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The Transcriptional Control of Female Puberty

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Abstract

The initiation of mammalian puberty requires a sustained increase in pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus. This increase is brought about by coordinated changes in transsynaptic and glial-neuronal communication, consisting of an increase in neuronal and glial stimulatory inputs to the GnRH neuronal network and the loss of transsynaptic inhibitory influences. GnRH secretion is stimulated by transsynaptic inputs provided by excitatory amino acids (glutamate) and at least one peptide (kisspeptin), and by glial inputs provided by growth factors and small bioactive molecules. The inhibitory input to GnRH neurons is mostly transsynaptic and provided by GABAergic and opiateergic neurons; however, GABA has also been shown to directly excite GnRH neurons. There are many genes involved in the control of these cellular networks, and hence in the control of the pubertal process as a whole. Our laboratory has proposed the concept that these genes are arranged in overlapping networks internally organized in a hierarchical fashion. According to this concept, the highest level of intra-network control is provided by transcriptional regulators that, by directing expression of key subordinate genes, impose genetic coordination to the neuronal and glial subsets involved in initiating the pubertal process. More recently, we have begun to explore the concept that a more dynamic and encompassing level of integrative coordination is provided by epigenetic mechanisms.

Keywords

hypothalamus; GnRH neurons; epigenetics; glial-neuronal communication; transcriptional regulation; timing of puberty

1. Introduction

There is little doubt that different regulatory systems contribute to controlling the pubertal process in mammals. The key, decisive, event required for puberty to occur is an increase in pulsatile gonadotrophin-releasing hormone (GnRH) release from GnRH neurons. While in primates these neurosecretory cells are mostly located in the medial basal hypothalamus, they reside exclusively in the preoptic area of rodents (Ojeda and Skinner, 2006; Plant and Witchel, 2006). It is also clear that the pubertal increase in GnRH secretion is initiated and maintained by changes in transsynaptic (Kordon et al., 1994; Ojeda and Terasawa, 2002) and

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glial (Ojeda et al., 2003; Ojeda and Terasawa, 2002) inputs to the GnRH neuronal network. The transsynaptic changes consist of a coordinated increase in excitatory inputs and a reduction in inhibitory influences (Ojeda and Terasawa, 2002; Plant and Witchel, 2006; Terasawa and Fernandez, 2001); the glial component of the system is mostly facilitatory, and provided by growth factors and small diffusible molecules that directly or indirectly stimulate GnRH secretion (Ojeda et al., 2003; Ojeda and Skinner, 2006).

In recent years, new components of each of these regulatory systems have been identified. Thus, in addition to glutamatergic neurons (Brann, 1995; Ojeda and Skinner, 2006; Plant and Witchel, 2006), which are widely distributed throughout the basal forebrain, the hypothalamus contains two discrete subsets of neurons that produce the peptide kisspeptin (also termed metastin), a powerful stimulator of GnRH release (Kauffman et al., 2007; Oakley et al., 2009; Shahab et al., 2005). In rodents, one group of kisspeptin neurons is located in the periventricular region of the anteroventral periventricular nucleus (AVPV), and the other in the periventricular region of the arcuate nucleus (ARC) (Clarkson et al., 2009). Kisspeptin neurons of the ARC produce two additional peptides: neurokinin B (TAC3) (Navarro et al., 2009), which is encoded by a gene recently shown to be required for puberty to occur (Topaloglu et al., 2008), and dynorphin, an opioid peptide that inhibits GnRH secretion (Kinoshita et al., 1982; Navarro et al., 2009; Schulz et al., 1981). Altogether, these observations indicate that the excitatory transsynaptic regulation of GnRH secretion is provided by neurons that use glutamate, kisspeptin (Dungan et al., 2006; Ojeda and Skinner, 2006), and perhaps neurokinin B (Topaloglu et al., 2008), for transsynaptic communication.

