Molecular and Epigenetic Mechanism Regulating Hypothalamic *Kiss1* Gene Expression in Mammals

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Key Words

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Abstract

After the discovery of hypothalamic kisspeptin encoded by the *Kiss1* gene, the central mechanism regulating gonadotropin-releasing hormone (GnRH) secretion, and hence gonadotropin secretion, is gradually being unraveled. This has increased our understanding of the central mechanism regulating puberty and subsequent reproductive performance in mammals. Recently, emerging evidence has indicated the molecular and epigenetic mechanism regulating hypothalamic *Kiss1* gene expression. Here we compile data regarding DNA and histone modifications in the *Kiss1* promoter region and provide a hypothetical scheme of the molecular and epigenetic mechanism regulating *Kiss1* gene expression in two populations of hypothalamic kisspeptin neurons, which govern puberty and subsequent reproductive performance via GnRH/gonadotropin secretion.

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Introduction

Proper regulation of gene expression in each cell type sharing the same genetic blueprint is controlled by the binding of different transcription factors and epigenetic modifications. The past decade has seen remarkable progress in our understanding of epigenetic regulation of gene expression in mammals during embryo development, in each cell type and at various disease states [1–5]. Generally, epigenetic modifications fall into two broad categories: DNA methylation and histone modifications. DNA methylation in cytosines of the CpG dinucleotides of the promoter region is perhaps the most extensively studied epigenetic modification in mammals and is generally linked to the repression of gene expression. DNA methylation is catalyzed by a family of DNA methyltransferases [6] and recruits methyl-CpG binding domain proteins in combination with histone deacetylases to transform chromatin to a repressive state [3]. On the other hand, posttranslational modifications of histone proteins such as lysine acetylation and methylation can lead to either gene activation or repression

[7]. Acetylation at lysines 9 and 14 of histone H3 protein (H3K9/14 acetylation) and trimethylation at lysine 4 of histone protein (H3K4 trimethylation) are classified as activating and permissive histone modifications, respectively [7]. Those are catalyzed by histone acetyltransferases and histone methyltransferase, respectively, and both decrease the interaction between DNA and histones, and hence loosen the nucleosome structure, which is open to transcriptional factors [8]. Conversely, histone deacetylation by histone deacetylases and H3K9/27 trimethylation leads to a general repression of gene expression [7].

In the past few years, emerging evidence has indicated that epigenetic mechanisms regulate gene expression of hypothalamic neuropeptides controlling feeding and reproduction. A previous study showing epigenetic regulation of the *Pomc* gene encoding proopiomelanocortin expression [9] demonstrated an inverse relationship between DNA methylation and *Pomc* gene expression, and a mechanism linking maternal undernutrition to obesity in the offspring. The epigenetic regulation of the *Gnrh1* gene encoding gonadotropin-releasing hormone (GnRH) expression was reviewed elsewhere by Kurian and Terasawa [10], who demonstrated an inverse relationship between DNA methylation and *Gnrh1* gene expression during the peripubertal period. Likewise, understanding of the epigenetic regulation of the *Kiss1* gene encoding kisspeptin (initially named metastin) expression has become increasingly clear [11–14].

This review focuses on the recent progress in our understanding of the molecular and epigenetic mechanisms regulating *Kiss1* gene expression in the rodent hypothalamus during the estrous cycle and at the time of puberty onset. Estrogenic regulation of hypothalamic *Kiss1* gene expression and its interaction with histone modifications will be the main topics discussed. The epigenetic contribution to the sexual dimorphism of *Kiss1* gene expression in rodents will also be discussed.

