the expression of *Agrp* and *Pomc*. In the case of *Agrp*, Isl1 binds other two transcriptional activators, Bsx and Nr3c1 (the glucocorticoid receptor, GR) on *Agrp* regulatory sequences (Nasif et al. 2015; Lee et al. 2016).

Other non-neuroendocrine neurons of the ARH and VMH

Nuclear receptor gene *Nr5a2* (*LRH-1*) is required to activate *Kiss1* expression in the ARH (not elsewhere) through binding to its regulatory sequences (Atkin et al. 2013).

The proliferation of the progenitors of *Nr5a1* (*SF-1*)-expressing neurons of the VMH depends on a dosage-dependent function of *Ascl1* and the fate determination of the same progenitors requires *Ascl1* activation of *Neurog3*. *Nr5a1* (*SF-1*), in turn, is required for the terminal differentiation of the entire VMH (Dellovade et al. 2000; Shinoda et al. 1995; Tran et al. 2003).

Sending axons to fenestrated capillaries: regulation of the neuroendocrine (neurosecretory) phenotype

How do the axons of the magnocellular and parvocellular neurons know to go to the fenestrated capillaries of the median eminence and the posterior hypophysis? First of all, some well-known molecules like Netrin (*Ntn1*) (Deiner and Sretavan 1999) very likely act through a classical Netrin receptor, DCC (Low et al. 2012) are involved in this process. Other proteins involved in axonal guidance and neuronal migration are the semaphorins and their receptors, the neuropilins and plexins (Fiore and Püschel 2003; Püschel 2002). As mentioned previously, PlexinC expression is activated (and the PlexinA's inhibited) by *Sim1* and could contribute to appropriate guidance of magnocellular axons in the median eminence (Xu and Fan 2007).

Some factors have very specific effects on certain neuroendocrine axons leading to adapt the function of the hypophysis to the environment perinatally. The protein insulin-like growth factor I (IGF-1) is able to stimulate axonal outgrowth by *Ghrh*-expressing hypothalamic neurons in the early postnatal mouse; underfed pups show low levels of IGF-1 and less Ghrh axons reach the median eminence (Decourtye et al. 2017).

That morphogens can act as axonal navigation regulators at a later developmental stage has been known for some time, in the spinal cord (Bourikas et al. 2005; Bovolenta 2005; Salinas 2003; Sánchez-Camacho et al. 2005) and in the developing retina (Kahn et al. 2017; Sánchez-Camacho and Bovolenta 2008; Zhao et al. 2012). Shh exerts an influence on axonal guidance through interaction with the Wnt signaling cascade (Zuñiga and Stoeckli 2017).

There is experimental evidence from the chick embryo model that Shh, BMPs and FGFs help neuroendocrine axons to find their way to the hypophysis (Liu et al. 2013; Liu and Placzek 2014).

Another intriguing aspect of the neurosecretory phenotype is the formation of the so-called neurovascular interface, the point where neurosecretory axon terminals contact fenestrated capillaries. It has been shown in zebrafish that Oxt "leaked" from developing magnocellular axons as they proceed towards the posterior hypophysis can influence the endothelial cells of embryonic blood vessels to generate new buds that grow in their direction (Gutnick et al. 2011).

Effector genes

Developmental transcription factors exert their phenotypic effects by activating effector genes whose products directly affect the behavior of the developing cell. Those can be proteins involved in cell movement, cell adhesion, axonal outgrowth and pathfinding and synaptogenesis. We know considerably less about these than about the upstream transcriptional regulators.

The Notch pathway

Notch receptors expressed by progenitor cells are able to bind specific membrane-bound ligands from neighboring cells; in this way becoming activated, translocating to the nucleus and activating expression of specific genes involved in proliferation and fate determination (Artavanis-Tsakonas et al. 1999; Bhat 2014; Olave et al. 1998; Shimizu et al. 2000; Struhl and Adachi 1998). The Notch pathway is active in the hypothalamic VZ and is involved in the general proliferation of ARH progenitors as well as in fate determination (Aujla et al. 2013, 2015; Biehl and Raetzman 2015), reviewed in Biehl and Raetzman (2017). RBPJ-κ, an essential transcriptional cofactor of *Notch* (Nam et al. 2003), plays a role in *Notch*-dependent repression of *Ascl1* by ARH progenitors. This results in appropriate numbers of *Pomc*-, *Npy*-expressing and *Kiss1*-expressing neurons in the ARH (unchecked *Ascl1* function would favor *Pomc* and *Npy* fates at the expense of the *Kiss1* fate) (Aujla et al. 2013).

