General and Comparative Endocrinology 192 (2013) 237–245

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Insight from the lamprey genome: Glimpsing early vertebrate development via neuroendocrine-associated genes and shared synteny of gonadotropin-releasing hormone (GnRH)

Wayne A. Decatur [a], Jeffrey A. Hall [a], Jeramiah J. Smith [b], Weiming Li [c], Stacia A. Sower [a,*]

[a] Center for Molecular and Comparative Endocrinology and Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH 03824, USA
[b] Department of Biology, University of Kentucky, Lexington, KY, USA
[c] Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI, USA

ARTICLE INFO

Article history:
Available online 12 June 2013

Keywords:
Evolution
Paralogs
Gnathostomes
Agnathans
Orthologs
Phylogenetics

ABSTRACT

Study of the ancient lineage of jawless vertebrates is key to understanding the origins of vertebrate biology. The establishment of the neuroendocrine system with the hypothalamic–pituitary axis at its crux is of particular interest. Key neuroendocrine hormones in this system include the pivotal gonadotropin-releasing hormones (GnRHs) responsible for controlling reproduction via the pituitary. Previous data incorporating several lines of evidence showed all known vertebrate GnRHs were grouped into four paralogous lineages: GnRH1, 2, 3 and 4; with proposed evolutionary paths. Using the currently available lamprey genome assembly, we searched genes of the neuroendocrine system and summarize here the details representing the state of the current lamprey genome assembly. Additionally, we have analyzed in greater detail the evolutionary history of the GnRHs based on the information of the genomic neighborhood of the paralogs in lamprey as compared to other gnathostomes. Significantly, the current evidence suggests that two genome duplication events (both 1R and 2R) that generated the different fish and tetrapod paralogs took place before the divergence of the ancestral agnathans and gnathostome lineages. Syntenic analysis supports this evidence in that the previously-classified type IV GnRHs in lamprey (lGnRH-I and -III) share a common ancestry with GnRH2 and 3, and thus are no longer considered type IV GnRHs. Given the single amino acid difference between lGnRH-II and GnRH2 we propose that a GnRH2-like gene existed before the lamprey/gnathostome split giving rise to lGnRH-II and GnRH2. Furthermore, paralogous type 3 genes (lGnRH-I/III and GnRH3) evolved divergent structure/function in lamprey and gnathostome lineages.

1. Introduction: The dawn of vertebrates

The Cambrian explosion, which featured the divergence of protochordates and proto-vertebrates, encompasses undoubtedly some of the most profound changes occurring in the life of our planet. Numerous and varied biological innovations arose during those tens of millions of years of evolution. Ohno (1970) was the first to propose that these significant changes were associated with genome duplications, and a growing body of knowledge has since accumulated in support of his hypothesis (see Van de Peer et al., 2009, 2010 and references therein). One of the most conspicuous pieces of evidence of the degree and the nature of these duplications comes from comparing the amphioxus genome to the human genome, revealing that the human genome is essentially a core set of protochordate segments multiplied four times and shuffled (Putnam et al., 2008). It has been proposed that the most parsimonious explanation of the mapping of the amphioxus core genome is that sometime after the chordates branched from the lineage that led to humans, two rounds of whole genome duplication occurred, referred to individually as 1R and 2R, manifesting a potential quadruple set of genes. Most of the duplicates produced by these rounds of whole genome duplication (WGD) are redundant and indeed the vast majority was lost with only a small percent (~20%) retained as genes evolving in parallel, or paralogs (Putnam et al., 2008; Smith et al., 2013).

Much of the impetus and importance of the lamprey genome project derives from the critical placement of the lamprey along the path of the development of vertebrates. The exact timing of the two rounds of whole genome duplication relative to basal

Abbreviations: GnRHs, gonadotropin-releasing hormones; lGnRH, lamprey GnRH; WGD, whole genome duplication; 1R, 1st round WGD; 2R, 2nd round of WGD; 3R, teleost-specific 3rd round of WGD; bp, base pair; kb, kilobase; NPY, neuropeptide Y.

* Corresponding author. Address: 316 Rudman Hall, 46 College Road, University of New Hampshire, Durham, NH 03824-3544, USA.
E-mail address: sasower@unh.edu (S.A. Sower).

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http://dx.doi.org/10.1016/j.ygcen.2013.05.020

vertebrates has been in question (Kuraku et al., 2009); the recent prevailing view is that the second round (2R) took place sometime after the agnathans (jawless hagfish and lamprey) diverged from the lineage that led to the later-evolved, jawed vertebrates, the gnathostomes (see for example, Andreakis et al., 2011; Dores, 2011; Parmentier et al., 2011), but see Shimeld and Donoghue (2012) for an alternative scenario. Significantly, the current evidence suggests that two genome duplication events (both 1R and 2R) that generated the different fish and tetrapod paralogs took place before the divergence of the ancestral agnathans and gnathostome lineages (Smith et al., 2013).