***Kiss1* Gene Expression in Two Populations of Hypothalamic Kisspeptin Neurons and the Role of Estrogen Signaling**

Accumulating evidence indicates that kisspeptin neurons are located in two hypothalamic regions and play a critical role as a master regulator of pulsatile and surge-mode GnRH secretion in all mammalian species examined to date [15–24]. Cyclic changes and the estrogenic regulation of hypothalamic *Kiss1* gene expression are well

demonstrated in the mammalian hypothalamus, including rodents [15, 16, 25] and sheep [26–28]. *Kiss1* gene expression is high at proestrus and is positively controlled by estrogen in the anteroventral periventricular nucleus (AVPV) and periventricular nucleus (PeN) continuum (also known as the rostral periventricular area of the third ventricle) in rodents [15, 16]. On the other hand, *Kiss1* gene expression in the arcuate nucleus (ARC) is high at diestrus and is negatively controlled by estrogen in rodents [15, 16, 25]. Both populations of kisspeptin neurons express estrogen receptor- α (ER α) [15, 16, 25, 29]. ER α knockout mice failed to duplicate those estrogen-dependent changes in *Kiss1* gene expression in both the AVPV and the ARC, whereas ER β knockout mice did [15]. These findings suggest that estrogen-ER α signaling bidirectionally controls *Kiss1* gene expression in a brain region-specific manner and raise the following question: how does estradiol-ER α signaling bidirectionally control *Kiss1* gene expression in the AVPV and ARC?

Classic estrogen response element (ERE)-dependent and non-classic ERE-independent signaling pathways provide a clue to understanding the bidirectional regulation of *Kiss1* gene expression by estrogen. Jameson and colleagues [30] generated a mutated ER α lacking the DNA binding capacity to the ERE and demonstrated that estradiol exerts opposite effects on the classic ERE-dependent and non-classic ERE-independent signaling pathways: namely, estradiol stimulated ERE reporter expression via the interaction of wild-type ER α and suppressed AP-1 reporter expression via the interaction of mutated ER α as well as wild-type ER α [30]. In addition, NF- κ B and Sp1, as well as Ap-1, reportedly interact with wild-type ER α and inhibit their response element-driven reporter expression [31–33]. These findings suggest that the estrogen-ER α complex exerts a bidirectional influence on gene expression via ERE-dependent and -independent pathways; the latter transcriptional factors play a role as a DNA tethering partner or corepressor. Using non-classic ER α knock-in (NERKI) mice carrying mutated ER α , Gottsch et al. [34] demonstrated that estradiol failed to exert a positive influence on *Kiss1* gene expression in the AVPV, whereas estradiol successfully exerted a negative influence on *Kiss1* gene expression in the ARC. In addition, Huijbregts and de Roux [35] demonstrated that estradiol suppressed *Kiss1* promoter activity in a breast cancer cell line bearing luciferase reporter conjugated with the proximal promoter region of human *KISS1* gene devoid of ERE. These in vivo and in vitro studies suggest that two separate ERE-dependent and ERE-independent pathways are responsible for estrogenic regula-

tion of *Kiss1* gene expression in the AVPV and ARC, respectively: estradiol-bound ER α seems to directly bind to the ERE of the *Kiss1* promoter region and then upregulates *Kiss1* gene expression in the AVPV, whereas estradiol-bound ER α seems to bind to the non-ERE response element via interaction with other transcriptional factor(s) and then downregulates *Kiss1* gene expression in the ARC. The question of which transcriptional factor(s), in combination with ER α , play(s) a critical role in estrogenic suppression of ARC *Kiss1* gene expression remains to be answered.

