# Chapter 2
# Structure, Synthesis, and Phylogeny of Kisspeptin and its Receptor

**Shinji Kanda and Yoshitaka Oka**

**Abstract** The kisspeptin system is considered to be essential for successful mammalian reproduction. In addition to the Kiss1 peptide, Kiss2, the product of *kiss2* (the *kiss1* paralogue), has also been shown to activate kisspeptin receptor signaling pathways in nonmammalian species. Furthermore, in nonmammalian species, there are two subtypes of receptors, Gpr54-1 (known as GPR54 or Kiss1R in mammals) and Gpr54-2. Although complete understanding of the two kisspeptin—two kisspeptin receptor systems in vertebrates is not so simple, a careful examination of the phylogeny of their genes may provide insights into the functional generality and differences among the kisspeptin systems in different animal phyla. In this chapter, we first discuss the structure of kisspeptin ligands, Kiss1 and Kiss2, and their characteristics as physiologically active peptides. Then, we discuss the evolutionary traits of *kiss1* and *kiss2* genes and their receptor genes, *gpr54-1* and *gpr54-2*. It appears that each animal species has selected either *kiss1* or *kiss2* rather randomly, leading us to propose that some of the important characteristics of kisspeptin neurons, such as steroid sensitivity and the anatomical relationship with the hypophysiotropic GnRH1 neurons, may be the keys to understanding the general functions of different kisspeptin neuronal populations throughout vertebrates. Species differences in *kiss1*/*kiss2* may also provide insights into the evolutionary mechanisms of paralogous gene-expressing neuronal systems. Finally, because kisspeptins belong to one of the members of the RFamide peptide families, we discuss the functional divergence of kisspeptins from the other RFamide peptides, which may be explained from phylogenetic viewpoints.

S. Kanda, Ph.D • Y. Oka, Ph.D. (✉)
Department of Biological Sciences, Graduate School of Science, University of Tokyo,
7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan
e-mail: okay@biol.s.u-tokyo.ac.jp

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## Introduction

As introduced in Chap. 1, kisspeptin is now considered to be an essential component of the central regulation of reproduction, because the lack of *Kiss1* or kisspeptin receptor (*Kiss1r* or *Gpr54*) genes causes hypogonadotropic hypogonadism in humans and rodents. On the other hand, accumulating evidence suggests that *kiss2*, a paralogous gene for *kiss1*, widely exists in the vast majority of vertebrates, although this gene appears to have been lost in placental mammals. Because the peptide products of these paralogous genes, Kiss1 and Kiss2,[1] show the same extent of receptor activation for Gpr54, these genes, which probably have arisen by genome-wide duplication, should be considered as "kisspeptins" from both receptor affinity and phylogenetic viewpoints.

In this chapter, we will introduce the structure and phylogeny of kisspeptin peptides first. Then, projections and steroid sensitivity of the kisspeptin-expressing neurons will be discussed. Finally, because it is known that kisspeptins, Kiss1 and Kiss2, belong to the RFamide family, we also discuss the characteristics of kisspeptins as members of the RFamide family peptides.

## Structure of Kisspeptin

Like other peptide neurotransmitters/neuromodulators, kisspeptin is initially translated into long kisspeptin precursors, which are cleaved or processed to form shorter mature peptides. For instance, the human kisspeptin is first translated to prepro-kisspeptin (Kisspeptin-145, consisting of 145 amino acids), including the signal peptide to be loaded to the peptide vesicles. The peptide is then proteolytically cleaved at the site next to the dibasic residues by subtilisin-like convertase, and the C terminal-RFG is amidated by carboxypeptidase, as in the processing of GnRH peptides [1]. Because several other kisspeptin peptides shorter than 54 amino acid residues have also been found, it is suggested that the peptides are degraded from the N terminus to produce shorter but still active peptides (Fig. 2.1a). From human placental extracts, for example, kisspeptin-54, -14, and -13 have been purified [1, 2]. Although the relative potency for their activation of the receptor Gpr54 varies slightly when the N terminal amino acid length changes, it was suggested that 10 amino acid residues from the C terminal RY-$NH_2$ (for instance, YNWNSFGLRY-$NH_2$ for rodent *Kiss1*, and YNWNSFGLRF-$NH_2$ for primates; see Fig. 2.1b) are essential and sufficient for the activation of Gpr54 signaling pathways. For Kiss2, a kisspeptin-12 isoform (SKFNFNPFGLRF-$NH_2$) has been isolated from *Xenopus laevis*

[1] According to the Zebrafish Information Network, ZFIN; http://zfin.org/zfinfo/nomen.html, we will italicize gene names, such as *kiss1* and *kiss2*, and romanize protein and peptide name, such as Kiss1 and Kiss2 in this chapter. We will call the receptor for kisspeptins as "GPR54" because of the promiscuous nature of ligands and receptors for RF amide families, including kisspeptin. For details, see Kanda and Oka [37].