Proteins involved in migration and axonal extension

Plxnc1 (acting downstream *Sim1*) and *Ntn1* have been mentioned previously. *Slit2*, an extracellular matrix protein with chemorepulsive properties that regulates axon guidance at the midline (crossing vs not crossing, commissure formation) (Chédotal 2007). *Slit2* expression in the hypothalamus is missing in *Nkx2-1* mutants (Marin et al. 2002). Another chemorepellent protein involved in axonal guidance, *semaphorin 3A* (*sema3a*), is decreased in *Nkx2-1* mutants (Kawano et al. 2003).

In *Rax* mouse mutants, some phenotypical characteristics suggest a lack of appropriate expression of cell adhesion molecules (Lu et al. 2013; Orquera et al. 2016) suggesting that this transcription factor regulates specific aggregation of certain neurons.

Principles of neuropeptidergic development in the hypothalamus

On the basis of the available data on hypothalamic neuroendocrine development, few generalizations are possible. Mouse mutant analysis reveals a puzzling heterogeneity in the neuroendocrine nuclei and lineages. This suggests that the GRNs

and other mechanisms regulating the generation of specific neuropeptidergic neurons (neuroendocrine or non-neuroendocrine) have evolved to a large degree independently and at different times. A few principles can however be gleaned, some of them relative to progenitor domains and some to the organization of GRNs.

Progenitor domains

One very intriguing novel insight refers to the strategy of different and often spatially separate progenitor domains generating specific neuroendocrine subpopulations that meet each other through complex tangential migration routes to form the heterogeneous neurosecretory nuclei (Diaz et al. 2014; Morales-Delgado et al. 2011, 2014). The most striking example of such migration is provided by the *Gnrh* neurons migrating from the nasal placode along the vomeronasal nerve (Schwarting et al. 2007). The tangential migrations postulated for other neuroendocrine populations are shorter and less clear and could be partially due to a simple change in position due to the general growth of the neural tube. The principle, however, is undeniable, that the hypothalamic VZ can be seen as a patchwork of combinations of gene expression delimiting diverse progenitor domains and that some of these generate specific neuropeptidergic subpopulations that later change position and form in many cases neuronal aggregates of heterogeneous origin (Diaz et al. 2014). Such progenitor domains express some major transcription factors whose mutation intriguingly causes differential phenotypes, leading to reflexion about how the neuroepithelium itself acquires diversity.

Different progenitor domains (Fig. 2)

The simplest case is represented by two different progenitor domains generating neurons with the same neuropeptide but only one of them generates the neurosecretory version (Fig. 2a, b). This could be attributed to accidental convergence of regulatory pathways onto one neuropeptide gene. In Fig. 2(a),

