



# Molecular and evolutionary aspects of the protochordate digestive system

Satoshi Nakayama<sup>1</sup> · Toshio Sekiguchi<sup>2</sup> · Michio Ogasawara<sup>1</sup>

Received: 29 November 2018 / Accepted: 12 April 2019 / Published online: 2 May 2019  
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

## Abstract

The digestive system is a functional unit consisting of an endodermal tubular structure (alimentary canal) and accessory organs that function in nutrition processing in most triploblastic animals. Various morphologies and apparatuses are formed depending on the phylogenetical relationship and food habits of the specific species. Nutrition processing and morphogenesis of the alimentary canal and accessory organs have both been investigated in vertebrates, mainly humans and mammals. When attempting to understand the evolutionary processes that led to the vertebrate digestive system, however, it is useful to examine other chordates, specifically protochordates, which share fundamental functional and morphogenetic molecules with vertebrates, which also possess non-duplicated genomes. In protochordates, basic anatomical and physiological studies have mainly described the characteristic traits of suspension feeders. Recent progress in genome sequencing has allowed researchers to comprehensively detail protochordate genes and has compared the genetic backgrounds among chordate nutrition processing and alimentary canal/accessory organ systems based on genomic information. Gene expression analyses have revealed spatiotemporal gene expression profiles in protochordate alimentary canals. Additionally, to investigate the basis of morphological diversity in the chordate alimentary canal and accessory organs, evolutionary developmental research has examined developmental transcription factors related to morphogenesis and anterior-posterior patterning of the alimentary canal and accessory organs. In this review, we summarize the current knowledge of molecules involved in nutrition processing and the development of the alimentary canal and accessory organs with innate immune and endocrine roles in protochordates and we explore the molecular basis for understanding the evolution of the chordate digestive system.

**Keywords** Digestive system · Lancelets · Tunicates · Gene expression · Evolution

## Introduction

The digestive system is a functional unit. The digestive system functionally performs nutrition processing and morphologically consists of an epithelial tubular structure (alimentary canal) and accessory organs in most triploblastic animals, which are heterotrophic organisms (Ruppert et al. 2003; Schmidt-Rhaesa 2007). The morphology of the alimentary canal and accessory organs varies greatly among triploblastic animals depending

upon habitat (aquatic, amphibious, or terrestrial) and food habits (carnivorous, herbivorous, or omnivorous) (Fig. 1). Alimentary canals with accessory organs, however, have shared functional sequential processing stages for nutrition processing, including ingestion, digestion, absorption and elimination that occur from the mouth to the anus (Reece et al. 2011).

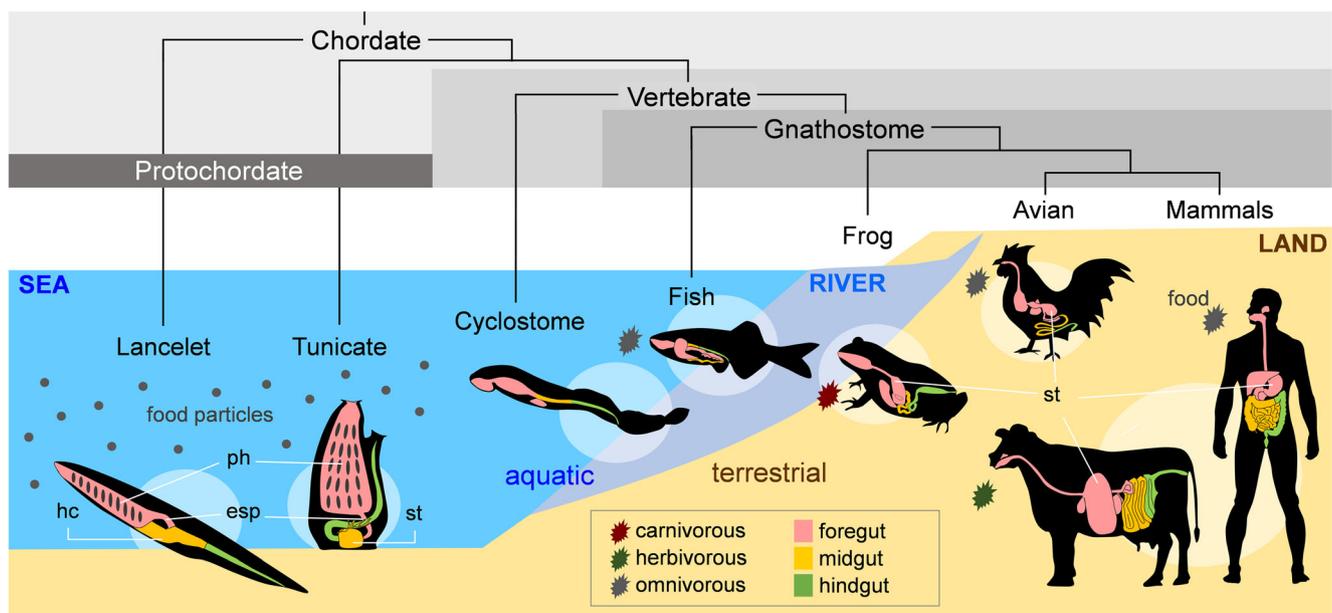
Developmentally, the alimentary canal and accessory organs are formed as endodermal derivatives (Schmidt-Rhaesa 2007). Generally, the endoderm is specified into three regions (foregut, midgut and hindgut). Each region is subdivided into several digestive compartments, such as the pharynx, esophagus, stomach and intestine, with the addition of accessory organs including the pancreas and liver, that exhibit various shapes in each animal group. Although the nomenclature for each digestive compartment is based on the homologous structure in closely related animals, occasionally, a specific name is used in a certain animal group and some traits are given the same name in spite of the fact that their shape is greatly divergent and unclear