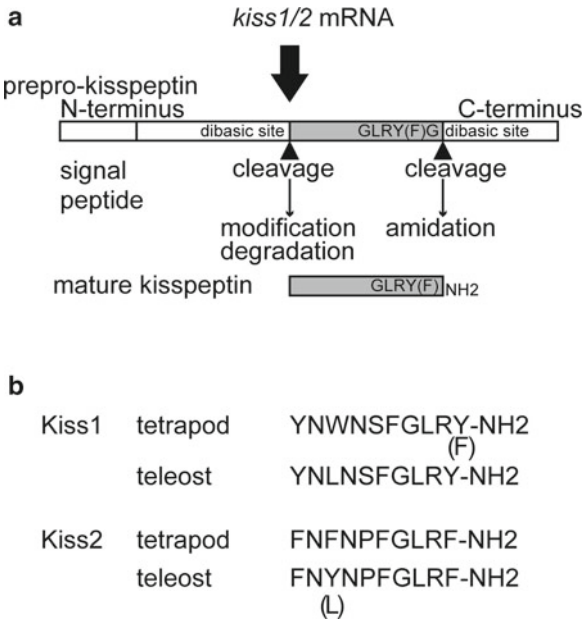


**Fig. 2.1** Schematic illustration of Kiss1/Kiss2 peptide maturation process and conserved peptide core sequence in vertebrates. (**a**) Prepro-kisspeptin molecules are cleaved into shorter kisspeptin by the dibasic cleavage sites. The C terminus of kisspeptin is amidated to the characteristic RF or RY motif. After cleavage, amidation occurs at C terminus, and degradation and/or modification such as pyroglutamate formation may occur at N terminus. (**b**) A summary of core sequence of Kiss1 and Kiss2 in vertebrates. Note that tyrosine and tryptophan possess similar side-chain

brains [3]. From the prediction of a cleavage site and subsequent binding assay studies, it has been shown that the 10 amino acid "core sequence" is essential and sufficient for the full activation of Gpr54 by Kiss1 and Kiss2 throughout vertebrates in general [3–5] (Fig. 2.2). Consequently, many researchers refer to the peptides that possess the highly conserved 10 amino acid core sequence as "kisspeptins" and have used kisspeptin-10 as kisspeptin in many studies. However, not many studies have purified "native" forms of kisspeptins in various vertebrate species, and we should therefore be careful about the interpretation of physiological experiments using only the kp-10 as kisspeptin ligands, since there may be some other physiological functions that are slightly different when conveyed by the natural peptides.

## Evolution of Kisspeptins and Their Receptors

### *Phylogeny of Kiss1 and Kiss2 Genes*

For proteins that possess longer amino acid residues, sequence similarity of proteins can be used for the construction of phylogenetic trees rather easily. However, shorter

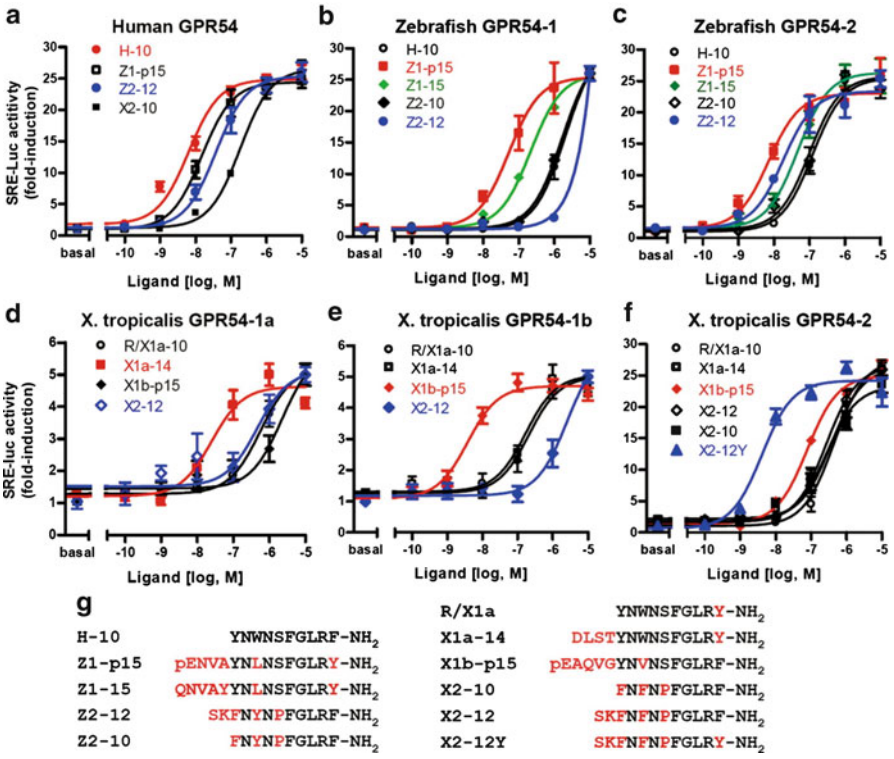


**Fig. 2.2** Both Kiss1 and Kiss2 activate both Gpr54-1 and Gpr54-2 in *Xenopus* and zebrafish. Although the relative potency differs among different receptor subtypes, Kiss1 or Kiss2 longer than 10 amino acid residues show activation at physiological concentrations, suggesting that Kiss1 and Kiss2 are the ligands for Gpr54-1 and Gpr54-2. Luciferase assay. Adapted with permission from Lee YR, Tsunekawa K, Moon MJ, Um HN, Hwang JI, et al. (2009) Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates. Endocrinology 150: 2837–2846. 2009 © Endocrine Society

peptides, including the kisspeptins, share only a small number of conservative sequences in common. Therefore, to unveil the phylogenetic relationship of certain genes among or within species, especially for shorter peptides, the synteny analysis will often give us powerful evidence for such relationship. It can be used to predict if certain genes in the other species are the homologous gene or just analogous ones by chance.