Epigenetic Mechanism of Estrogenic Regulation of *Kiss1* Gene Expression

Analyses of the histone modulation of the *Kiss1* promoter region provide a clue to understanding the region-specific pattern of hypothalamic *Kiss1* gene expression. Tomikawa et al. [11] showed that H3K9/14 acetylation, an activating histone H3 modification, in the *Kiss1* promoter region is closely associated with an increase in *Kiss1* gene expression in both the AVPV and the ARC of mice. Specifically, histone H3K9/14 acetylation and ER α binding in the AVPV *Kiss1* promoter region were induced by estradiol and were positively associated with an increase in *Kiss1* gene expression in this nucleus. Furthermore, histone deacetylation inhibitor induced *Kiss1* expression in murine hypothalamic cell lines in vitro [11]. These findings suggest that histone H3K9/14 acetylation plays a critical role in inducing AVPV *Kiss1* gene expression at proestrus in mice. Indeed, the levels of histone H3K9/14 acetylation in the *Kiss1* promoter region are low at diestrus but increase at proestrus in the AVPV in mice. Estradiol also enhanced formation of the chromatin loop between the promoter and 3' downstream regions of the *Kiss1* gene, suggesting that the 3' downstream region of the *Kiss1* gene plays a key role in regulating *Kiss1* gene expression as an enhancer in the AVPV. Taken in the light of the NERKI mice described previously [34] and previous studies showing mechanisms of estradiol-induced histone acetylation [36, 37], it is likely that the estrogen-ER α complex on the ERE of the *Kiss1* promoter region induces histone acetylation of the *Kiss1* promoter region via recruitment of histone acetyltransferases and/or reduction of histone deacetylases, and that, subsequently, the chromatin loop between the promoter and 3' downstream regions of the *Kiss1* gene seems to be formed. This is a hypothetical scenario, and further studies are

needed to clarify the molecular cascade controlling estradiol-induced AVPV *Kiss1* gene expression.

In contrast with the AVPV, histone H3K9/14 acetylation in the ARC *Kiss1* promoter was increased by ovariectomy and reduced by estradiol treatment in mice [11], indicating that histone H3K9/14 acetylation of the *Kiss1* promoter is positively associated with *Kiss1* gene expression in the ARC in the absence of estrogen. Goto et al. [14] found ARC-specific chromatin loop formation between the promoter region and the 5' upstream region of the *Kiss1* gene, suggesting that the 5' upstream region of the *Kiss1* gene plays a key role in upregulating *Kiss1* gene expression as an enhancer in the ARC. This study also showed a binding of unoccupied ER α and putative transcriptional factor binding sites in the ARC-specific putative enhancer region of the *Kiss1* locus. The molecular mechanism regulating histone acetylation and chromatin loop formation of the ARC *Kiss1* gene in the absence of estrogen should be investigated in the future. It should be noted that either unoccupied or occupied ER α may be unnecessary for ARC *Kiss1* gene expression, because ARC *Kiss1* gene expression in ER α knockout mice was largely higher than in wild-type controls [15].

The hypothesis that region-specific enhancers of the *Kiss1* gene are involved in the induction of *Kiss1* gene expression in the AVPV and ARC is supported by the results of in vivo reporter assays using *Kiss1*-GFP reporter transgenic mice. The assay revealed that the 3' downstream region of the *Kiss1* locus contributes to induction of estrogen-induced AVPV *Kiss1* gene expression [11], while the 5' upstream region of the *Kiss1* locus is conducive to ARC *Kiss1* gene expression [14]. More specifically, chromatin loop formation between the *Kiss1* promoter region and the 3' downstream region in the AVPV or the 5' upstream region in the ARC, respectively, may act as a switch, which could turn on *Kiss1* gene expression. Figure 1a shows schematic illustrations of a putative mechanism involved in estrogen-induced AVPV *Kiss1* gene expression: the localization of the AVPV-specific enhancer of the *Kiss1* gene as well as the formation of the chromatin loop between the *Kiss1* promoter region and the 3' downstream region in the AVPV in the presence of estrogen. On the other hand, figure 1b demonstrates a possible mechanism for ARC *Kiss1* gene expression in the absence of estrogen: an ARC-specific enhancer located in the 5' upstream regions of the *Kiss1* gene may form multiple chromatin loops with the *Kiss1* promoter in the ARC.

Chromatin looping has been known to play a crucial role in gene repression as well as expression [38]. Tomi-

kawa et al. [11] showed that estrogen enhanced multiple chromatin loop formation between the *Kiss1* promoter region and the 3' downstream region in the ARC. It is, thus, tempting to speculate that estrogen-induced multiple chromatin loop formation may contribute to the estrogen-negative feedback action on ARC *Kiss1* gene expression (fig. 1b). Taken together with the results from NERKI mice, an ERE-independent pathway such as interaction of c-Jun and ER α would induce such ARC-specific multiple chromatin looping in the presence of estrogen. Further studies also need to clarify this issue.