---

✉ Michio Ogasawara  
ogasawara@faculty.chiba-u.jp

<sup>1</sup> The Graduate School of Science, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan

<sup>2</sup> The Noto Marine Laboratory, Division of Marine Environmental Studies, Institute of Nature and Environmental Technology, Kanazawa University, Hosu-gun, Ishikawa 927-0553, Japan



**Fig. 1** Phylogenetic relationships of chordates and their habitat, food habit and morphology of the alimentary canal and accessory organs. Chordates consist of lancelets, tunicates and vertebrates. Lancelets and tunicates are collectively called “protochordates”. The habitats of chordates are diverse (aquatic, amphibious, or terrestrial) and therefore their food habits are also divergent (carnivorous, herbivorous, or omnivorous). The alimentary

canal and accessory organs are developmentally divided into three regions (foregut, midgut and hindgut). The morphology of the alimentary canal and accessory organs varies depending on their phylogenetic relationship and food habit. esp, esophagus; hc, hepatic cecum; ph, pharynx; st, stomach

homology (e.g., the stomach). Therefore, morphologies and nomenclature of the alimentary canal and accessory organs can be confusing and the evolution of digestive systems among animal groups should be examined with care, particularly among different taxa.

To investigate the evolution of digestive systems, knowledge of phylogenetically varied animals is useful; however, among the deuterostomes, studies of the digestive system have been mostly carried out in mammals. Therefore, descriptions of the digestive system in general biology textbooks are typically focused on vertebrates, while descriptions of the digestive system of other animals are limited. This biased knowledge limits the evolutionary understanding of the digestive system (Reece et al. 2011). Thus, further study in invertebrates provides us with new insight into the evolution of this organ system.

Protochordates (lancelets and tunicates) are invertebrate chordates that are closely related phylogenetically to vertebrates (Dehal et al. 2002; Putnam et al. 2008). Molecular phylogenetics studies demonstrate that tunicates are a sister group of vertebrates and lancelets (also called amphioxus), with the tunicates and the vertebrates, constitute the phylum Chordata (Putnam et al. 2008). Protochordates with non-duplicated genomes that arose prior to the whole genome duplication in the vertebrate lineage have been emerging as a key animal group for evolutionary research on the origin of vertebrates. Additionally, the number of protochordate species whose genomes have been sequenced has increased in recent years. Thus, genome-wide and molecular research on the evolution

of vertebrate traits is progressing (Dehal et al. 2002; Putnam et al. 2008; Brozovic et al. 2018).