In genetics, synteny describes the physical co-localization of genes in a certain genetic locus. Recent synteny analysis of kisspeptin genes proposes that *kiss1* and *kiss2* are paralogous to each other and that they have arisen as a result of gene duplication at the locus level. The synteny analysis of *kiss1* and *kiss2* genes in several vertebrate species strongly suggested that *kiss1* and *kiss2* are duplicated together with some surrounding genes such as *golt1a*/*b*, *plekha5*/*6*, *pik3c2b*/*cg*, and *etnk1*/*2*

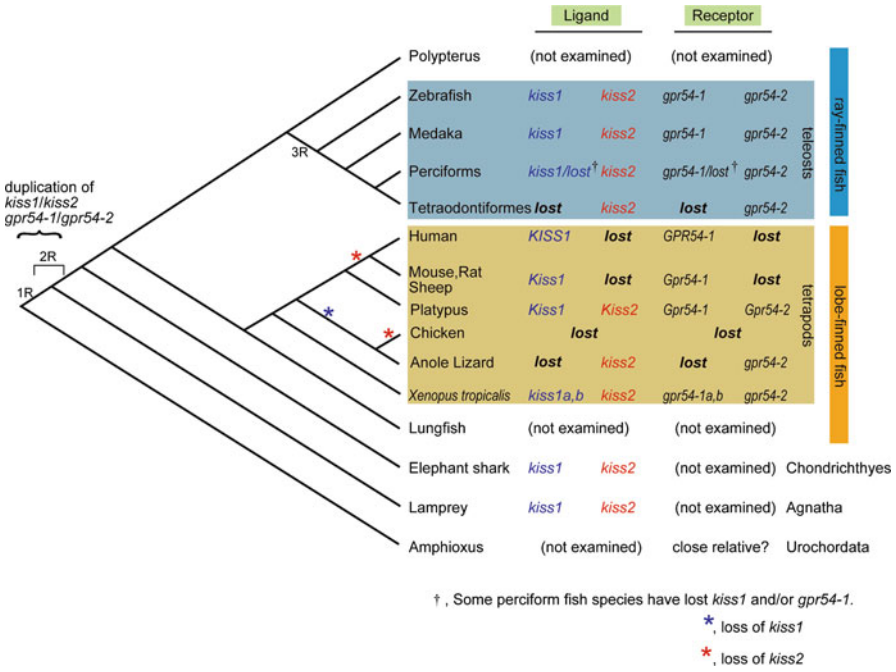


**Fig. 2.3** Summary of ligand and receptor genes for kisspeptin systems (*kiss1*/*kiss2*) along the vertebrate lineage. *kiss1* and *kiss2* are suggested to be duplicated before the emergence of lamprey, probably due to the whole genome duplication of the ancestral vertebrate. It is suggested that *kiss2* and *gpr54-2* were lost in marsupial and placental mammals after the divergence from the monotreme during mammalian evolution. Some teleost species have lost *gpr54-1*, but no teleosts have lost *gpr54-2*, suggesting the significance of gpr54-2 in the regulation of teleost reproduction, which is opposite to the case in mammals. It is also consistent with the higher level and wider distribution of expression of *gpr54-2* compared to that of *gpr54-1* in teleost brains. The loss of *kiss1* (*blue*) or *kiss2* (*red*) is indicated by *asterisks* (figure as originally published in Kanda S and Oka Y (2012) Evolutionary insights into the steroid sensitive *kiss1* and *kiss2* neurons in the vertebrate brain. Front. Endocrin. 3: doi: 10.3389/fendo.2012.00028)

[6, 7], because these genes are located in the same locus as their paralogues, suggesting that they have been duplicated at the locus level. Because the phylogenetically old vertebrate, lamprey, has both *kiss1* and *kiss2* (see Fig. 2.3), the duplication of the locus probably occurred in the basal vertebrate. Presumably, the 1R or 2R whole genome duplication[2] produced the paralogous system of *kiss1* and *kiss2*. It is speculated that these peptides shared their receptor Gpr54 in each species in the long evolutionary history, as discussed below.

[2]In the vertebrate lineage, whole genome duplication (WGD) events [8] are considered to have taken place three times in teleosts (1R-3R) and twice (1R and 2R) in tetrapods [9].