Role of Epigenetic Silencing for Pubertal Changes in ARC *Kiss1* Gene Expression and GnRH Secretion

In mammals, sexual maturation at the onset of puberty is considered to be timed by an increase in GnRH/gonadotropin secretion. The central mechanism regulating pubertal changes in GnRH secretion has been extensively studied. As Ojeda and colleagues reviewed elsewhere [39, 40], pubertal changes in GnRH secretion depend on both stimulatory and inhibitory inputs from neurons and glial cells. Kisspeptin is now considered a powerful stimulator of the pubertal increase in GnRH/gonadotropin secretion in mammals, because pubertal failure is known to be caused by loss-of-function mutations of the *KISS1* gene – as well as its cognate receptor, the *GPR54* gene – in human [41–43] and rodent models [24, 42, 44–49]. Also, a pubertal increase in *Kiss1* gene expression was evident in both the AVPV and the ARC in rodents [17, 50, 51]. In particular, ARC kisspeptin neurons seem to be responsible for the pubertal increase in GnRH/gonadotropin secretion, because ovariectomy increased ARC *Kiss1* gene expression along with an increase in luteinizing hormone (LH) pulses, and estradiol replacement strongly suppressed them in prepubertal rats [50]. A study by Mayer et al. [52] showing that kisspeptin neuron-specific ER α knockout mice exhibited an advanced puberty onset suggests that prepubertal suppression of GnRH/LH secretion is mainly due to a direct action of estrogen on ARC *Kiss1* gene expression. This result is consistent with our recent results showing that an estrogen microimplant in the ARC suppressed pulsatile LH secretion in prepubertal rats [53]. It should be noted that estradiol seems to exert a potent inhibitory effect on ARC *Kiss1* gene expression via both direct and indirect pathways. Our recent study demonstrated that the medial preoptic area is also involved in estrogen-in-

Fig. 1. Schematic illustration indicating the localization of region-specific enhancers of the murine *Kiss1* gene and formation of the chromatin loop between the *Kiss1* promoter region and enhancers. The solid boxes and red, blue, and yellow lines indicate three exons, the ARC-specific enhancer, the AVPV-specific enhancer, and the promoter region of the murine *Kiss1* locus, respectively. **a** The estrogen-ER α complex seems to play a critical role in chromatin loop formation between the *Kiss1* promoter region and the 3' downstream region in the AVPV via the ERE-dependent pathway. **b** In the absence of estrogen, unknown transcriptional factors may induce chromatin loop formation between the *Kiss1* promoter region and the 5' upstream region in the ARC. In the presence of estrogen, the 3' downstream region of the *Kiss1* locus may serve as a repressor of the *Kiss1* gene via the ERE-independent pathway.

duced suppression of LH pulses in prepubertal rats [53], suggesting that estrogen-responsive neurons in the preoptic area contribute the prepubertal suppression of GnRH/LH secretion.

Ojeda and colleagues [12] demonstrated that polycomb epigenetic silencing is responsible for the pubertal changes in ARC *Kiss1* gene expression. Their chromatin immunoprecipitation assay showed a pubertal decrease in the binding of EED, a component of polycomb repressive complex 2 [54], in the *Kiss1* promoter region, and overexpression of EED resulted in suppression of *Kiss1*

gene expression and subsequent GnRH secretion in rats. These results suggest that the polycomb repressive complex 2 plays a critical role in suppressing ARC *Kiss1* gene expression and, hence, GnRH/gonadotropin secretion before the onset of puberty. It is well known that the polycomb repressive complex 2 exerts histone methyltransferase activity, resulting in histone H3K27 trimethylation, an inhibiting histone H3 modification [55]. Indeed, Lomniczi et al. [12] showed that in rats, H3K27 trimethylation in the *Kiss1* promoter region decreased at the first proestrus, as opposed to the prepubertal period. They also demonstrated that increases in two activating histone H3 modifications (H3K4 trimethylation and H3K9/14 acetylation) in the *Kiss1* promoter region preceded the decrease in H3K27 trimethylation at the onset of puberty in female rats. Taken together, the relief from polycomb silencing and activating histone H3 modifications may play critical roles in triggering *Kiss1* gene expression at the onset of puberty in rats.