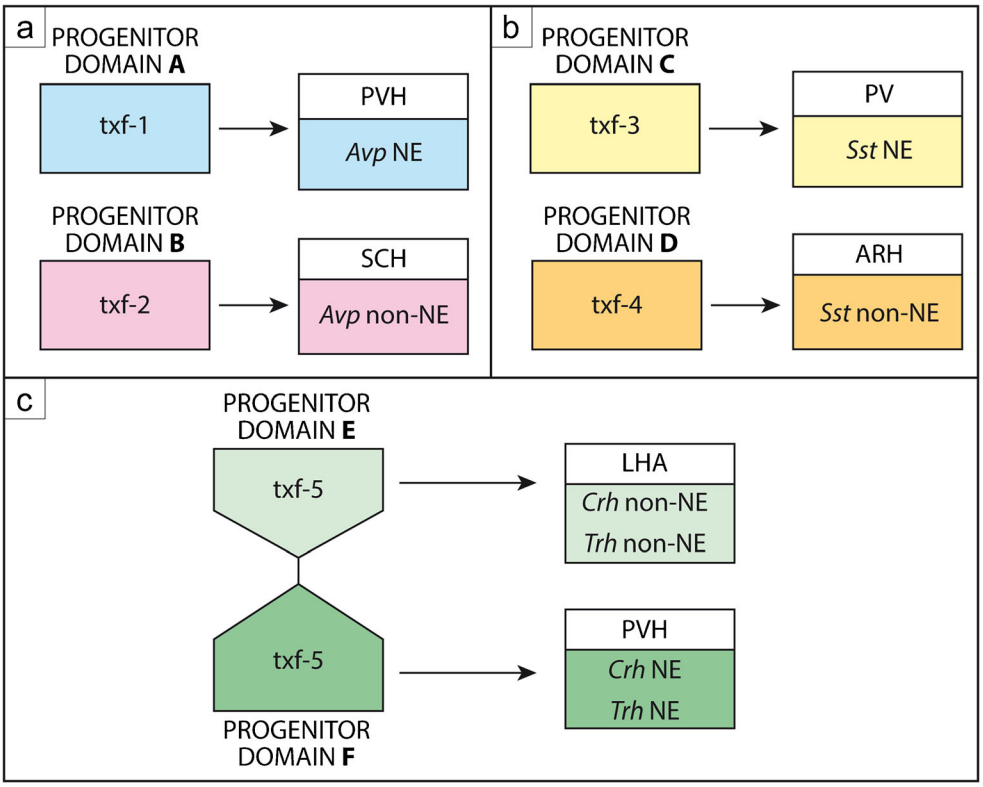

Fig. 2 Some hypotheses about progenitor domains (I). **a**, **b** Completely different progenitor domains expressing different transcription factors generate either neuroendocrine or non-neuroendocrine versions of neurons expressing a classical neurohormone. The regulators are different and the downstream neuropeptide is the same by accident, not by evolution. Eliminating the main regulator (targeted mutation) would cancel expression of the neuropeptide in one region, not the other. In **a**, two different progenitor domains generate either the neurosecretory *Avp* neurons of the PVH or the non-neurosecretory *Avp* of the SCH. In **b**, two different progenitor domains generate the neurosecretory *Sst* neurons of the PV or the non-neurosecretory *Sst* neurons of the ARH. **c** A phylogenetically older progenitor domain E, under the control of transcription factor txf-5, generates neuropeptidergic, non-neuroendocrine neurons (here the *Crh* and the *Trh* neurons of the LHA). During evolution, a second progenitor domain F evolves from E and separates, conserving *txf-5* as a major regulator but acquiring others that confer the neuroendocrine phenotype (here the *Crh* and the *Trh* neuroendocrine neurons of the PVH). Elimination of *txf-5* cancels expression of *Crh* and *Trh* in both regions

the example of *Avp* cells that are neurosecretory in the PVH but not in the SCH. The ones residing in the PVH are affected by mutation of either *Otp*, *Sim1*, or *Pou3f2* (*Brn2*), while the SCH ones are not (Acampora et al. 1999; Wang and Lufkin 2000; Schonemann et al. 1995). In Fig. 2(b), a similar example is shown, relative to two kinds of *Sst*-expressing neurons, neurosecretory in the PV and non-neurosecretory in the ARH (Kawano et al. 1982; Kawano and Daikoku 1988). As shown previously for *Avp*-expressing cells, both *Sst*-expressing populations are differentially affected by the mutation of upstream regulators. Moreover, in this case, we know that the two subpopulations originate in two different progenitor domains (Daikoku et al. 1983; Morales-Delgado et al. 2011).

The opposite case is shown in Fig. 2(c), where the same transcriptional regulator can be used to activate expression of one certain neuropeptide in neuroendocrine and non-neuroendocrine cells, as is the case for *Crh* and *Trh*, which require expression of *Otp* in neuroendocrine (PVH) and non-neuroendocrine (LHA) neurons and are equally affected by *Otp* mutation (Acampora et al. 1999; Wang and Lufkin 2000). This suggests that progenitor domains may be phylogenetically derived from each other and later lose some potentiality down the GRN.