## *Phylogeny of gpr54-1 and gpr54-2*

It has also been generally accepted that the gene for the kisspeptin receptors, *gpr54*, has also been duplicated before the divergence of teleosts and tetrapods, as shown in Fig. 2.3 (see ref. [37]). Recent studies have shown by in vitro luciferase reporter assays that both Kiss1 and Kiss2 ligands activate both Gpr54-1 and Gpr54-2, and that these genes have been duplicated early in the vertebrate lineage [7, 8]. Thus, in the mammalian lineage, *Kiss1* and *Gpr54* are the only kisspeptin and kisspeptin receptor, because *Kiss2* and *Gpr54-2* were lost after divergence from the monotremes.

Some studies refer to *gpr54-1* and *gpr54-2* as *kiss1r* and *kiss2r* in accordance with [9] and because Gpr54-2 has relatively higher affinity for Kiss2 in zebrafish. However, because it has been clearly shown that both Kiss1 and Kiss2 bind to and activate both Gpr54-1 and Gpr54-2 [3–5], and, in contrast, the one-to-one relationship between kisspeptin (Kiss1 or Kiss2) and the receptor (Gpr54-1 or Gpr54-2) has not been unequivocally demonstrated by either anatomical or physiological methods, we simply call them *gpr54-1* and *gpr54-2* in this chapter. We are of the opinion that we should wait for the anatomical and/or physiological demonstration of the projection of Kiss1/Kiss2 neurons and the distribution of kisspeptin receptors before we can refer to them as *kiss1r* or *kiss2r*.

Figure 2.3 summarizes, as a phylogenetic tree, the expression of *kiss1/2* and *gpr54-1/-2* in some representative species. Interestingly, *gpr54-2* is suggested to play more critical roles in teleosts, because some species lack *gpr54-1*, and broader distribution of *gpr54-2* expressions in the brain has been reported in some teleosts. In contrast, it is interesting that *gpr54-2* has been lost in mammals during evolution, and *gpr54-1* appears to have taken its place. Although there has been no study to systematically examine the distribution or cellular localization of *gpr54-1* or *gpr54-2* in the nonmammalian tetrapod brain, the inverse situation in teleosts and mammals is intriguing. Likewise, it is interesting that wider variety of species in teleosts appear to possess *kiss2* compared to *kiss1*. Moreover, *kiss1* is lost in reptiles, while *kiss2* remains intact. Thus, except for mammals, *kiss2* appears to be more widely conserved throughout vertebrate species. However, the contribution of *kiss1* or *kiss2* to the central regulation of the hypothalamic–pituitary–gonadal (HPG) axis should not be evaluated only by the existence or absence of the gene(s) in the phylogenetic tree, because the loss of the gene can be functionally compensated for by the other genes, especially by close relative genes.

By taking the phylogenetic tree into account, it is clear that both the genes for ligands and receptors have been duplicated at least before the divergence of teleosts and tetrapods. In other words, the common ancestor of teleosts and tetrapods are considered to have possessed two ligands and two receptors. Except for the complete loss of the kisspeptin system in avian species, reported in chicken and zebrafinch [7], the genes for at least one ligand and one receptor remained in each vertebrate species. The losses of genes seem to have taken place randomly, but, as described above, there appears to be some basic rules for the gene loss by natural selection in each branch. In the long history of vertebrate evolution, it appears that

either one of the genes, *kiss1* or *kiss2*, and *gpr54-1* or *gpr54-2*, acquired predominant functions in the HPG axis regulation, which were different among branches. That is because Kiss1 and Kiss2 show similar binding activity to Gpr54-1 and Gpr54-2 immediately after their divergence. Interestingly, there is an observed tendency for the species that lost Gpr54-1 to also have lost Kiss1. Obviously, much more extensive analyses using different species in different phylogenetic branches are needed to verify the general significance of this phenomenon.

## Functional Evolution of Kiss1 and Kiss2

As described above, the kisspeptin system is well conserved among vertebrate species, except for the avian species. However, the general physiological functions of kisspeptin in vertebrates still remain to be elucidated. To date, numerous studies have reported on the involvement of kisspeptin(s) in the regulation of the HPG axis. In mice, kisspeptins have been reported to act on GnRH neurons directly, by acting on some intrinsic ion channels to produce strong persistent depolarization [10–15] (see Chap. 6). From the initial reports that loss of *Kiss1* or *GPR54* genes in human and rodents disrupts puberty and leads to hypogonadotropic hypogonadism [16–18], and Kiss1 peptide administration induces LH release by activating Gpr54 localized in GnRH1 neurons [11, 19, 20], it is clear that the kisspeptin-Gpr54 system plays a critical role in the regulation of HPG axis at least in placental mammals (reviewed in ref. [21]). On the other hand, there are fewer studies supporting similar regulatory mechanisms of the kisspeptin system in nonmammalian vertebrates. Moreover, it should be noted that the avian lineage can reproduce in spite of the fact that they lack both the *kiss1* and *kiss2* systems (see Fig. 2.3). Therefore, there are presumably other mechanisms in addition to the kisspeptin system for the central regulation of reproduction, at least in birds.