In this review, we summarize the current knowledge of protochordate digestive systems in view of recently accumulated protochordate genomic information, as this information increases our understanding of vertebrate digestive system evolution. We first present current anatomical, histological and physiological knowledge as fundamental information. Then, we examine the recent state of genome-wide research from molecular and evolutionary perspectives, with a special focus on the digestive system genes related to the nutrition processing and morphogenesis of the alimentary canal and accessory organs (i.e., differentiation marker genes and developmental transcription factor genes). We also describe the spatiotemporal expression patterns of digestive and absorptive differentiation marker genes and developmental transcription factor genes in protochordates. Finally, we discuss the evolution of the chordate digestive system based on the current knowledge of gene expression profiles in protochordates.

## Anatomical and histological features of the protochordate alimentary canal and accessory organs

Lancelets and tunicates are marine chordates and they capture food particles in seawater through the pharynx as suspension feeders (Satoh 2009) (Fig. 1). The morphology of the

alimentary canal and accessory organs of protochordates varies among protochordate species; however, the sequence of digestive compartments of the alimentary canal along the anterior-posterior axis (mouth, pharynx, esophagus, stomach/chamber, intestine and anus) is shared in protochordates (Fig. 1).

### Lancelets possess a straight alimentary canal

Lancelets, which are fish-like marine chordates, possess an alimentary canal that exhibits morphologically less diversity among species. The alimentary canal extends linearly with several digestive compartments along the anterior-posterior body axis (Kaji et al. 2013). The pharynx is the domain responsible for filtering food particles suspended in seawater and it occupies half of the animal's body length (Riisgård and Svane 1999; Nielsen et al. 2007). In the pharynx, a branchial sac (also called the branchial basket or pharyngeal basket) with numerous gill slits (also called pharyngeal slits) plus an endostyle are formed for filtering. The endostyle is a unique structure for mucus secretion in the protochordate pharynx. The post-pharyngeal region is divided into the esophagus, the midgut, the hepatic cecum and the hindgut. Occasionally, the midgut is called a stomach but the midgut does not appear to exhibit a distinct swelling structure like the vertebrate stomach. The hepatic cecum (also called the hepatic diverticulum or midgut diverticulum), which is a structure not found in vertebrates, arises from the midgut and expands anteriorly along the right side of the pharynx (Barrington 1937; Ruppert et al. 2003; Kaji et al. 2013). The boundary region between the midgut and hindgut is termed the ilio-colon ring and is covered with cilia to promote food transport. The hindgut runs straight and opens at the anus.

Histologically, the lancelets' alimentary canal appears to mostly consist of a single endodermal epithelial layer, while the vertebrate alimentary canal consists of multiple cellular layers: the mucosa layer, submucosa layer and muscularis layer, which are derived from the endoderm, mesoderm and ectoderm, respectively. Sphincters, however, are found at both the velum and anus (Ruppert 1997). In the endodermal epithelium, two types of cells are found in the hepatic cecum and midgut (Barrington 1937). In addition, phagocytic cells are observed in the hepatic cecum and midgut (van Weel 1937; He et al. 2018). However, an understanding of other types of cells and their distributions are unclear.

### Tunicates possess morphologically diverse alimentary canals and accessory organs

Tunicates possess morphologically diverse alimentary canals and accessory organs in relation to their lifestyle (swimming, free-floating, sessile, solitary, or colonial) (Ruppert et al. 2003). In recent phylogenomic analyses, tunicates are divided into three (or four) major clades by recent phylogenomic

analyses: (1) Appendicularia, (2) Thaliacea + Phlebobranchia + Aplousobranchia and (3) Stolidobranchia (Kocot et al. 2018; Delsuc et al. 2018). In this section, we focus on appendicularians, thaliaceans and ascidians (phlebobranchs, aplousobranchs and stolidobranchs).

Appendicularians, known as free-swimming tunicates and the sister group of all other tunicates, possess a different suspension feeding mode compared with that of other tunicates that use a unique mucus net called a "house." They do not need to filter food particles in the pharynx and they possess only a single pair of gill pores instead of numerous gill slits in the pharynx (Brena et al. 2003; Burighel et al. 2005; Onuma et al. 2017). The endostyle is generally formed on the ventral side of the pharynx. The pharynx leads to the esophagus and it connects to the stomach. The stomach is divided into the left stomach and right stomach in some species (Brena et al. 2003). The intestine opens to the exterior at the anus in the ventral side of the trunk.