Finally, neurons expressing the same non-neuroendocrine neuropeptide can be born under different regulation, in two progenitor domains distant from each other and settle in different hypothalamic nuclei. *Kiss1*-expressing neurons in the ARH and in the PV offer a good example, since they originate in separate domains and differentiate through unique lineages and mechanisms (reviewed in Biehl and Raetzman 2017).

Does one progenitor domain generate magnocellular and parvocellular neurons? (Fig. 3a)

The *Pou3f2* (*Brn2*) null mutant phenotype, where magnocellular (either *Avp*- or *Oxt*-expressing) as well as one kind of parvocellular (*Crh*-expressing) neurons are missing from the PVH and SO (Nakai et al. 1995; Schonemann et al. 1995), shows that magnocellular GRNs are not strictly separated from parvocellular GRNs. Can progenitors in the VZ sequentially generate different cell types? Or is each different cell type generated by one kind of progenitor? The *Pou3f2* (*Brn2*)-expressing cells would then originate in three different contiguous "micro-progenitor domains" of the VZ formed by progenitors fate specified by very similar GRNs and may be gradually detached from a phylogenetically ancient progenitor domain able to produce only one kind of cell. The hypothesis of contiguous very restricted progenitor domains can be applied elsewhere in the hypothalamus. For instance, one relatively restricted neuroepithelial region generates MCH-expressing neurons with a certain axonal projection pattern on E11.5 and again MCH-expressing neurons with completely different projections on E12.5 (Brischoux et al. 2002).

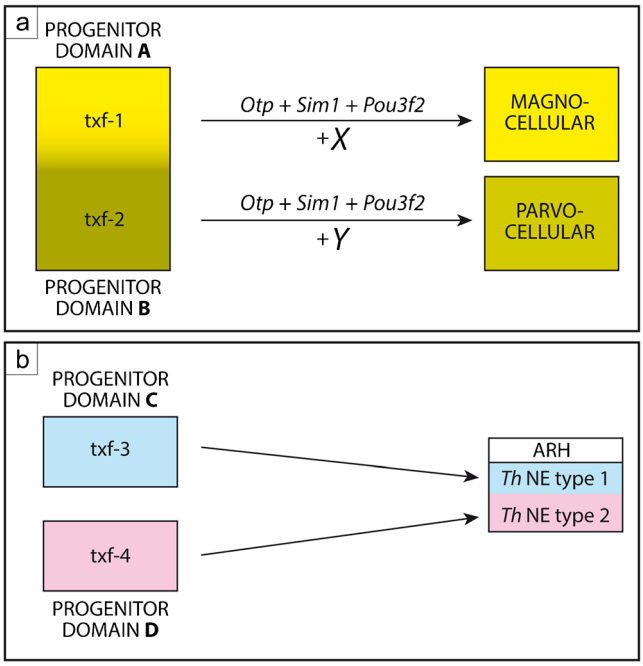

Fig. 3 Some hypotheses about progenitor domains (II). **a** Very restricted, contiguous progenitor domains expressing several common plus some domain-specific regulators conferring specific traits to the neuronal progeny. **b** Convergence: parvocellular neurons expressing the same neuropeptide originate in different domains under different regulators but migrate to converge in one specific hypothalamic nucleus. It should be possible to mutate one of the regulators and observe the corresponding abnormal phenotype on one of the subpopulations not the other

Convergent tangential migration from diverse progenitor domains (Fig. 3b)

Here, the example is the heterogeneous *Th*-expressing neuronal population of the ARH, where some *Th*-expressing neurons coexpress *Ghrh* and some *Th*-expressing neurons coexpress the dopamine transporter DAT (*Slc6a3*) (Meister et al. 1986; Phelps et al. 2003; Yip et al. 2017) and neurons expressing *Ghrh* and/or *Th* seem to migrate from at least two progenitor domains, one of them coincident with the presumptive ARH (Diaz et al. 2014; Morales-Delgado et al. 2014).