Studies in teleost kisspeptin systems seem to have yielded more complex situations. There are conflicting results that either support the presence of co-expression of *gpr54* in GnRH1 neurons in European seabass [22] and a tilapia *Oreochromis niloticus* [23] or their absence in another species of tilapia *Astatotilapia burtoni* [24]. Our unpublished results in medaka also showed the absence of *gpr54* mRNA in GnRH1 neurons. Thus, the results of previous reports suggest that the situation is different among different species. Studies of exogenous administration of kisspeptins have been performed both in mammals and teleosts. In mammals, to our knowledge, all the studies to date showed an increase in plasma LH [20, 25–34]. In contrast to the wealth of knowledge in mammals, a much smaller number of studies have been performed in nonmammalian vertebrates. Kiss1 and/or Kiss2 increased LH mRNA or serum LH concentration in zebrafish [35], sea bass [6], and goldfish [5]. The experimental conditions for the occurrence of a rise in LH induced by kisspeptin, and the time of LH/FSH rise after administration of kisspeptin, vary among the different studies. Thus, in teleosts, the situation is somewhat different from those obtained from the placental mammals, where kisspeptin is essential for reproduction.

To our knowledge, there has been no report on the kisspeptin neuronal systems of nonmammalian species other than teleosts and placental mammals, and there is a big gap between these two branches of animals. In fact, during the long history of vertebrate phylogeny, birds are the only species that have lost both *kiss1* and *kiss2*, suggesting that the kisspeptin system may have increased genetic fitness in each species. Thus, the identification of the general functions in vertebrates, including the regulation on HPG axis, is yet to be concluded.

## Steroid Sensitivity of Kisspeptin Neurons Is Conserved Among Vertebrates

As discussed above, the kisspeptin system has been suggested to play important roles in the regulation of reproduction in mammals, but not in birds, while it may also be involved in the regulation of some reproductive functions in teleosts. From the survey of literature on the functional aspects of kisspeptin in vertebrates, the apparent conservative nature of the kisspeptin-Gpr54 system undoubtedly suggests considerable contribution of kisspeptin on "evolutionary fitness." Interestingly, the sex steroid sensitivity of kisspeptin-expressing neurons appears to be well conserved among teleosts [36–39] and mammals [40–44], suggesting that this sex steroid sensitive nature of kisspeptin neurons was already present before the divergence of teleosts and tetrapods; the steroid sensitivity is likely to be a general feature of all the kisspeptin systems throughout vertebrates. As described in the previous section, in placental mammals, the *Kiss1* neurons are strongly suggested to mediate sex steroid feedback effects; these neurons receive sex steroid signals from the gonads and directly and/or indirectly modulate the activity of GnRH1 neurons [10–15]. The mediators of sex steroid feedback, i.e., the neurons that directly receive sex steroids and control the release of GnRH, have long been searched for, because GnRH neurons themselves lack estrogen receptor alpha (reviewed in ref. [45]), which is essential for the sex steroid control of reproduction in mammals [46–48]. Because *Kiss1* neurons in mammals express ER alpha, and *Kiss1* mRNA expression in many placental mammalian species is negatively regulated in the arcuate nucleus and positively regulated in the anteroventral periventricular nucleus (AVPV)/preoptic area (POA) [20, 40–44, 49, 50], the *Kiss1* neurons are the most plausible candidate as the "missing link" in the steroid feedback mechanism. From several lines of recent experimental evidence, it is now hypothesized that the arcuate kisspeptin neurons and the AVPV kisspeptin neurons are involved in negative and positive feedback, respectively [40, 51] (see Chap. 13).

In nonmammalian species, the sex steroid sensitivity of kisspeptin neurons has been demonstrated experimentally only in teleosts, medaka, and goldfish [36, 39]. In medaka, among several populations of *kiss1* and *kiss2* neurons in the brain, only the *kiss1* neurons in a hypothalamic nucleus, nucleus ventralis tuberis (NVT), show sex steroid sensitivity; NVT *kiss1* expression is positively regulated by gonadal steroids, probably directly via sex steroid hormone receptors [36, 39]. On the other