Ojeda and colleagues [12] also demonstrated that intraperitoneal administration of 5-azacytidine, a DNA methyltransferase inhibitor, delayed puberty onset in rats and depressed the hypothalamic-pituitary axis. This interpretation was supported by findings that administration of kisspeptin, GnRH, or equine chorionic gonadotropin overrides the suppression of GnRH, LH, or estradiol secretion in 5-azacytidine-treated animals, respectively. The 5-azacytidine treatment suppressed methylation of CpG dinucleotides of the putative *Kiss1* promoter region (flanking region of the *Kiss1* transcriptional start site), suggesting that methylation of the putative *Kiss1* promoter region is required for the pubertal increase in *Kiss1* gene expression. In several cancer cells, an inverse relationship was found between DNA methylation in the CpG island of the putative *KISS1* promoter and *KISS1* gene expression [56, 57]. However, this inverse relationship may be absent, along with the disappearance of the CpG island in the putative *Kiss1* promoter, in rodents, as already pointed out elsewhere [58]. Indeed, bisulfite sequencing by our and other groups has revealed that the putative *Kiss1* promoter region appeared hypermethylated: Tomikawa et al. [11] showed that methylation levels were comparable between *Kiss1*-expressing cells obtained from the AVPV or ARC and non-*Kiss1*-expressing cells obtained from the cerebral cortex; Kauffman and colleagues [13] demonstrated that methylation levels were greater in AVPV tissue obtained from females than males. Taken together, putative *Kiss1* promoter methylation may be required for proper *Kiss1* gene expression in the rodent hypothalamus. *Kiss1* promoter

methylation in the ARC seems to be established before the onset of puberty, since Ojeda and colleagues [12] showed that the methylation levels are comparable between pre- and postpubertal periods. A likely explanation is that *Kiss1* promoter methylation is established before birth, because medial basal hypothalamus *Kiss1* gene expression was found during late embryonic development and was highest at 18.5 embryonic days in rats [59] and 16.5 embryonic days in mice [60, 61]. It should be noted that bisulfite sequencing cannot discriminate methylcytosine from hydroxymethylcytosine, an intermediate in the demethylation of methylated CpG dinucleotides [62], suggesting that our and other previous studies may have overestimated *Kiss1* promoter methylation. A previous study [63] showed that several methyl-CpG binding proteins do not recognize hydroxymethylcytosine, suggesting that hydroxymethylcytosine may serve to displace those bindings and relieve transcription silencing. Further studies, thus, are warranted to analyze DNA methylation and hydroxymethylation in the *Kiss1* promoter region.

Epigenetic Regulation of Sexual Dimorphic AVPV *Kiss1* Gene Expression

Kauffman and colleagues [13] explored epigenetic contributions to sexually dimorphic AVPV *Kiss1* gene expression in mice. In rodents, a sex difference in AVPV kisspeptin neurons is responsible for the sex difference in the mechanism generating a GnRH/LH surge [17–19]. Sexual dimorphism in AVPV *Kiss1* gene expression and GnRH/gonadotropin secretion seems to be caused by an organizational effect of estrogen converted from testicular androgen, because neonatal administration of estrogen or aromatizable androgen resulted in a reduction of AVPV *Kiss1* gene expression and disruption of GnRH/LH surge generation in female rats [18, 19, 64]. This consideration is also supported by the finding that neonatal castration preserves AVPV *Kiss1* gene expression as well as an estradiol-induced GnRH/LH surge in male rats [19, 64]. Recently, Kauffman and colleagues [13] examined the contribution of histone modification to *Kiss1* gene expression in mice and showed that neonatal administration of valproic acid, a histone deacetylase inhibitor [65], failed to override sex dimorphic AVPV *Kiss1* gene expression. On the other hand, neonatal administration of valproic acid increased the number of AVPV kisspeptin neurons in both sexes [13], as is the case with *Bax* knock-out mice [66]. Based on these results, it is suggested that