With regard to the inhibitory transsynaptic circuitry controlling GnRH release, it appears clear that GABAergic and opiateergic neurons are central players [reviewed in (Terasawa and Fernandez, 2001)]. However, a rapidly accumulating body of evidence is beginning to strengthen the concept that a product of the RFamide-related peptide gene (*RFRP*), which is the mammalian ortholog of the peptide gonadotrophin-inhibiting hormone (GnIH) in birds (Ebling and Luckman, 2008), is a physiological inhibitor of GnRH neurons in mammals (Ducret et al., 2009; Gibson et al., 2008; Tsutsui et al., 2010). These observations suggest that RFRP-containing neurons provide an additional, and perhaps important, transsynaptic mode of inhibitory control to GnRH neurons during reproductive maturation. Of these three inhibitory systems, the RFamide-component seems to be the simplest, because it is composed of one or two peptides (RFRP1 and RFRP3) and a single receptor termed GPR147 (Hinuma et al., 2000; Tsutsui et al., 2010), which appears to be expressed in GnRH neurons (Ducret et al., 2009). The GABA controlling system is endowed with a higher level of complexity, because GABA can inhibit GnRH secretion indirectly, via effects exerted on neurons connected to the GnRH neuronal network (Ojeda and Skinner, 2006; Terasawa and Fernandez, 2001), but can also stimulate GnRH neurons directly through activation of GABA_A receptors (DeFazio et al., 2002; Moenter and DeFazio, 2005). The opioid inhibitory system is even more complex, because opiateergic neurons employ different peptides and several receptors to inhibit GnRH release (Kordon et al., 1994); as in the case of GABAergic inputs, opiateergic inhibition may be exerted directly on GnRH neurons (Dudas and Merchenthaler, 2006) or indirectly on neurons involved in the stimulatory control of the GnRH neuronal network, such as kisspeptin neurons (Navarro et al., 2009).

Other studies have shown that, in addition to transsynaptic inputs, the pubertal activation of GnRH secretion also requires information from glial cells (Ojeda et al., 2000; Ojeda and Terasawa, 2002). Both astrocytes and ependymoglia cells lining the ventral surface of the third ventricle (called tanycytes) produce cell-cell signalling molecules that stimulate GnRH release, and that are necessary for the timing of puberty [reviewed in (Lomniczi and Ojeda, 2009)]. Glial cells contribute to the pubertal activation of GnRH secretion via two complementary mechanisms. One of them involves growth factors of at least four different

families. Transforming growth factor-beta (TGF β), of the TGF β superfamily, is recognized by cell-membrane receptors endowed with serine-threonine kinase activity and that are located on GnRH neurons (Prevot et al., 2000). Upon binding, TGF β enhances GnRH gene expression and GnRH secretion (reviewed in (Mahesh et al., 2006;Prevot, 2002)). Growth factors of the other three families, including the epidermal growth factor (EGF) family, basic fibroblast growth factor (bFGF), and insulin-like growth factor 1 (IGF-I), are recognized by receptors with tyrosine kinase activity. Some of these receptors (FGFR, IGF-1R) are expressed in GnRH neurons, but erbB receptors (which recognize EGF and EGF-like peptides) are mostly expressed on glial cells themselves. Genetic disruption of erbB receptors delays female sexual development due, at least in part, to impaired erbB ligand-induced glial prostaglandin E₂ (PGE₂) release (Lomniczi and Ojeda, 2009). While growth factors of glial origin set in motion glia-to-neuron signaling pathways, at least one neuron-to-glia regulatory pathway initiated by glutamatergic neurons, has been shown to facilitate astrocytic signaling mediated by erbB receptors (Dziedzic et al., 2003).

The second mechanism of glia-to-GnRH neuron communication involves plastic rearrangement in cell adhesiveness. The adhesion of glial cells to GnRH neurons appears to require at least three different cell-cell communications systems. One is thought to be provided by the sialylated form of the neural cell adhesion molecule NCAM (PSA-NCAM) (Parkash and Kaur, 2005;Perera et al., 1993). PSA-NCAM is abundant in brain regions endowed with a high degree of postnatal plasticity (Gascon et al., 2007), such as the medial basal hypothalamus-median eminence (ME) region (Perera et al., 1993). PSA-NCAM is especially abundant in GnRH nerve terminals and glial cells of the ME (Parkash and Kaur, 2005), suggesting an involvement in glia-GnRH nerve terminal adhesiveness. Another adhesive system employs Synaptic Cell Adhesion Molecule 1 (SynCAM1) (Ojeda et al., 2008), and the other is based on the interaction of neuronal contactin with glial Receptor-like Protein Tyrosine Phosphatase- β (RPTP β) (Parent et al., 2007). Because in all three cases the participating proteins contain intracellular domains with signaling capabilities, it is likely that the interaction of glial cells with GnRH neurons may not only involve secreted bioactive molecules, but also the activation of cell-cell signaling mechanisms set in motion by these and, likely other, adhesive molecules (reviewed in (Lomniczi and Ojeda, 2009).