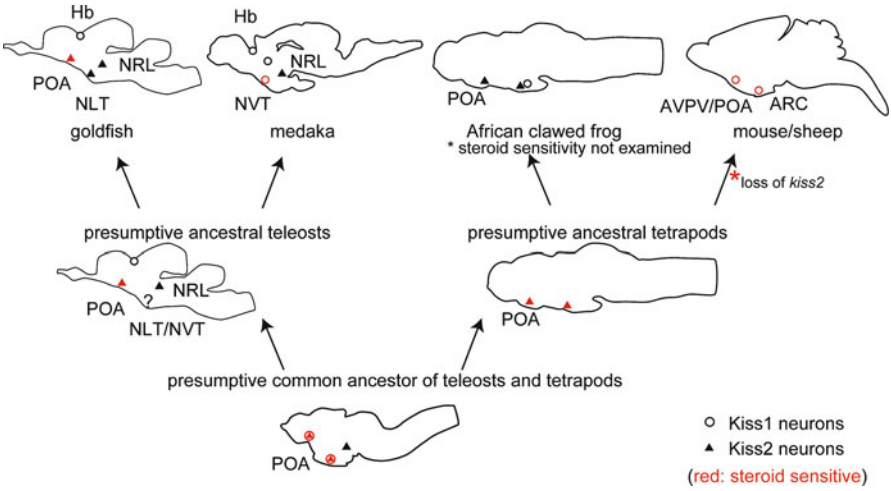


**Fig. 2.4** Schematic illustrations for the distribution of *kiss1* and *kiss2* neurons in vertebrate brains, including some hypotheses. Because *kiss1* and *kiss2* are duplicated paralogues, they are considered to have been co-expressed in the same neurons in the common ancestor of teleosts and tetrapods. Given that both amphibians and teleosts express *kiss2* in POA, the ancestral teleosts and ancestral tetrapods should have expressed *kiss2*. Because *Kiss2* was lost in the mammalian lineage, we hypothesize that *Kiss1* began to be expressed where *Kiss2* used to be expressed, to compensate for the loss of *Kiss2* during mammalian evolution. *Open circles* indicate *kiss1*, and *filled triangles* indicate *kiss2* neurons. *Circles*/*triangles* in *red* are *kiss1*/*kiss2* neurons that are steroid sensitive (figure as originally published in Kanda S and Oka Y (2012) Evolutionary insights into the steroid sensitive *kiss1* and *kiss2* neurons in the vertebrate brain. Front. Endocrin. 3: doi: 10.3389/fendo.2012.00028)

hand, in goldfish, which lack *kiss1* neurons in NVT, POA *kiss2* neurons are the only population of kisspeptin neurons that shows steroid sensitive kisspeptin mRNA expression in the brain [37]. These POA *kiss2* neurons are also positively regulated by gonadal steroids, as in the NVT *kiss1* neurons in medaka, and thus there has been no report of negative regulation of kisspeptin expression in teleost brain so far. Considering the report that positive or negative steroid feedback regulation can be rather easily changed by the composition of co-expressing transcription factors [52], the important common feature of the vertebrate kisspeptin neurons may be that steroid sensitive kisspeptin neurons are localized in NVT and POA, which are anatomically similar to arcuate and POA/AVPV in mammals, respectively. Although the precise homology of brain nuclei between mammalian and nonmammalian (especially teleost) kisspeptin neurons should be carefully discussed, the presence or absence of sex steroid sensitivity in each nucleus may be one of the strongest pieces of evidence to argue such homology.

An evolutionary working hypothesis of kisspeptin neuronal systems in vertebrates is shown in Fig. 2.4. In this hypothesis, *kiss1*- and *kiss2*-expressing neurons are differentially distributed in the brains of mammal and other vertebrates. It is suggested that the loss of *Kiss2* gene during mammalian evolution has been probably been

compensated functionally by the closely related *Kiss1* gene (for detailed discussion, see ref. [38]). From the arguments about the sex steroid sensitivity of kisspeptin neurons thus far, it is suggested that this property of kisspeptin neurons is one of the most general and important properties among the vertebrate kisspeptin systems; it may have been already acquired before the divergence of teleosts and tetrapods.

## Axonal Projections of the Kisspeptin Neurons and the Distribution of Kisspeptin Receptors

### *Projections of Kiss1 Neurons in Mammals*

In mammalian species, several studies have analyzed anatomical relationships between the Kiss1 neurons and the other components in the HPG axis, most importantly, the GnRH1 neurons. Recently, Clarkson et al. detailed the projections of Kiss1 neurons in the mouse brain [53]. It was shown that Kiss1-immunoreactive (ir) fibers were abundant in the ventral aspect of the lateral septum and the hypothalamus, running in periventricular and ventral retrochiasmatic pathways, except for the suprachiasmatic and ventromedial nuclei. Moreover, Kiss1-ir fibers were observed in the internal zone of the median eminence, but not in its external layer where GnRH and other hypophysiotropic hormone-containing axons are proposed to terminate. In addition, a small number of kisspeptin fibers were also observed outside the hypothalamus, in the bed nucleus of the stria terminalis, subfornical organ, medial amygdala, paraventricular thalamus, periaqueductal grey and locus coeruleus. These findings are consistent with a study in the rat brain using different antiserum, although there were some discrepancies in the distribution outside the hypothalamus [54]. In mammalian species, heavy projections to some hypothalamic and preoptic nuclei now seem to be the general consensus on the distribution of Kiss1 neurons. Moreover, Kiss1 fibers have been shown to project to the median eminence, and make close contacts with GnRH1 fibers in mammals [55–57]. Because GnRH1 neurons express *gpr54* in mammals, Kiss1 neurons are hypothesized to stimulate GnRH release from GnRH axons at the nerve terminals in or near the median eminence.