Fig. 2. Schematic illustration indicating a hypothetical scenario for the regulation of AVPV *Kiss1* gene expression in the rodent hypothalamus in the absence or presence of estrogen in adults. Solid circles and boxes represent methylated CpG dinucleotides and exons of the *Kiss1* gene, respectively. In the absence of estrogen, unoccupied ERα may not be capable of binding to the *Kiss1* promoter region (1), and histone deacetyltransferases (HDAC) may continuously suppress histone acetylation of the *Kiss1* promoter (2). At proestrus, the estrogen-ERα complex seems to bind to ERE in

the hypermethylated *Kiss1* promoter region (3) and enhance histone H3 acetylation of the *Kiss1* promoter region via recruitment of histone acetyltransferases (HAT; 4) and reduction of HDAC (5). This activating histone modification may facilitate chromatin loop formation between the promoter and 3' downstream regions of the *Kiss1* locus, resulting in an upregulation of AVPV *Kiss1* gene expression to consequently induce a preovulatory GnRH/gonadotropin surge in females.

histone acetylation and Bax-induced apoptosis contribute to the overall development of AVPV kisspeptin neurons in both sexes.

In the same study, Kauffman and colleagues [13] also examined DNA methylation in the whole region of the *Kiss1* locus and elaborated that CpG dinucleotides located in the promoter region and the first intron of the *Kiss1* gene appeared hypermethylated, and that the CpG island in exon 3 and its upstream region appeared hypomethylated in the AVPV. Interestingly, some of the CpG dinucleotides in the putative promoter and first intron of the *Kiss1* locus showed sexual dimorphism in terms of DNA methylation: DNA methylation levels were higher in females than in males, as was the case with AVPV *Kiss1* gene expression in mice. Again, *Kiss1* promoter methylation may be required for proper *Kiss1* gene expression in the rodent brain, as opposed to suppression of gene expression. An in silico analysis identified putative repressor binding sites near some of the sexually dimorphic CpG dinucleotides of the *Kiss1* promoter

region, suggesting that transcriptional repressors may contribute to the repression of AVPV/PeN *Kiss1* gene expression in males [13]. DNA methylation of the *Kiss1* gene body may be involved in AVPV/PeN *Kiss1* gene expression in a different manner, since emerging evidence indicates that gene body methylation has an impact on mRNA splicing [67].

Possible Molecular and Epigenetic Mechanisms Regulating *Kiss1* Gene Expression, and Unanswered Questions

A possible mechanism underlying estrogen-induced AVPV *Kiss1* gene expression and, hence, the GnRH/gonadotropin surge is illustrated in figure 2. Based on the results currently available, we speculate that in the absence of estrogens, unoccupied ERα may not be capable of binding to the *Kiss1* promoter region ('1' in fig. 2) and histone deacetyltransferases may continuously suppress

Fig. 3. Schematic illustration indicating a hypothetical scenario for the regulation of ARC *Kiss1* gene expression in the rodent hypothalamus during pubertal transition. Solid circles and boxes represent methylated CpG dinucleotides and exons of the *Kiss1* gene, respectively. **a** At the prepubertal stage, polycomb repressive complex 2 (PRC2) seems to bind to the hypermethylated *Kiss1* promoter to repress ARC *Kiss1* gene expression. The estrogen-ERα complex, interacting with c-Jun or some other transcription factor, may also play a role in prepubertal restraint of ARC *Kiss1* gene expression. The contribution of histone deacetyltransferases (HDAC) on the prepubertal restraint of *Kiss1* expression remains obscure, although the histone acetylation level of the *Kiss1* promoter region is likely low before puberty. **b** At the onset of puberty and thereafter,