2. The neuroendocrine control of puberty involves many genes with different functions

Several studies have suggested that no isolated pathway or cellular subset is solely responsible for the neuroendocrine control of puberty (Eaves et al., 2004;Gajdos et al., 2008;Krewson et al., 2004;Ojeda et al., 2006;Seminara and Crowley, Jr., 2001). Instead, the initiation of puberty may be controlled by regulatory gene networks composed of multiple functional modules (Ojeda et al., 2006). For instance, mutations affecting different genes, including *GNRHR* (Bedecarrats and Kaiser, 2007), *GPR54* (de Roux et al., 2003;Seminara et al., 2003), *KISS1* (Lapatto et al., 2007), *TAC3* and *TACR3* (Topaloglu et al., 2008), result in pubertal failure. Recent genome-wide association studies demonstrated an association of a sequence variation in *LIN28B* (a gene encoding an RNA-binding protein) with the age at menarche (He et al., 2009;Ong et al., 2009;Perry et al., 2009;Sulem et al., 2009), and showed that variants of at least 10 other genes are also associated with early menarche (Perry et al., 2009;Sulem et al., 2009). Very recently, it was reported that – complementing the observations in humans - mice overexpressing *Lin28a* have delayed puberty (Zhu et al., 2010). Notwithstanding the importance of this finding, it is currently unknown if *Lin28a* overexpression delays puberty due to a general effect on body metabolism or via a mechanism involving an alteration in GnRH secretion. Studies from our laboratory identified and functionally characterized several other components of the regulatory system controlling the onset of female puberty, including novel molecules required for glutamate

release (Choi et al., 2008; Ha et al., 2008), homeostatic maintenance of GnRH neuron excitability (Garcia-Rudaz et al., 2008), unidirectional glia-to-GnRH neuron signaling (Lomniczi et al., 2006), and glia-GnRH neuron adhesive communication (Parent et al., 2007; Sandau et al., 2009). We also identified transcriptional regulators of the pubertal process, including the POU-domain gene *Oct2* (Ojeda et al., 1999), the homeodomain gene *Ttf1/Nkx2.1* (Mastronardi et al., 2006), and a novel gene provisionally termed chromosome 14 open reading frame 4 (C14ORF4) (Rampazzo et al., 2000), which we termed *Eap1* (Enhanced At Puberty1) (Heger et al., 2007). Based on a variety of experimental approaches, including the use of antisense oligodeoxynucleotides (Ojeda et al., 1999), Cre-loxP-mediated, neuron-specific conditional gene deletion (Mastronardi et al., 2006) and siRNA-mediated region-specific knock-down of gene expression (Heger et al., 2007) we proposed that these genes occupy a central position in the hierarchical arrangement of networks controlling the pubertal process (Ojeda et al., 2006). Although in most of these studies the endpoints were somatic manifestations of puberty, such as vaginal opening and age at first ovulation, the inference was that GnRH secretion had been affected by manipulating the expression of these genes selectively in the hypothalamus.

3. Genes controlling puberty are organized in functional networks

From the above considerations it may be concluded that there are genes required for the acquisition of reproductive function, such as *GPR54*, *Kiss1* and *TAC3*, and genes that contribute to defining the correct timing of the pubertal process, such as those encoding molecules involved in the flow of glia-neuron communication, transsynaptic information, glia-GnRH neuron adhesive behavior, and intracellular signaling. Others, such as *OCT2*, *TTF1/NKX2.1*, *EAP1*, and *LIN28B* appear to be involved in the transcriptional control of puberty. As such, they are expected to control the expression of subordinate genes that are necessary for the neuron-to neuron and glia-to neuron regulation of GnRH secretion at puberty [reviewed in (Ojeda et al., 2006; Ojeda et al., 2010)].