The establishment of the hypothalamic–pituitary axis is one of the critical innovations that arose in vertebrates (Sower et al., 2009). The presence of the hypothalamic–pituitary axis in extant agnathans (lamprey and hagfish) and gnathostome (jawed vertebrate) lineages suggests that this system arose in the vertebrate stem lineage before the divergence of these deep lineages, ~500 million years ago. Central to this neuroendocrine system are the hypothalamic decapeptide hormones, the gonadotropin-releasing hormones (GnRHs), derived from roughly 90-amino acid precursor polypeptides. Previous data incorporating phylogenetic analysis, function, neural distribution, and developmental origin showed that all known vertebrate GnRHs are grouped into four paralogous lineages: GnRH1, 2, 3 and 4 (Kavanaugh et al., 2008; Kim et al., 2012, 2011; Roch et al., 2011; Silver et al., 2004; Tostivint, 2011). Lampreys possess three GnRHs, abbreviated lGnRH-I, -II, and -III, and it was suggested that lGnRH-I and -III formed a fourth grouping of GnRHs (Kavanaugh et al., 2008; Silver et al., 2004; Suzuki et al., 2000). The identification of two additional invertebrate GnRHs and one in Annelid suggested a fifth grouping of GnRHs (Aplysia, octopus, limpet, annelid GnRH). Against this backdrop of various forms and organisms, syntenic analysis has emerged as particularly insightful for developing a proposed evolutionary history of the family of peptides, at least for the bony fish and tetrapods of the vertebrates (Kim et al., 2011; Tostivint, 2011). Recently we have used the availability of the lamprey genome assembly to extend this history to include the dawn of the vertebrates

Here we present a summary of many curated lamprey neuroendocrine genes in the current genome assembly and use a more in-depth investigation of one of the families, the gonadotropin releasing hormones and their receptors, to illustrate the power and limitations presented by the current assembly.

2. The lamprey genome at a glance

The structure and content of the lamprey genome is interesting in its own right, regardless of the precise placement of this basal vertebrate relative to hagfish and chordate species (Smith et al., 2013). The genome has a particularly high GC-base content and the number and diversity of repetitive elements among its approximately 200 chromosomes (2*n*; Smith et al., 2010) is truly remarkable. Moreover, other work revealed extensive programmed genome rearrangement in the lamprey characterized by the deletion of ~20% of germline DNA (hundreds of megabases) from somatic tissues (Smith et al., 2009, 2012). This dramatic programmed elimination of genetic content happens during embryonic development and is the first example of broad-scale programmed rearrangement of a vertebrate genome. The current genome assembly (Smith et al., 2013) is restricted to somatic cells with a lamprey liver serving as the source material. The unique features of the lamprey genome overlap substantially with those that conspire to encumber assembly of long contiguous sequences. Although steps were taken to optimize assembly in light of this challenge and more than half the currently assembly is on contigs longer than 174 kb (N50 = 174 kb) (Smith et al., 2013), it is not possible at this time to assemble the lamprey sequence into chromosomes; it is presently represented as ~25,000 scaffolds. Despite this, the current assembly provides unparalleled resolution of gene content and structure of this evolutionarily important genome. Additional features of the lamprey genome are discussed in the next two sections with the bulk of the insights reserved for the discussion of the GnRH genes below in Section 5, as these genes lend themselves particularly well to illustrating key points of the lamprey genome assembly and its revelatory nature.

3. General inferences about vertebrate whole genome duplications

The lamprey genome project was anticipated to address one of the most fundamental questions remaining about vertebrate evolution: the timing of whole genome duplication (WGD) events. Did the frequency and distribution of paralogs look more like that seen in amphioxus, placing the timing of both duplication events after the divergence of the lamprey and gnathostome lineages? Or does the distribution of lamprey paralogs look like that seen in higher vertebrates? Or does the frequency and distribution of paralogs show only one round of duplication occurring between the protochordates and the lamprey lineage? Several approaches at assessing this question all indicate that the lamprey appears to have posses a complement of ancient paralogs similar to that of gnathostomes, suggesting a similar history of duplication (Smith et al., 2013). The question now becomes how soon after 2R did the split of the lamprey lineage from the gnathostome lineage occur. The answer has profound effects on the linkage (or lack thereof) of subsequent resolution of the paralogous genes. As discussed further in Section 5, this time is likely small (relative evolutionary/geologic time) and this has significant implications for assessing orthologs of lamprey and gnathostome genes.