### *Projections of Kiss1/Kiss2 Neurons in Teleosts*

There are a limited number of neuroanatomical studies detailing the axonal projections of Kiss1 and Kiss2 neurons and the distribution of Gpr54 in nonmammalian species, and most of the studies have been carried out in teleosts. As described below, the projections of Kiss1 and Kiss2 neurons in nonmammalian species are complicated; the steroid sensitive Kiss1 neurons in medaka project to POA, but only Kiss2 neurons project to POA in zebrafish. On the other hand, the distribution of receptor subtypes show rather consistent results in most teleost studies so far;

*gpr54-2* seems to have significant expression in POA in zebrafish and medaka, while *gpr54-1* has been lost in some species.

In zebrafish, Kiss1 neurons, located in the habenula, were demonstrated to project to the interpeduncular and raphe nuclei. On the other hand, Kiss2 neurons, whose cell bodies were shown to be localized in the dorsal and ventral hypothalamus, widely projected to the ventral telencephalon, POA, thalamus, and ventral/caudal hypothalamus, suggesting that Kiss2 neurons mainly function as a homeostatic regulator in this species [58]. *Gpr54-2* was shown to be predominantly expressed in POA, ventral telencephalon, hypothalamus, and several nuclei of the brain. However, *gpr54-1* was only expressed in habenula. Close apposition of Kiss2-ir fibers to GnRH1 neurons was also observed in zebrafish, although the authors did not examine the co-expression of *gpr54* in GnRH neurons.

On the other hand, in medaka, in addition to the habenulo-interpeduncular pathway, the Kiss1 neurons were shown to project many fibers to the POA, ventral telencephalon, and hypothalamus, but not to the rest of the brain [59], which is consistent with our recent results of immunohistochemical demonstration of the projections of Kiss1 neurons in the Kiss1-EGFP medaka established by us (Shimada et al., unpublished observations). This predominant distribution of Kiss1 fibers in the hypothalamus and POA correspond to the dense distribution of *kiss1* mRNA expressing neurons in the hypothalamus of medaka, whereas zebrafish lacks such Kiss1 neurons in the hypothalamus. Because the Kiss1 neurons in the medaka hypothalamic nucleus NVT show high sex steroid sensitivity [36] it is suggested that the release of Kiss1 in the POA, hypothalamus, and ventral telencephalon will also vary according to the breeding state. Furthermore, the receptor distribution was also examined for *gpr54-1*and *-2* by in situ hybridization, and *gpr54-2* was shown to be widely expressed in the medaka brain, especially in regions that are involved in homeostatic regulation, consistent with the Kiss1 neuron projections. On the other hand, as in the case of zebrafish, the expression of *gpr54-1* was practically confined to the habenula and POA (Kanda et al., unpublished data). Recently, indirect effects of kisspeptin on non-hypophysiotropic GnRH3 neurons was reported [60]. Further studies of hypophysiotropic and non-hypophysiotropic function of kisspeptin neurons are important for the understandings of physiological functions of kisspeptin systems.

In a Cichlid fish, *A. burtoni*, the distribution of kisspeptin receptor was examined by in situ hybridization [24]. The authors reported that the fish lacked *gpr54-1*. The cells expressing *gpr54-2* were localized in hypothalamus, POA, and ventral telencephalon, which is similar to the results of zebrafish and medaka. In addition, *gpr54-2* was shown to be expressed in the dorsal telencephalon and some other brain regions, with the most prominent expression in the olfactory bulb. Since *gpr54-2* was not expressed in the GnRH1 neurons, the authors suggested the existence of non-GnRH1 neurons that express *gpr54-2* and may exhibit sensitivity to kisspeptin, according to the social state or sexual maturation of the fish [24].

From these studies, it may be suggested that *gpr54-2* is likely to play a wide variety of roles in teleosts, because *gpr54-1* positive cells are localized mainly in habenula and other restricted regions in the brain in medaka and zebrafish, or even absent in some species [24, 61–63], whereas there is no report of teleost species that lack *gpr54-2*. Therefore, it may be suggested that *gpr54-1* became predominant in

the mammalian lineage (probably tetrapod lineage), whereas *gpr54-2* became predominant in the teleost lineage. To date, there is no in situ hybridization study of *gpr54* in amphibians. Such studies may give us clues to understanding the history of selection of *gpr54-1* or *-2* during vertebrate evolution.

## Kisspeptin as a Peptide Belonging to the RFamide Family of Peptide

A phylogenic tree for the RFamide family of peptides is shown in Fig. 2.5. As shown in the figure, the kisspeptin genes have already diverged from the other RFamide families and form an independent branch. The first identification of