Using a high-throughput approach (DNA arrays) we provided evidence for the existence of an additional gene network that, according to our results, contributes to the hypothalamic control of puberty (Roth et al., 2007). Although the genes composing this network have diverse cellular functions, they share the common feature of being first identified as involved in tumor suppression/tumor formation (Roth et al., 2007). Because of this, we termed the network “TSG” (for Tumor Suppressor Gene) network. Quantitative PCR studies verified the array results (Parent et al., 2008; Roth et al., 2007) and in silico analysis of transcription factor recognition sites present in gene promoters indicated that *Kiss1*, *Gpr54*, and *SynCAM1*, are subordinate genes of the network. Interestingly, before the role of kisspeptin in the control of puberty was discovered, the *Kiss1* gene was known as a suppressor of tumor metastases (Ohtaki et al., 2001; Steeg et al., 2003).

Cis-regulatory analysis of shared TSG binding sites predicted the existence of five central hubs (*Cdp/Cutl1*, *Maf*, *p53*, *Yy1*, and *Usf2*) controlling the TSG network at the transcriptional level (Roth et al., 2007). This analysis involves the computer-assisted inspection of the 5'flanking region of the genes of interest for predicted transcription factor binding sites using databases such as TransFac (Matys et al., 2003; Wingender et al., 1996) and P-MATCH (<http://www.gene-regulation.com/pub/programs.html>), the establishment of directional links among genes encoding transcription factors to all genes containing a binding site for those transcription factors, and the visualization of these interactions using the on-line tool CytoScape (<http://www.expasy.org>). Several hubs were identified and found to be connected to both subordinate genes encoding proteins required for intracellular signaling and cell-cell communication, and to other presumptive non-TSG upper-echelon genes (*Oct2*, *Ttf1*, and *Eap1*) involved in the transcriptional regulation of the pubertal

process. Immunohistochemistry and *in situ* hybridization analysis demonstrated that these three genes, as well as other subordinate nodes of the network, are expressed in neuronal and/or glial subsets involved in the control of GnRH secretion, including GnRH neurons themselves (Heger et al., 2007; Mastronardi et al., 2006; Ojeda et al., 2008; Roth et al., 2007). As it now stands, the current model of this TSG network is imperfect and needs to be fully validated by functional studies. Such studies are necessary to not only verify *in silico* predictions, but also to provide a more accurate and comprehensive architecture of the network. Also in need of definition is the identity of the neuronal and glial subsets expressing each putative central hub, and the possibility that individual central hubs have different, cell-specific, puberty-related developmental patterns of expression. Following this line of reasoning, we have now obtained evidence that the *Kiss1* gene is not only controlled by TTF1 and *Eap1*, two non-TSG transcriptional regulators (Heger et al., 2007; Mastronardi et al., 2006), but also by CUTL1 and YY1 (Mueller, Dietzel, Tefs, Kiess, Danne, Loche, Lomniczi, Ojeda and Heger, in preparation), two putative central hubs of the TSG network.

It is likely that the TSG network is just one of several sub-networks involved in the control of puberty. As indicated above, *Oct2*, *Ttf1*, and *Eap1* may form part of another, functionally connected network, because they do not behave as TSGs. This notion is supported by the absence of a discernible pattern of *EAP1* under-expression in a panel of human tumors (Heger and Ojeda, unpublished). *Lin28b*, on the other hand, may be a hub of the TSG network, because of its role in puberty and its well-established contribution to cancer biology (Viswanathan et al., 2009). Based on both *in silico* models and experimental data, we envision a controlling system in which transcriptional regulators are shared by different neuronal and glial subsets, with sets of subordinate genes specifically expressed in particular cellular subsets.

It is tempting to speculate that these transcriptional control systems extend to genes encoding enzymes involved in processing precursors of puberty-related peptides, such as kisspeptin, dynorphin, RFRP1/3, etc. Likewise, little is known about the transcriptional machinery operating in hypothalamic astrocytes to control the production of growth factors (e.g. TGF α , neuregulins), and small molecules (e.g., prostaglandin E₂, glutamate, ATP) involved in stimulating GnRH release. Of all of these factors, only TGF α expression has been shown to be controlled by a gene (*Oct2*) involved in the transcriptional regulation of puberty.