4. Lamprey neuroendocrine genes in the genome assembly

The repertoire of lamprey neuroendocrine genes is of particular interest since this group of factors contributed significantly to the presumptively massive increase in regulatory complexity that characterizes the shift from invertebrate chordates to vertebrates. Given the potential importance of this subset of genes, we examined the lamprey genome assembly and annotated several for inclusion in the lamprey genome project consortium release, in addition to those isolated by our lab and others. Our curated listing of these genes (Table 1) in the genome assembly is not exhaustive and primarily serves here as a basis for discussion of certain gene families, as well as features of the current lamprey genome assembly.

Examination of this subset of lamprey genes raises two salient points regarding the structure and content of the lamprey genome assembly. Although half of the assembly is in contigs of 174 kb or longer, Table 1 reflects that a number of much smaller scaffolds are also present. This can be seen by noting that many of the scaffolds listed in Table 1 are no longer than several kb, for example, 4 kb for GnRH receptor 3 and neuropeptide Y (NPY), 7 kb for somatostatin receptor 4; the value of the length of several of the scaffolds in base pairs is the number in parentheses following the project consortium scaffold designation. Given the numerous genes, and therefore associated exons, represented in the table, it is not unusual that several genes, or portions, fall on scaffolds at the small end of the range. In fact, the lamprey neuroendocrine gene families have more members than a typical gene family and thus there is a greater chance a member will lie on a small scaffold. The greater representation of parologs among the neuroendocrine genes is

Table 1
Lamprey Neuroendocrine genes in the context of the genome assembly.

	Genbank accession	Ensembl Accession[a]	Ref.	Detail in genome assembly	Consortium scaffold (size in bp)	Ensembl scaffold	Position
GnRH & Receptors							
Gonadotropin-releasing hormone I precursor	AF144481.1	ENSPMAG00000000251	Suzuki et al. (2000)	Found	176 (302,093)	GL476504	258,947–267,580
Gonadotropin-releasing hormone II mRNA	DQ457017.1	ENSPMAG00000010330	Kavanaugh et al. (2008)	Found	821 (95,111)	GL477149	91,718–92,408
Gonadotropin-releasing hormone III precursor	AY052628.1	ENSPMAG00000000250	Silver et al. (2004)	Found	176 (302,093)	GL476504	273,420–280,447
Gonadotropin-releasing hormone receptor 1 mRNA	AF439802.1	ENSPMAG00000009199 (partial)	Silver et al. (2005)	Found	17,086 (5012) exon 1,2	GL493405	1362–3509
				exon 1, 5'missing 14bp	10,311 (9708) exon 3	GL486639	5731–6753
GnRH receptor 2	HM641828	ENSPMAG00000003314(partial)	Joseph et al. (2012)	exon 2, 3 found exon 1 missing	7065 (16,526)	GL483393	10,180–10,384 7976–8578
GnRH receptor 3	HM641829	ENSPMAG00000010331(partial)	Joseph et al. (2012)	exon 1,2 found	7513 (14,552) exon 1	GL483841	12,774–13,418
				Exon 3 missing	19,484 (4031) exon 2	GL495795	954–1173
GpH/GTH & Receptors							
Glycoprotein hormone alpha 1 (cga)				Not found			
Glycoprotein hormone alpha 2	FJ265881.2	ENSPMAG00000002562	Dos Santos et al. (2009), Dos Santos et al. (2011)	Found	62 (792,450)	GL476390	400,119–402,428
Gonadotropin II beta subunit (cgbb)	AY730276.1	ENSPMAG00000007737	Sower et al. (2006)	Found	459 (134,261) 2 exons	GL476787	2762–5781
Glycoprotein hormone-beta5 (gpb5)	BN001271.1	ENSPMAG00000009915	Dos Santos et al. (2009)	Found 5' end, missing 189 bp of CDS (last 62 amino acids)	6853 (343,847)	GL483181	155,145–155,477
Testicular glycoprotein hormone receptor I precursor, mRNA (lGpHR I)	AY750688.2	ENSPMAG00000006134	Freamat et al. (2006)	missing first 197 bp(5')	6853 (343,847)	GL483181	159,644–181,679
				Missing 69 bp – 574–641 of mRNA			
Glycoprotein hormone receptor II mRNA (lGpHR II)	AY750689.2	ENSPMAG00000005673	Freamat and Sower (2008)	Missing 245 bp 5'	4268 (26,044)	GL480596	5181–15861
				15 bp gap in 3' exon			
Growth hormone family							
growth hormone	AB081461.1	ENSPMAG00000001744(partial)	Kawauchi et al. (2002)	Found exons 1,2,4,5	1, 2 on 2826 (983,188)	GL479154	271–2159
				exon 3 missing	4,5 on 24812 (37,778)	GL501072	2222–6049
Insulin-like growth factor precursor	AB081462.1	ENSPMAG00000002950	Kawauchi et al. (2002)	Found	295 (511,735)	GL476623	128,985–140,899
Growth hormone secretagogue (ghrelin) receptor		ENSPMAG00000006581	Cruz and Smith (2008)	Found	6204 (19,579)	GL482532	609–10,680
Somatostatin receptor 1[b]		ENSPMAG00000010125		Found	661 (146,424)	GL476989	42,137–43,228
Somatostatin receptor 4[b]		ENSPMAG00000000513		Found	12,776 (7352)	GL489102	299–1011
Kisspeptin family							
Kiss1	EB722290; EB722291		Lee et al. (2009)	Found	8553 (12,356)	GL484881	3144–6294
Kiss 2			Lee et al. (2009)	Found	987 (269,378)	GL477315	209,828–209,932
Kiss1 receptor (GPR54)		ENSPMAG00000001451		Found	1829 (100,293)	GL478157	14,221–35,167
Melanotropin and corticotropin							
POC mRNA for proopiocortin	D55628.1	ENSPMAG00000004886	Takahashi et al. (1995)	Found	4233 (26,611)	GL480561	13,341–15,664
Proopiomelanotropin mRNA	D55629.1	ENSPMAG00000000198	Takahashi et al. (1995)	missing last 105 bps of CDS	3365 (56,931)	GL479693	1–2428
Corticoid receptor mRNA	AY028457.1	ENSPMAG00000005858	Thornton (2001)	Found	933 (82,918)	GL477261	3134–78,930
Progestogin receptor							