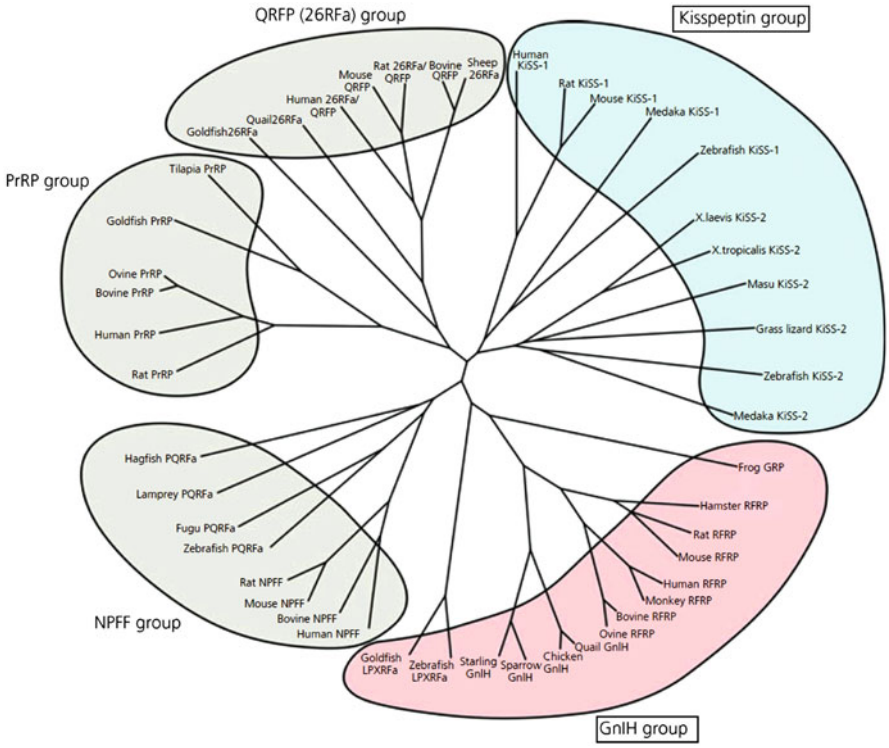


**Fig. 2.5** A phylogenetic tree of RFamide peptide family, including *kiss1* and *kiss2*. *npff* group and *gnih* (*rfrp*) group share the same receptor, Gpr147 and 74. In addition, Kiss1 and Kiss2 have been shown to activate Gpr147 and Gpr74 in some species as well. Comprehensive understanding of the promiscuous relationship of ligands and receptors in the RFamide family of peptides is necessary for the future studies. Adapted from Tsutsui et al., Discovery and Evolutionary History of Gonadotrophin-Inhibitory Hormone and Kisspeptin: New Key Neuropeptides Controlling Reproduction. J Neuroendron 716–727. 2010 © The British Society for Neuroendocrinology. With permission from John Wiley and Sons

RFamide peptide in animals goes back to the finding of FMRFamide in sunray Venus clam *Macrocallista nimbosa* [64]. Many RFamides have been identified in invertebrates since then [65], although their evolutionary relationship to the vertebrate RFamide family is yet to be elucidated. Among the vertebrate RFamide family, it is suggested that *kiss1*, *kiss2*, *rfrp*/*gnih*, and *npfff*/*pqrf* had already diverged in the basal vertebrate, because these four genes have been identified in lamprey [3, 66–68]. Like the paralogous relationship of *kiss1* and *kiss2*, *rfrp* and *npff* are also considered to be duplicated during the whole genome duplication, because they are closely located to the HoxA and HoxC cluster respectively, which are supposed to be duplicated in the whole genome duplication [69]. On the other hand, *prrp* and *qrfp* (*26rf*) have been identified only in teleosts and tetrapods so far, and the presence of these genes in the ancestral vertebrate is still argued. Thus, including *kiss1* and *kiss2*, the RFamide family has already been listed in the early evolution of vertebrate lineage.

It is important to note that their receptors are also close to one another both in sequence and binding capacity for the relative ligands. In fact, human Kiss1 is shown to activate Gpr74 and Gpr147 [70, 71], suggesting the promiscuous relationship between the ligand and receptor among the RFamide group. Interestingly, in spite of this promiscuous relationship, the properties of the receptors are completely different; Gpr54 couples to Gq [2], whereas Gpr147 and Gpr74 both couple to Gi and Gs [72]. In fact, in contrast to kisspeptin, RFRP has been shown to inhibit reproduction [73–79]. Thus, the effect of in vivo or in vitro administration of peptides should be considered with caution, because of the pharmacological side effects via different types of receptors. In the central nervous system, the precise information on the projection of each neuron makes it possible to discriminate the promiscuous ligand–receptor relationship of RFamide and their receptor family.

## Conclusions

In this chapter, the structure, function, and phylogeny of kisspeptin and Gpr54, and projection and steroid sensitivity of kisspeptin neurons were discussed. It is a complex but interesting situation that the RFamide family of peptides show promiscuous ligand–receptor relationships, thereby making the results of in vivo pharmacological experiments not always possible to tell the natural or physiological effects of RFamides in the central nervous system. Comprehensive understanding of the morphological/anatomical and physiological characteristics of the neurons, receptor distributions, and ontogenic expression may explain the kisspeptin functions at the organismal level and the mechanisms of kisspeptin actions in the brain. Moreover, the *kiss1* and *kiss2* genes, and the neurons expressing these genes, may be considered as the model for the study of paralogous gene functions, because the genes have avoided strong selection pressure in vertebrates other than mammals, unlike the GnRH system. Consequently, studies of kisspeptin may open a new era of understanding the physiological functions of paralogous genes in addition to a better understanding of neuroendocrine systems.

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