A strategy that can be used to assess the involvement of transcription factors in the control of the pubertal process is the use of gain-of-function or loss-of-function approaches (Davidson et al., 2002; Ideker et al., 2001). We have employed gain-of-function approaches to define the involvement of astroglial cells (Ma et al., 1994; Prevot et al., 2003; Rage et al., 1997; Sandau et al., 2009) and specific neuronal subsets (Bilger et al., 2001; Heger et al., 2003) in the hypothalamic control of puberty, and loss-of-function strategies to identify three upstream transcriptional regulators of the pubertal process (Heger et al., 2007; Mastronardi et al., 2006; Ojeda et al., 1999), and four subordinate genes involved in neuron-neuron communication (Choi et al., 2008; Garcia-Rudaz et al., 2008; Ha et al., 2008; Sandau et al., 2009). Because lentiviruses (Tiscornia et al., 2003) can efficiently deliver small interfering (si)RNAs to tissues or cells of interest (Garcia-Rudaz et al., 2007; Heger et al., 2007), we have used this delivery system to determine if decreasing the production of *EAP1* would alter the onset of female puberty and adult reproductive cyclicity (Heger et al., 2007). We observed that *Eap1* siRNA-producing lentiviral particles injected bilaterally into the preoptic area (POA) of juvenile 23-day-old rats results in delayed puberty, disrupted estrous cyclicity, reduced plasma LH, FSH and estradiol levels, and delayed growth of ovarian follicles. We conducted additional experiments to determine if *EAP1* is also important for the hypothalamic control of menstrual cyclicity in nonhuman

primates (Dissen et al., 2009), and found that knocking down *EAP1* expression in the arcuate nucleus (ARC) of the hypothalamus abolished menstrual cyclicity (Dissen, Lomniczi, Heger, and Ojeda, in preparation). These results indicate that *EAP1* is crucial for the ARC to maintain menstrual cyclicity in higher primates. Because *EAP1* has an intrinsic repressive activity (Heger et al., 2007), *EAP1* knock-down may delay puberty and prevent reproductive cyclicity by relieving an *EAP1* repressive influence on inhibitory systems controlling GnRH output. These inhibitory systems may be inherent to the transsynaptic control of GnRH neurons (e.g., opioid peptides, GABA, GnIH) or – as detailed below – involve more encompassing regulatory systems, able to impose a broader level of transcriptional repression to the gene networks controlling the pubertal process.

4. The transcriptional repression of puberty

Though not yet proven, emerging evidence is beginning to surface suggesting that puberty may not occur earlier because it is held in check by a developmental program involving transcriptional/posttranscriptional repression of genes that are stimulatory to the pubertal process. For instance, nucleotide polymorphisms near the *LIN28B* gene in chromosome 6(q21) are associated with earlier puberty and shorter stature in girls (Ong et al., 2009; Perry et al., 2009; Sulem et al., 2009). *LIN28B* and its homolog *LIN28* (located on a different chromosome) encode RNA-binding proteins that control gene expression via posttranscriptional regulation (Moss et al., 1997). However, only sequence variation in *LIN28B* is associated with early menarche (Ong et al., 2009). Increasing *Lin28b* expression in *C. Elegans* (Moss et al., 1997) and *Lin28a* in mice (Zhu et al., 2010) leads to developmental delay. *Lin28b* exerts this effect by preventing the early expression of genes, which should normally be activated at a subsequent phase of development (Ambros and Horvitz, 1984; Moss et al., 1997). Mammalian *LIN28A* and *B* control cellular function by blocking the maturation of *let-7* miRNA precursors into mature miRNAs. An excess of *LIN28B* has been shown to reduce production of this miRNA family leading to derepression of *let-7* miRNA target genes (Viswanathan et al., 2009). Because some of these targets are oncogenic (for instance *K-Ras* and *c-Myc*), *let-7* miRNAs are considered to be TSGs (Viswanathan et al., 2009). Considering that *Lin28a* overexpression in mice results in delayed puberty (Zhu et al., 2010), both *Lin28* and *let-7* miRNAs can be considered as repressive components of the TSG network controlling the onset of puberty. As such, *Lin28a* and/or *Lin28b* expression would be expected to decline in the hypothalamus at the time of puberty, resulting in derepression of *let-7* miRNA maturation. A reduction in *Lin28b* mRNA abundance does, in fact, occur both in the agonadal monkey hypothalamus at the expected time of puberty (Matagne, Ojeda and Plant, unpublished), and in ovary-intact female rats during prepubertal development (Tena-Sempene, Ojeda et al, unpublished observations). In this latter study, we also observed that *let-7* miRNA levels increase in the hypothalamus as *Lin28b* mRNA abundance decreases.