(*continued on next page*)

Table 1 (*continued*)

	Genbank accession	Ensembl Accession[a]	Ref.	Detail in genome assembly	Consortium scaffold (size in bp)	Ensembl scaffold	Position
progestin receptor mRNA	AY028458.2	ENSPMAG00000004366(partial)	Thornton (2001)	Found	96 (317,340) exons 1-4	GL476424	297,512–316,908
				exon 1, 5'missing 11bp	2473 (92,703) exon 5	GL478801	11,425–11,586
Secretin family							
Proglucagon I precursor, mRNA	AF159707.1	ENSPMAG00000002186(partial)	Irwin et al. (1999)	missing last 277 bp	4775 (23,725)	GL481103	17,707–22,694
Proglucagon II precursor, mRNA	AF159708.1	ENSPMAG00000005961	Irwin et al. (1999)	Found	1627 (125,973)	GL477955	64,390–71,824
Neuropeptide Y family							
PMY	AY823512.1		Montpetit et al. (2005)	Found 3' end (86 bp of CDS) + 3'UTR; last two exons	24883 (789,298)	GL501143	785,281–786,496
NPY	AY823514.1	ENSPMAG00000010376(partial)	Montpetit et al. (2005)	Found	exon 1(5'UTR) on 24854 (108,404)	GL501114	106,307–106,424
				missing last 107 bps	exon 2 on 17951 (4670)	GL494269	88–295
PYY	AY823513.1	ENSPMAG00000007688	Montpetit et al. (2005)	Found	996 (82,217)	GL477324	72,174–75,650
Neuropeptide Y receptor Y1[c]		ENSPMAG00000009963	Pérez-Fernández et al. (2013), Salaneck et al. (2001), Xu et al. (2012)	Found	5090 (22,077)	GL481418	9833–10,936
Neuropeptide Y receptor Y5[c]	GQ429289.1	ENSPMAG00000009978	Xu et al. (2012)	Found	1961 (80,451)	GL478289	63,825–65,414
Neuropeptide Y receptor Y8[c]			Pérez-Fernández et al. (2013)	Missing			
Estrogen family							
Aromastase (cyp19)				Not found			
Estrogen Receptor 1	AY028456; AB626148*from arctic lamprey		Thornton (2001)	Found	3884 (177,925) exon 1	GL480212	158,326–157,988
					11,183 (8734) exon 2	GL487511	3419–3668
					76 (790,685) exons 3-8	GL476404	504,720–590,818
Estrogen Receptor 1 homolog (partial/ pseudogene)		ENSPMAG00000007579		Found	24924 (105,483)	GL501184	27,264–43,592
Estrogen Receptor 2	AB626149*from arctic lamprey	ENSPMAG00000008530		Found	286 (1,026,879)	GL476614	518,779–536,522
Estrogen-related receptor gamma		ENSPMAG00000002463		Found	24868 (61,197)	GL501128	21,434–36,771
Estrogen-related receptor gamma isoform 3		ENSPMAG00000008137		Found	7386 (14,760)	GL483714	804–4621
COUP-TF (NR2F1)		ENSPMAG00000000046	Klinge (1999)	Found	1746 (367,241)	GL478074	192,316–147,758
Prolactin							
Prolactin				Not found[d]			
Prolactin releasing hormone receptor		ENSPMAG00000010253		Found	162 (381,134)	GL476490	205,363–206,355
RFamide peptide family							
Petromyzon marinus PQRFa mRNA	AB233469.1		Osugi et al. (2006)	Missing 1st exon (first 145 bps of CDS) & last 61 bps of 5'UTR	4168 (27,323)	GL480496	487–2458
Petromyzon marinus LPXRfamide mRNA (GnIH)	AB661773		Osugi et al. (2012)	Found	270 (2,382,300)	GL476598	913,830–917,957
Vasopressin/oxytocin family							
Vasotocin precursor	FJ195978.1	ENSPMAG00000000237(partial)	Gwee et al. (2009)	Found	10,824 (9121)	GL487152	2528–8634
Arginine vasopressin receptor 1B		ENSPMAG00000007650		Found	2017 (59,428)	GL478345	24,256–33,787
Vasotocin/oxytocin receptor		ENSPMAG00000001242		Found	807 (600,329)	GL477135	197,258–202,768
Vasotocin/oxytocin receptor		ENSPMAG00000009764		Found	3133 (165,448)	GL479461	3925–14,587

