PUBERTY IN 2013

Unravelling the mystery of puberty

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In 2013, considerable progress was made towards deciphering the molecular foundations of puberty. Loss of transcriptional repression was identified as a core mechanism underlying the onset of puberty, and this loss was found to be precipitated by epigenetic cues. It was also discovered that nutritional deprivation delays puberty by repressing reproductive neuroendocrine function.

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During the past decade, substantial efforts have been made to identify the genes that, operating within the neuroendocrine brain, might ultimately be responsible for the initiation of puberty. The picture that is emerging is one of complexity but also of exquisite coordination and functional integration. The discovery that mutations in *KISS1R* (which encodes the receptor for kisspeptins) cause hypothalamic hypogonadism[1,2] drove the identification of various components of the system that regulates puberty and the complexities of the interactions within this system. These investigative efforts led to the realization that the initiation of puberty requires the involvement of many other genes, such as *TAC3*, *TAC3R* and *LEPR*.[3,4] These genes also seem to be organized into functionally connected networks that affect various steps along the neuroendocrine cascade that sets the pubertal process in motion.[3]

A prevailing view of the initiation of puberty is that during the prepubertal period, neurons secreting gonadotropin-releasing hormone (GnRH) are subjected to persistent trans-synaptic inhibition. When this inhibition is lifted, GnRH secretion increases, which leads to puberty.[5] As loss of inhibitory inputs alone would not suffice to set puberty in motion, it was recognized that a gain in excitatory inputs to GnRH neurons is required.[5] Kisspeptin neurons have emerged as a major component of this excitatory input, as kisspeptin signalling is necessary for puberty to occur.[1,2] Kisspeptin neurons of the arcuate nucleus (ARC) in the hypothalamus seem to be essential for pulsatile GnRH release in both sexes. However, whilst kisspeptin neurons are almost absent in the anteroventral periventricular nucleus (AVPV) of the hypothalamus of male individuals, they are required for the preovulatory surge of gonadotropins in female individuals.[6]

Does this finding mean that a change in inhibitory inputs is irrelevant to puberty? On the contrary, in 2013 we learned that a basic mechanism of inhibition–activation does indeed exist, but it operates at a transcriptional level. This mechanism not only affects the output of a primary excitatory system (kisspeptin neurons) that controls pulsatile GnRH secretion, but importantly, is subjected to epigenetic regulation.[7] In 2013, Lomniczi *et al.*[7] showed that *Kiss1* expression in the ARC of female rats is subjected to a repressive state exerted by the polycomb group (a protein complex). At the initiation of puberty, promoter DNA methylation of two key members of this complex (*Eed* and *Cbx7*) increases, expression of both genes decreases, and association of their protein products with the *Kiss1* promoter declines. This change to the promoter coincides with an increase in the expression of *Kiss1* and changes in the chromatin status of the *Kiss1* promoter. Marked increases occur in the levels of histone H3 Lys4 trimethylation and histone H3 acetylated at Lys9 and Lys14, two histone marks associated with gene activation. A gradual loss was found in levels of histone H3 Lys27 trimethylation, a repressive histone modification catalysed by the polycomb group complex. Inhibition of DNA methylation prevented the peripubertal decrease in *Eed* and *Cbx7* expression and the removal of EED and CBX7 from the *Kiss1* promoter, which resulted in puberty not occurring. A direct causal relationship between polycomb-mediated inhibition and the timing of puberty was established by the finding that overexpression of EED in prepubertal female rats blunted pulsatile GnRH release, delayed the initiation of puberty and compromised fertility.[7]

These findings raised the important question of why mutations in genes that encode transcriptional repressors had never been reported in humans with disorders of puberty. A definitive answer to this question was provided just 3 months after the publication of the Lomniczi paper by the demonstration that mutations in *MKRN3* are responsible for several cases of familial central precocious puberty.[8] *MKRN3* is a maternally imprinted gene, that is, the copy of the gene derived from the mother is not expressed. Abreu *et al.*[8] studied a cohort of 40 patients with central precocious puberty from 15 families and observed that the *MKRN3* gene had deleterious mutations in five of these families. *MKRN3* is located on chromosome 15q11.2,[9] the region in which the mutations that cause Prader–Willi syndrome occur; however, the findings of Abreu and colleagues suggest that mutations in *MKRN3* are responsible for the precocious puberty seen in these patients

Key advances

- Transcription of *Kiss1*, a gene required for the initiation of puberty, is repressed in the ARC of the rat hypothalamus before puberty by the polycomb group transcriptional silencing complex[7]
- This repression is lifted at the initiation of female puberty by epigenetic cues, including changes in DNA methylation and gaining histone marks associated with gene activation[7]
- Inactivating mutations of *MKRN3*, a gene predicted to have transcriptional repressive activity, results in precocious puberty in both girls and boys[8]
- Loss of *MKRN3* function underlies many cases of familial pubertal precocity[8]
- Circulating levels of FGF21, a growth factor of liver origin, increase in response to fasting and delay female puberty by suppressing the preovulatory surge of gonadotropins[10]
- FGF21 exerts this effect by inhibiting expression of the gene that encodes vasopressin in SCN neurons, resulting in loss of this stimulatory input to kisspeptin neurons of the AVPV[10]

but not for other features of Prader–Willi syndrome, such as developmental delay.