It is likely that there are other potential mechanisms of transcriptional/posttranscriptional repression that may be involved in controlling the timing of puberty. For instance, we recently observed that expression of a family of genes encoding Zinc-finger (ZNF)-containing proteins changes in the hypothalamus of castrated male nonhuman primate at the expected time of puberty (Matagne et al., 2009). The expression profiles of these genes was inversely correlated to the prepubertal changes in LH output, i.e., it was elevated during the late juvenile period when GnRH secretion is turned off, and low as GnRH neuronal activity increases at the initiation of puberty. ZNF genes encode proteins that function as transcriptional repressors (Urrutia, 2003; Vogel et al., 2006). In other ongoing high-throughput studies, we identified two families of transcriptional repressors expressed in the pre and peripubertal female rat hypothalamus (Lomniczi et al., 2010). Intriguingly, expression of genes belonging to one of these families decreases at puberty, while that of

members from the other family increases. Family 1 consists of genes of the POZ-ZF (poxvirus and Zinc finger) family of transcriptional regulators, also known as BTB (broad complex, Tramtrack, bric-à-brac) (Kelly and Daniel, 2006). POZ-ZF genes are transcriptional repressors implicated in the control of a variety of developmental processes; because alterations of their function result in tumorigenesis and developmental disorders, many of them are considered to be TSGs (Kelly and Daniel, 2006). We observed that expression of a subset of these genes, known as the POK (POZ and Krüppel) subfamily, *increases* in the hypothalamus of female rats at puberty, suggesting that POK proteins may be repressing downstream repressors of the pubertal process. Family 2 consists of genes of the Polycomb group (PcG). The PcG silencing complex is considered as a master regulator of genomic programs, because it acts at different stages of development to define which sets of genes are active and which ones are quiescent (Kohler and Villar, 2008; Schwartz and Pirrotta, 2007; Simon and Kingston, 2009). We found that expression of key members of this complex *decreases* in the hypothalamus at puberty, suggesting that PcG proteins might provide a repressive influence on the initiation of puberty by inhibiting the expression of downstream genes whose activation is ultimately required for puberty to occur. Like POK genes, genes of the PcG silencing complex can be considered as TSGs, because they have tumor suppressor activity (Classen et al., 2009; Kelly and Daniel, 2006; Martinez et al., 2009). Based on these considerations, the concept of a TSG network controlling puberty can be refined by postulating that the network's core is composed of both trans-activational and repressive nodes. While the former move the process along by facilitating the sequential activation of key stimulatory events, the latter may impose a repressive, and likely encompassing, tone to the system, so that premature reproductive maturation is prevented. Obviously, these preliminary observations will need to be thoroughly tested before they can be employed to support the idea that transcriptional repression is an integral component of the developmental program controlling mammalian puberty at a hypothalamic level.