Pineal opsin							
Pineal gland-specific opsin	AH006524.1	ENSPMAG00000007441(partial)	Yokoyama and Zhang (1997)	Found 5′ end	6496 (17,161) exon 1,2,3	GL482824	663–9412
Parathyroid hormone family							
Parathyroid hormone (PTH)			Pinheiro et al. (2012)	Found	283 (394,134)	GL476611	12,907–13,110
Tuberoinfundibular peptide 39 (TIP39, PTH2)			Pinheiro et al. (2012)	Found	987 (269,378)	GL477315	197,502–197,681
Parathyroid hormone-related protein			Pinheiro et al. (2012)	Found	283 (394,134)	GL476611	27,573–27,704
PTH 1 Receptor		ENSPMAG00000003038	Pinheiro et al. (2012)	Found	8 (776,138)	GL476336	196,042–234,273
PTH 2 Receptor		ENSPMAG00000001351	Pinheiro et al. (2012)	Found	4265 (590,707)	GL480593	40,218–49,961
PTH 3 Receptor			Pinheiro et al. (2012)	Not found			

[a] 'Partial' noted in parentheses means the Ensembl designated gene entry only represents a part of the entire gene.
[b] The specific assignments are made based on percent identity, and in the case of Receptor 1, limited shared synteny data for genes such as LRR1. Others have indicated, rightly so, the shared synteny data cannot contribute to firm ortholog assignation given the current assembly (Ocampo Daza et al., 2012).
[c] The specific designations follow the prevailing assignments for the corresponding sequences in the cited references.
[d] Searched against Human, Sturgeon, Eel orthologs; additionally, (Huang et al., 2009) similarly reported no identification in the genomic sequences).

because this class of genes, along with those associated with transcription, signal transduction, development, neurons and others facilitating substantial biological innovations, are retained in modern genomes at a higher frequency than average genes (Putnam et al., 2008; Smith et al., 2013).

5. The GnRH genes illustrate critical features of the lamprey genome assembly and its revelatory nature

In recent reviews of GnRH and their respective receptors, focusing primarily on gnathostomes (Guilgur et al., 2006; Kah et al., 2007; Okubo and Nagahama, 2008) and predating availability of the lamprey genome (Kim et al., 2012, 2011; Roch et al., 2011; Tostivint, 2011), various scenarios on the evolutionary relationships among GnRHs were proposed. Two such papers are of particular relevance to the discussion herein since they used available genomic information for several bony fish and tetrapods to recently propose new schemes of vertebrate evolution based on the chromosomal organization of the GnRH genes (Kim et al., 2011; Tostivint, 2011). Looking at the conservation of the genes located adjacent the GnRH genes, or shared synteny, in a wide assortment of teleosts and tetrapods, they were able to suggest clear evolutionary paths for the various GnRH isoforms recognized in later-evolved vertebrates. Significant insights shared by both were: (1) that the GnRHs in all these organisms fall into three lineages of paralogs with a fourth paralog evidenced as a non-functioning vestige, i.e. 'lost', in a conserved chromosomal location; and (2) that tetrapods have lost GnRH3 orthologs yet the flanking regions remain obvious despite the absence of a function. With regard to the agnathans and GnRH, the latest hypothesis had been that likely due to a genome/gene duplication event, an ancestral gene gave rise to two lineages of GnRHs—the gnathostome GnRH and lamprey GnRH-II; the gene duplication events that generated the different fish and tetrapod paralogous groups may have taken place within the gnathostome lineage, after its divergence from the ancestral agnathans (Kavanaugh et al., 2008; Sower et al., 2009). The invertebrate GnRHs suggested a fifth grouping of GnRHs that had reinforced the fourth grouping (group IV) of lGnRH-I and -III (Tsai and Zhang, 2008; Zhang et al., 2008). Subsequently, Kim and colleagues (2011) incorporated the lamprey data as representing the fourth paralog, GnRH4, lost in other vertebrates (Kim et al., 2011; Tostivint, 2011).