Developmental GRNs

Some of the heterogeneity and complexity shown by the genetic regulation of neuroendocrine development could be explained by the general principles ruling the workings of developmental GRNs (Davidson et al. 2002).

First of all, the temporal factor. The arrow of time is embedded into the developmental GRNs, which proceed "inexorably forward in developmental time" (Davidson et al. 2002), thanks to genes that either repress their own expression directly or indirectly or cause some previous target genes to become inaccessible to activation. Spatio-temporal, CreER-based conditional inactivation of key regulators reveals the

gradual progress of developmental GRNs. A case in point is the conditional inactivation of *Rax* (already commented here) that makes it possible to follow *Rax* functions sequentially (Orquera et al. 2016).

Another GRN property that contributes to complexity is the redeployment or using the same transcription factor in different branches of one GRN and in different GRNs. It is precisely the time-dependent property (see previously) that allows for redeployment. If, as the developmental GRN progresses, previous targets become impervious to activation, then particularly efficient regulator proteins can be used again to activate new targets. For instance, *Nkx2-1* is essential during hypothalamic development and, after birth, it acquires new targets with important hypothalamic functions (Lee et al. 2001; Son et al. 2003). *Dbx1* is expressed upstream *Bsx* in the hierarchy that specifies orexigenic neurons (*Npy* + *Agrp* expressing) of the ARH but *Dbx1* is also necessary for expression of *Pmch*, *Hcrt* and *Lhx9* in the LHA. *Isl1*, which activates *Ghrh* expression in ARH neuroendocrine cells through direct interaction with its regulatory sequences, is used by other cell types to activate expression of *Pomc* (anorexigenic neurons), *Agrp* and *Npy* (orexigenic neurons) and *Sst* (in ARH non-neuroendocrine neurons).

Finally, another interesting conclusion is the importance of the interaction of the GRNs with the cellular environment. The GRNs elicit from the environment responses that affect their function going forward. A good example is the inhibition of *Ascl1* function by the *Notch* pathway, in order to generate the right numbers of *Kiss1*-expressing neurons in the ARH (Aujla et al. 2013). Another example is the positive influence of brain-derived neurotrophic factor (BDNF) on the expression of *Trh* in the developing PVH (Ubieta et al. 2007; Wang et al. 2016).

Abbreviations 3V, third ventricle; ac, anterior commissure; *Agrp*, agouti related neuropeptide; AHA, anterior hypothalamic area; ANT, anterior region of the hypothalamus; ARH, nucleus arcuatus of the hypothalamus; Avp, arginine vasopressin; *Cartpt*, CART (cocaine- and amphetamine-regulated transcript protein) prepropeptide; CNS, central nervous system; *Crh*, corticotropin releasing hormone; DMH, dorsomedial nucleus of the hypothalamus; *Ghrh*, growth hormone releasing hormone; *Gnrh*, gonadotropin releasing hormone; GRN, genomic regulatory network; hp., hypophysis; *Kiss1*, kisspeptin (KiSS-1 metastasis-suppressor); LHA, lateral hypothalamic area; MAM, mamillary region of the hypothalamus; MBO, mamillary body; MCH, melanin-concentrating hormone; ME, median eminence; α-MSH, alpha-melanocyte-stimulating hormone; NE, neuroendocrine (neurosecretory); non-NE, non-neuroendocrine (non-neurosecretory); Npy, neuropeptide Y; *Oxt*, oxytocin; PMA, premamillary area; POA, preoptic region of the hypothalamus; PTh, prethalamus; PV, periventricular nucleus; PVH, paraventricular nucleus of the hypothalamus; SCH, suprachiasmatic nucleus; SO, supraoptic nucleus; *Sst*, somatostatin; *Th*, tyrosine hydroxylase; *Trh*, thyrotropin releasing hormone; TUB, tuberal region of the hypothalamus; txf, transcription factor; VMH, ventromedial nucleus of the hypothalamus; VZ, ventricular zone; ZLI, zona limitans intrathalamica

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