5. The epigenetic control of puberty

While advancing our knowledge, the aforementioned findings were made only within the context of a genetic framework. As such, they do not explain how inherited, permanent changes in DNA sequence can regulate gene expression dynamically, while also imposing an encompassing level of coordination and transcriptional plasticity to gene sets, such as those controlling female reproductive development. It appears intuitively obvious that alternative mechanisms of control must exist. We believe that a powerful biological regulatory system that meets these requirements is epigenetics – i.e., those heritable changes in gene expression that occur without changing the primary nucleotide sequence of a gene (Herman and Baylin, 2003; Wolffe and Matzke, 1999). Chemical modification of DNA or chromatin-associated proteins, particularly histones, has a major influence on chromatin structure and gene expression. DNA can be modified by methylation of cytosine residues in CpG dinucleotides (Bjornsson et al., 2004; Jaenisch and Bird, 2003), and the N-terminal tails of histone proteins are subject to a wide range of different modifications, including acetylation, methylation, phosphorylation and ubiquitylation (Jenuwein and Allis, 2001; Kouzarides, 2007). Epigenetic mechanisms can not only provide gene-specific gate keeper functions (Garcia-Bassets et al., 2007), but are also endowed with an unsuspected degree of plasticity able to transiently change gene expression within hours (Miller and Sweatt, 2007), and even minutes (Kangaspeska et al., 2008; Metivier et al., 2008). Even more remarkable, epigenetic regulation of certain genes (Metivier et al., 2008), including the gene encoding ER α (Kangaspeska et al., 2008), is unexpectedly cyclic, exhibiting a periodicity that results in a rapid, tight and dynamic control of gene expression. It is now clear that epigenetic information is also essential for a variety of neural functions, including memory formation (Miller and Sweatt, 2007), recovery of learning and memory (Fischer et al., 2007), dendritic development (Wu et al., 2007), neuronal and behavioral plasticity

(Kumar et al., 2005), estrogen-induced gene expression (Perillo et al., 2008; Subramanian et al., 2008), glial-neuronal interactions (Shen et al., 2008), circadian rhythms (Nakahata et al., 2008), and sexual differentiation of the brain (McCarthy et al., 2009).

The potential contribution of epigenetics to the pubertal process has never been formally addressed. We have now obtained evidence (Lomniczi et al., 2010) suggesting that an epigenetic mechanism of *transcriptional repression* operating in the hypothalamus plays a significant role in timing the initiation of female puberty. We obtained results suggesting that the PcG group of transcriptional silencers is a major contributor to this repressive mechanism. We observed that hypothalamic expression of core components of the PcG complex decreases at puberty, and that this change is associated with acquisition of epigenetic silencing marks (DNA methylation, repressive histones) and loss of activating histone marks from their promoter regions. In additional studies, we explored the concept that PcG genes target for repression downstream genes directly involved in the stimulatory control of GnRH secretion at puberty. Using the *Kiss1* gene as a prototype, we found that PcG proteins interact with the 5' flanking region of this gene, and that the pubertal increase in *Kiss1* expression is accompanied by the acquisition of epigenetic modifications associated with gene activation, i.e. DNA demethylation, recruitment of activating histones and loss of repressive histones. These results support the notion that epigenetic mechanisms are integral components of the neuroendocrine process controlling female puberty.

6. Conclusions and perspectives

The observations discussed in this article support the notion that the onset of puberty depends on the contribution of more than one gene. There are many genes and many pathways that contribute to the process, but as seen in embryonic developmental processes, puberty also appears to be controlled at the transcriptional/post-transcriptional level by discrete groups of genes. “Activators” might move the process along by promoting key developmental events; “repressors” might prevent the untimely activation of activating genes (Fig. 1). We speculate that both inhibitory and stimulatory pathways of transcriptional/post-transcriptional regulation operate within neuronal and glial populations involved in controlling the onset of puberty, and that an encompassing level of regulation is provided by two layers of gene repression, acting in concert with epigenetic mechanisms. One of these layers, formed by transcriptional/posttranscriptional “repressors” (e.g. *PcG* genes, *Lin28b*, etc) may function to prevent the premature activation of puberty-inducing genes (such as *Kiss1*, *Tac3*, *Ttf1*, *Eap1*, *Oct2*, etc); the other layer, located more centrally in the network (e.g., *POK*, *ZNF* genes, and possibly others), may modulate in a temporally dynamic manner the expression of repressors (Fig. 2). As such, these more centrally located genes can be considered as “repressors of repressors”. Both layers may also directly control key subordinate genes of the network (such as those operating within kisspeptin, GABAergic, glutamatergic, opioid, and RFRP neurons, in addition to genes expressed in glial cells). The model also predicts that an encompassing level of coordination and transcriptional plasticity to these gene networks is provided, not by variations in DNA sequence, but by changes in epigenetic information (Lomniczi et al., 2010; Ojeda and Lomniczi, 2010), i.e. heritable changes in gene expression that occur without changing the primary nucleotide sequence of a gene. Much research is obviously needed to verify or challenge the validity of these concepts.