With the availability of the lamprey genome, we have been able to extend shared synteny analyses to better resolve the ancestral state of the GnRH genomic region and improve our understanding of the evolution of the GnRHs in vertebrates. Because of the overall small length and limited number of introns, the GnRH genes are particularly suited to analysis in this sort of genome assembly, as has been pointed out for the single-exon KCNA genes (Qiu et al., 2011). Analyses of conserved syntenic arrangements (Fig. 4 of Smith et al., 2013) in the regions surrounding GnRH genes in lamprey as compared to the corresponding regions in the gnathostomes medaka, chicken, and humans (Kim et al., 2011; Tostivint, 2011) revealed that the lamprey GnRH genes are located on two scaffolds with the two genes, lGnRH-I and -III that had been classified previously as group IV genes, occurring in tandem (ca. 7.5 kb ORF-less intergenic region in between). Specifically, regions of chicken chromosomes 6 and 4, and human chromosomes 10 and presumably ancestrally-linked regions of human 2 and 20 (Kim et al., 2011; Tostivint, 2011) share conserved synteny with the lamprey GnRH-linked genes, with the shared set of genes presumably present in the common ancestor. A representation of a region of medaka chromosome 15 is included in that figure (Fig. 4 of Smith et al., 2013) to emphasize the fact that although tetrapods have lost GnRH3 orthologs, as also noted by others (Kim et al.,

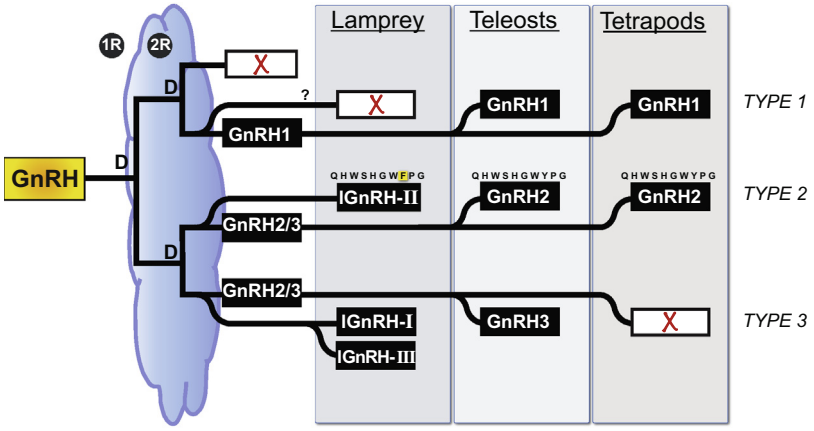

Fig. 1. Proposed scenario of the evolution of the GnRH gene family in vertebrates. The relative placement of the whole genome duplication events is indicated by 1R, 2R, and 3R. Open rectangles with X's indicate lost loci. In the interest of space, the evolution of vertebrates is significantly condensed with several taxa remaining unrepresented and time (left-to-right axis) not drawn to scale; it is similar to the paradigm used by Kim and colleagues (2011), but abridged. The cloud represents the ambiguity about the timing of the lamprey/gnathostome split relative to the last shared whole genome duplication and the degree of paralog resolution prior to the split. The altered lamprey branches are also placed differently relative the other taxa to accommodate the established names of the lineages. The question mark above the lost lamprey branch is to signal we don't know at this time when GnRH1 was lost in that lineage. The deduced amino acids corresponding to the lamprey GnRH II and GnRH2 (zebrafish and chicken) decapeptides are shown above the representative blocks with the single amino acid difference in lamprey highlighted. Importantly, although we have drawn the scenario where lamprey GnRH-II is orthologous to GnRH2; a scenario wherein lamprey GnRH-II and gnathostome GnRH3 share common ancestry (post duplication) is also plausible. Modified from Smith et al. (2013).

Table 2
Proposed shift in classification of GnRH genes among paralog groups based on the revision in light of the genomic neighborhood of the lamprey paralogs. Modified from previously published material (Gorbman and Sower, 2003; Silver et al., 2004; Smith et al., 2013).