PRC2 would be dissociated from the *Kiss1* promoter (1) and may possibly enhance activating histone modification of the *Kiss1* promoter region, such as histone H3K9/14 acetylation and H3K4 trimethylation (2), even in the presence of estrogen. The activating histone modifications likely drive ARC *Kiss1* gene expression to enhance GnRH/gonadotropin pulses. Histone H3K9/14 acetylation is especially upregulated in the absence of estrogen (3), resulting in chromatin loop formation between the promoter and 5' upstream regions of the *Kiss1* locus, further resulting in upregulation of *Kiss1* gene expression via unknown mechanisms (4). The mechanism regulating histone acetylation and chromatin loop formation of the *Kiss1* gene in the absence of estrogen also remains unknown.

histone acetylation of the *Kiss1* promoter ('2' in fig. 2). At the onset of puberty and thereafter, when the estrogen-ERα complex binds to the ERE in a hypermethylated *Kiss1* promoter region ('3' in fig. 2), it may enhance histone H3 acetylation of the *Kiss1* promoter region via recruitment of histone acetyltransferases ('4' in fig. 2) and reduction of histone deacetyltransferases ('5' in fig. 2). We further speculate that this activating histone modification forms the chromatin loop between the promoter region and the 3' downstream region of the *Kiss1* locus (fig. 1a), resulting in AVPV *Kiss1* gene expression and, hence, induction of the preovulatory GnRH/gonadotropin surge in females (fig. 2). It is worth noting that the mechanism underlying AVPV *Kiss1* gene expression seems conserved in males of nonrodent species. Our recent studies showed estrogen-induced activation of preoptic kisspeptin neurons (possibly equivalent

to AVPV kisspeptin neurons), which was accompanied by an LH surge in castrated male goats and monkeys [22, 23].

Likewise, a possible mechanism regulating ARC *Kiss1* gene expression before and after the onset of puberty is illustrated in figure 3. We imagine that polycomb repressive complex 2 and perhaps the estrogen-ERα complex, interacting with some other transcriptional factors such as c-Jun, bind to the hypermethylated *Kiss1* promoter to repress ARC *Kiss1* gene expression until the onset of puberty (fig. 3a). At the onset of puberty, polycomb repressive complex 2 reportedly dissociates from the *Kiss1* promoter ('1' in fig. 3b) and may possibly enhance histone H3K9/14 acetylation and H3K4 trimethylation in the *Kiss1* promoter region ('2' in fig. 3b), resulting in *Kiss1* gene expression for stimulating GnRH/gonadotropin pulses. In particular, his-

tone H3K9/14 acetylation ('3' in fig. 3b) seems to form the chromatin loop between the promoter and 5' upstream regions of the *Kiss1* locus in the absence of estrogen (fig. 1b), resulting in upregulation of ARC *Kiss1* gene expression via unknown mechanisms ('4' in fig. 3b). These are hypothetical scenarios, and further studies are needed to clarify the molecular and epigenetic cascades responsible for region-specific *Kiss1* gene expression and regulation.

Overall, the recent studies have increased our understanding of molecular and epigenetic mechanisms regulating *Kiss1* gene expression in the rodent hypothalamus. There are still important unanswered questions. For instance, are there activating and inhibiting histone modifications other than histone H3K9/14 acetylation that are involved in estrogen-induced *Kiss1* gene expression in the AVPV? How does the estrogen-ER α complex decrease histone acetylation in the *Kiss1* promoter region as

well as subsequent *Kiss1* gene expression in the ARC? Does the estrogen-ER α complex control histone modifications of the *Kiss1* locus in the peripubertal period? How does the estrogen-ER α complex alter *Kiss1* promoter methylation in the prenatal period in males? Further detailed analyses are required to clarify the molecular and epigenetic mechanisms regulating *Kiss1* expression in the AVPV and ARC in the future.

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