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Abbreviations

GnRH	Gonadotrophin-Releasing Hormone
AVPV	anteroventral periventricular nucleus
ARC	arcuate nucleus
RFRP	RFamide-related peptide gene
GnIH	gonadotrophin-inhibiting hormone
TGFβ	Transforming growth factor-beta
EGF	epidermal growth factor
bFGF	basic fibroblast growth factor
IGF-I	insulin-like growth factor 1
PGE₂	prostaglandin E ₂
NCAM	neural cell adhesion molecule
PSA-NCAM	polysialylated NCAM
ME	median eminence
SynCAM1	Synaptic Cell Adhesion Molecule 1
RPTPβ	Receptor-like Protein Tyrosine Phosphatase-β
Eap1	Enhanced At Puberty1
TSG	Tumor Suppressor Gene
POA	preoptic area
ARC	arcuate nucleus
ZNF	Zinc-finger
POZ-ZF	poxvirus and Zinc finger
BTB	broad complex - Tramtrack, bric-à-brac
PcG	Polycomb group
GABA	gamma amino butyric acid
GNRHR	gonadotrophin releasing hormone receptor
TAC3	G-protein coupled receptor 54, tachykinin 3
TAC3	tachykinin 3 receptor
POU	Pit1-Oct2-Unc86
TGFα	transforming growth factor alpha
LH	luteinizing hormone
FSH	follicle stimulating hormone

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Figure 1. The hypothetical transcriptional control of mammalian puberty by “activators” and “repressors” operating in the female neuroendocrine brain

According to this model, during early juvenile days there are sets of genes repressing downstream genes involved in advancing the pubertal process (for instance, the effectors *Kiss1* and *Tac3* and the transcriptional regulators *Tcf1*, *Oct2* and *Eap1*- collectively referred to as puberty-activating genes, PubAGs). In contrast, transcriptional repression of cellular systems inhibitory to puberty (e.g., dynorphin and RFRP neurons, and/or genes encoding prepropeptide processing enzymes in different inhibitory neuronal systems) would be negligible. At this time of development, trans-activation of PubAGs genes would be minimal, whereas trans-activation of puberty-delaying genes may be enhanced. The model predicts that the transcriptional inhibition of PubAGs is lifted at or before puberty, and replaced by increased trans-activation of gene expression. Simultaneously, the repression of puberty-delaying genes is expected to be enhanced. ← = stimulation; ⊥ = inhibition. Whether a similar, but “reversed”, mechanism operates in primates at the time of the infantile-juvenile transition, when GnRH secretion is turned off is a possibility that requires experimental consideration.

Figure 2. General organization of a hypothetical transcriptional complex controlling the activational arm of female puberty

According to this model the highest level of control is exerted by *repressors of repressors* (e.g., *POK*, *ZNF* genes); because these genes repress other repressors (e.g. *PcG* genes), their influence would be expected to be low during juvenile development, increasing at puberty. In contrast, the repressive influence of a second, less “central” level of control formed by “repressors” (e.g. *PcG* genes) would be higher during juvenile development than at puberty, because these genes function to prevent the premature activation of puberty activating genes (PubAGs). As a result of these changes in repressive tone, PubAG expression (e.g. *Kiss1*, *Tac3*, *TTF1*), would increase at and/or preceding the onset of puberty. Because *Eap1* predominantly represses gene expression, it may be considered as a second tier repressor, but more information is needed before an accurate assignment can be made. An additional level of regulation is provided by epigenetic mechanisms. According to existing information derived from studies performed in our laboratory, these mechanisms involve opposite changes in DNA methylation (DNA me) and association of modified histones to *PcG* and PubAG promoters (repr H3 = histones associated with gene repression; activ H3 = histones associated with gene activation). Not considered in this model is the possibility that *repressors of repressors* also modulate the activity of neuronal populations involved in the inhibitory control of puberty; should this be the case, an increased repressive influence of these factors at puberty would remove transsynaptic inhibitory influences on GnRH neurons, allowing stimulatory inputs to operate at full force. Lastly, this model does not consider “further upstream” mechanisms of control that may govern the expression of repressors of repressors, and that are likely to impose an even more encompassing regulation of the transcriptional cascade controlling puberty onset.