Paralog groups	Representative members[a]	
	Old view	New view
GnRH1	Mammal GnRH, Seabream GnRH-I	Mammal GnRH, Seabream GnRH-I
GnRH2	Chicken GnRH-II, lamprey GnRH-II	Chicken GnRH-II, lamprey GnRH-II
GnRH3	Salmon GnRH-III	Salmon GnRH-III, lamprey GnRH-I, lamprey GnRH-III
GnRH4	Lamprey GnRH-I, Lamprey GnRH-III	LOST

[a] In the interest of space, only a few, key representatives are provided for most paralog groupings. The representatives undergoing substantial shift are highlighted in bold.

2011; Tostivint, 2011), the flanking regions remain obvious despite the absence of a functioning version of this gene from the tetrapod lineage.

Our revised scenario for the evolution of the GnRH family of genes resulting from the analysis of conserved synteny between lamprey and gnathostomes (Fig. 1) reveals several salient features of vertebrate GnRH evolution. GnRH1 has been lost from lamprey, as it has in zebrafish (Kuo et al., 2005). In addition, our analysis corroborates recent views (Kim et al., 2011; Tostivint, 2011) that GnRH3 was lost in the tetrapod lineage and did not arise in the teleost lineage as a result of a third round of whole genome duplication (3R). We see no biochemical evidence in lamprey for an extant GnRH4-like paralog as proposed to have arisen from tetraploidizations in the early stages of vertebrate evolution (Tostivint, 2011), and we did not identify any obvious candidate from preliminary analysis of the genome, suggesting that the homolog of this gene was lost in the lamprey, similar to its loss in the other vertebrate lineages. With respect to the agnathans and GnRH, our analysis of the synteny agrees with the previous proposal in that lGnRH-I and -III resulted from a duplication event within the lamprey lineage (Kavanaugh et al., 2008). However, the data now suggest a substantially different view of the evolutionary history of the GnRH family in vertebrates. Significantly, the current evidence suggests that all of the genome duplication events that generated the different fish and tetrapod paralogous groups (Kuraku et al., 2009) likely took place before the divergence of the ancestral agnathans and gnathostome lineages and that the GnRHs in lamprey previously proposed (erroneously) as members of group IV (lGnRH-I and -III) share a more recent common ancestry with GnRH2 and 3 (Table 2). Given the single amino acid difference between mature lGnRH-II and GnRH2 we propose that a GnRH2-like gene existed before the lamprey/gnathostome split.

This situation illustrates several interesting facets of the lamprey genome relative to other later-evolved vertebrates. We found that it was hard to ascribe either the genomic neighborhood of lGnRH-II or the lGnRH-I/-III region as directly equivalent to GnRH2 or GnRH3 in gnathostomes. While at first glance this may seem to be simply due to the limitation of having data for primarily only one side of the lamprey GnRH genes, it is more likely caused by a more overarching phenomenon arising from the timing between the last shared whole genome duplication and the divergence of the lamprey and gnathostome lineages. In general, due to the apparent incomplete resolution of loss and retention of gene duplicates during the short time interval between the last whole genome duplication and the gnathostome/lamprey split, specific orthologs cannot be assigned unequivocally based on conserved synteny alone. In fact, there is not a strict 1:1 relationship, but a 2:2 relationship between lamprey and vertebrate genomic regions (Smith et al., 2013). This pattern is repeated throughout the lamprey genome and is presumably one of the factors contributing to the problem of assigning orthologs to lamprey based solely on phylogenetics (Qiu et al., 2011).

Finally, returning to our revised scenario for the evolution of the vertebrate GnRHs, we propose lGnRH-I/III are not type 4 GnRHS but that the paralogous lGnRH-I/III and GnRH3 evolved divergent structure/functions in lamprey and gnathostome lineages. The independent path of lamprey and gnathostome paralogs following a shared origin is a typical pattern revealed by the lamprey genome assembly and again reflects the juxtaposition of paralog resolution subsequent to the last shared whole genome duplication (2R) and the relatively short time frame between that event and the agnathan/gnathostome split as discussed above within this section.

Genomic data from the only other extant agnathan lineage, hagfish, should provide additional informative details and may ultimately identify a bona fide member of the GnRH4 group. Intriguingly, previous data suggest that hagfish express two GnRH-2/3-like peptides (Braun et al., 1995; Sower et al., 1995), and this would be consistent with the supposition that the tandem duplication that gave rise to lamprey GnRH-I, -III was a geologically 'recent' event, long after both the divergence ancestral agnathan and gnathostome lineages and the divergence of ancestral hagfish and lamprey lineages (Kavanaugh et al., 2008). The high degree of identity shared by lGnRH-I and -III (precursors ca. 70% identical) is also consistent with this same evolutionary scenario.