MKRN3 does not contain introns and encodes a protein with a RING finger domain and several zinc finger motifs,[9] which indicates the protein has activity similar to that of ubiquitin ligases. In this sense, MKRN3 is remarkably similar to IRF2BPL, another protein with a RING finger domain and that has been implicated in controlling the initiation of puberty and reproductive function.[3] The decline in hypothalamic expression of *Mkrn3* that was observed in mice during prepubertal development and the loss of *MKRN3* function in patients with precocious puberty strongly suggest that MKRN3 represses the initiation of puberty.[8] As such, these findings provide the first example of a gene that controls puberty by potentially repressing downstream targets instead of activating the GnRH neuronal network. The results of Abreu and co-workers are in keeping with the emerging notion that members of the zinc finger family of genes might be transcriptional repressors of the pubertal process in higher primates, including humans.[3] These findings also complement the notion put forward by Lomniczi *et al.*[7] that transcriptional repression is a core component of the neuroendocrine circuitry that regulates the timing of puberty.

The two aforementioned studies identify regulatory components that underlie the ability of the hypothalamus to govern pulsatile GnRH release, but do not address the process by which puberty is completed in female individuals via the first preovulatory surge in levels of gonadotropins. Clearly, this surge is elicited by an increase in GnRH release that is in turn induced by estrogen via a mechanism involving kisspeptin neurons of the AVPV. However, little was known about the pathways by which peripheral signals associated with metabolic activity regulated this process. Owen *et al.*[10] ended this state of affairs by showing that fibroblast growth factor 21 (FGF21), a circulating form of FGF that originates in the liver, is a peripheral signal that is secreted in response to short-term (<24 h) nutritional deprivation and inhibits the preovulatory surge in levels of gonadotropins by acting on vasopressin neurons of the suprachiasmatic nucleus (SCN). These investigators showed that FGF21 acts on the SCN via β-klotho FGF co-receptors to suppress vasopressin gene expression. This suppression results in loss of vasopressin-dependent stimulation of kisspeptin neurons in the AVPV.

Taken together, these studies[7,8,10] offer a tantalizing new view of the central and peripheral mechanisms responsible for initiating puberty (Figure 1). Instead of occurring at the level of trans-synaptic communication, the central control of puberty is provided by upstream mechanisms that involve gene silencing. Whilst this process has been mechanistically identified in the case of polycomb group genes, much remains to be done in the case of *MKRN3*. However, the structural features of this gene and its pattern of expression in the developing hypothalamus suggest that MKRN3—and possibly other gene products from the zinc finger gene family—might function much like polycomb group proteins as silencers of downstream genes that activate puberty, such as *KISS1*. The demonstration by Owen and colleagues that FGF21, as a peripheral signal, delays the completion of puberty by repressing vasopressin expression in SCN neurons agrees with the nascent concept that envisions transcriptional repression as a key mechanism controlling the initiation of mammalian puberty.

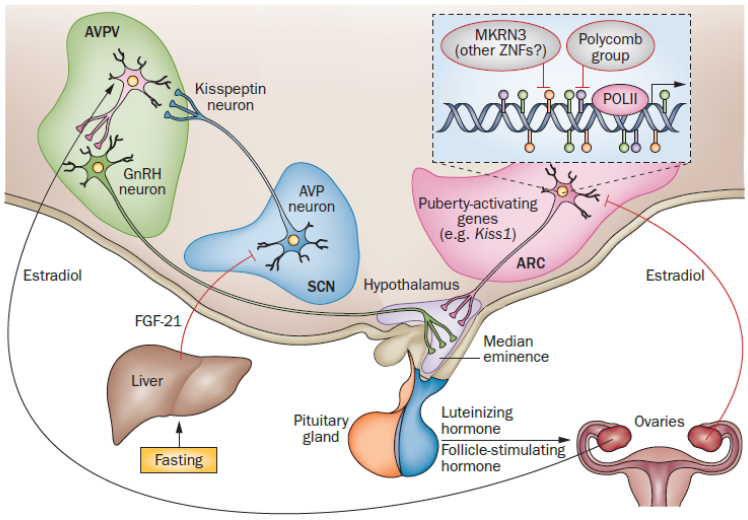

Figure 1 | The repressive control of puberty. In the ARC, two gene-silencing systems are involved; the polycomb group complex and MKRN3. At the AVPV level, the completion of puberty is delayed by FGF21. Abbreviations: ARC, arcuate nucleus; AVP, vasopressin; AVPV, anteroventral periventricular nucleus; FGF21, fibroblast growth factor 21; MKRN3, makorin ring finger protein 3; SCN, suprachiasmatic nucleus; ZNFs, member of the zinc finger family of proteins.

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Competing interests
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