6. The genome beyond GnRH

Despite the potential to reveal much about the molecular genetics of early vertebrates and details of individual gene families of interest, the analyses of genes in the current lamprey genome assembly may not always be as straightforward as seen for the GnRH genes. For example, in contrast to the situation for the GnRH peptide hormones, the situation of the receptors for the GnRH peptide hormones in the current lamprey genome is much different and more typical of several other average-sized, multi-exonic genes (Table 1). In fact, although half of the assembly is in scaffolds of 174 kb or longer, we have found the assembly limiting for investigating several of the neuroendocrine genes in lamprey. A similar issue was faced by Meyer and colleagues in their work resolving orthology of vertebrate genes, leading to their conclusion that single exon genes are the most amenable given the current state of the assembly at that time (Qiu et al., 2011). While all except for one of the lamprey genes for the GnRH peptide ligand possesses introns in the coding region (Kavanaugh et al., 2008), the genes themselves encode small precursors (ca. 90 amino acids) and serendipitously fall on scaffolds of much larger size (95 kb and 302 kb) than those of the receptors. The open reading frame for the GnRH receptors in lamprey are substantially larger (ca. 10-times) than those of the peptide precursor and span three exons and two introns, as found for the GnRH receptor genes of higher vertebrates and even some genes of the protochordate amphioxus (Tello and Sherwood, 2009). Syntenic analysis of the GnRH receptors is precluded at this time by this fractional representation (Sower et al., 2012). A similar fragmentary situation with the estrogen receptor 1 shows that this is not an isolated occurrence. The identified estrogen receptor 1 exons occur over at least three scaffolds and that still does not completely account for the expected sequence. And although the current assembly provides unparalleled resolution of gene content and structure of this evolutionarily important genome, incomplete representation of genes in the current assembly is one of the significantly limiting factors in working with the lamprey genome at this time. Notably though, other existing resources can be used to further resolve the structure of specific regions of interest (Nikitina et al., 2009; Smith et al., 2010).

7. Conclusion

The lamprey genome has provided much insight into the gene families and genetic makeup existing at the origins of the vertebrates. The process of analyzing the evolution of GnRH encapsulates much of the current possibilities and issues presented by the availability of the current lamprey genome assembly. The syntenic analysis of GnRH in the present study has provided a broader perspective than phylogenetic analysis alone and allows us to set forth a more encompassing scenario for the evolution of this gene family. This new scenario shows a higher conservation of the lamprey GnRHs with the gnathostome GnRH family than previously appreciated. It is marginally possible the lamprey may yet still have some more to reveal about the evolution of the vertebrate GnRH. While we do see individual genes that flank GnRH1 in fish on scaffolds in lamprey, there lacks contiguous flanking data to conclusively locate the GnRH1 lost 'ghost' locus if it exists in the somatic genome. The additional 20% of the genome that is only present in germline cells may hold information on the lost GnRH1 or may even reveal that it is present and perhaps only expressed in early development although there is no data to support this conjecture. There is also the chance additional lamprey genomic information could provide evidence on the vertebrate GnRH paralog GnRH4 that would have arisen as a result of two rounds of duplication, yet is absent in vertebrates, i.e. lost. Additional data from the lamprey germline tissues or a better sequenced and assembled somatic version may enable identifying a region where this gene resided in a lamprey ancestor, a 'ghost' locus. A candidate locus has been characterized in humans, as well other organisms (Kim et al., 2011; Tostivint, 2011), and it may be informative to compare more distantly diverged members to learn about the ancestral form and evolution.

Other gene families can be analyzed further in depth incorporating the neighboring genes in comparison to those in other vertebrates to elucidate their individual progression over the course of vertebrate evolution, keeping in mind the current genome assembly may impose some limitations. In a recent paper, the evolution of the cranial structures were examined in hagfish and lampreys and showed that they developed in the same way supporting a close phylogenetic relationship between these two groups (Janvier, 2013; Oisi et al., 2013). Considering the growing lines of evidence suggesting that cyclostomes are monophyletic (Heimberg et al., 2010; Janvier, 2010; Oisi et al., 2013), additional genomic analysis of lamprey and hagfish could contribute significantly to our understanding of the evolutionary relationship between these basal vertebrates compared to other vertebrates. In particular, the sequencing and annotation of the hagfish genome may shed light on the molecular evolution of neuroendocrine hormones and receptors.

8. Support

This work was supported by NSF-0849569 to SAS. Partial funding was provided by the New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2498.

9. Disclosure summary

The authors have nothing to disclose.

Acknowledgments

We would like to thank the Lamprey Genome Project Consortium and members of the Sower laboratory. We thank the anonymous reviewers for their constructive and helpful comments. This research was supported by NSF-0849569 to SAS. Partial funding was provided by the New Hampshire Agricultural Experiment Station. This is Scientific Contribution Number 2498.

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