Thaliaceans, which are free-floating tunicates, generally possess a branchial sac, also called the branchial basket or pharyngeal basket, with gill slits, also called pharyngeal slits and an endostyle in the large pharynx. This is followed by the esophagus, stomach and intestine as an alimentary canal (Piette and Lemaire 2015). As an accessory organ, they develop a pyloric gland at the boundary region between the stomach and intestine.

Ascidians (phlebobranchs, aplousobranchs and stolidobranchs), known as sessile tunicates, generally possess a U-shaped alimentary canal including the pharynx, esophagus, stomach and intestine (Ruppert et al. 2003). The pharynx includes a large branchial sac with numerous gill slits and an endostyle and leads to the esophagus. The stomach exhibits a divergent epithelial structure among ascidians (Koyama et al. 2012). For example, *Ciona intestinalis* possesses a folded epithelial stomach, while the stomach of *Cibacapsa gulosa* is smooth (Lescano et al. 2011). Ascidians also possess a hepatopancreas (also called a pyloric gland), which is a branching structure developed from the boundary between the stomach and intestine (Hirano and Nishida 2000; Orito et al. 2015).

Histological observations suggest that the tunicate alimentary canal consists mostly of a single endodermal epithelium layer and neither a muscular nor a tissue layer (including sphincters) is found in the alimentary canal. In an appendicularian *Oikopleura dioica*, three types of cells are observed in the endodermal epithelium: ciliated microvillar cells, globular cells and gastric band cells (Burighel et al. 2005). These cells show regional distributions in the alimentary canal. In an ascidian *Polyandrocarpa misakiensis*, several types of cells are found in the endodermal epithelium of the esophagus and stomach: absorptive cells, zymogenic cells, endocrine cells, ciliated mucous cells, undifferentiated cells and band cells (Koyama 2011; Koyama et al. 2012). These types of cells are found in other tunicates (Koyama 2011).

## Physiological studies of protochordate nutrition processing

Physiological aspects of nutrition processing differ between protochordates and vertebrates as protochordates are suspension feeders and their lifestyle and food habits differ from those of vertebrates. Physiological studies regarding the nutrition processing in protochordates are classified into studies on food habit, the suspension system in the pharynx, flow of food through the alimentary canal, digestive process and nutrient absorption.

### Lancelet suspension feeding system

Lancelets suck in seawater containing food particles (e.g., algae), which enter the pharynx and are trapped by mucus secreted from the endostyle. This combination of food particles and mucus is formed into a mucus cord by the movement of cilia in the pharynx and then the mucus cord is transported posteriorly to the midgut through the esophagus (Barrington 1937; Riisgård and Svane 1999; Nielsen et al. 2007). In the midgut, food particles within the mucus cord are mixed with digestive enzymes secreted from the cells of the hepatic cecum and midgut. Food particles are partially released from the mucus cord and digested intracellularly in the cells of the hepatic cecum epithelium (Barrington 1937; He et al. 2018). Food particles within mucus are transiently pooled around the ilio-colon ring and transported into the hindgut (Nakashima et al. 2018). Biochemical studies have detected the activities of carbohydrate-degrading enzymes and lipolytic enzymes in the hepatic cecum, midgut and hindgut and protease activity is detected in the hepatic cecum and hindgut (Barrington 1937).

### Tunicates possess diverse suspension feeding systems

Tunicates possess various suspension feeding systems depending on the lifestyle of the given species. Free-swimming appendicularians accumulate food particles in the house, which is mucus secreted outside the body (Satoh 2009; Onuma et al. 2017). Condensed food particles are captured in the pharynx by mucus. In the appendicularian gastrointestinal tract, the digestion and absorption processes are unclear.

Ascidians and thaliaceans, which are sessile or free-floating, take in seawater containing food particles and capture the food particles through the use of mucus nets that include von Willebrand factor-like proteins secreted from the endostyle (Petersen 2006; Sasaki et al. 2003; Nakashima et al. 2018). These filtered food particles pass through the esophagus as a mucus cord and are digested in the stomach (Yonge 1925; Nakashima et al. 2018). In addition to extracellular digestion, in some tunicates, intracellular digestion as lancelets was observed (Burighel and Cloney 1997; Cima and Brena 2002).

Biochemical studies have detected carbohydrase activity and lipolytic activity within the gastrointestinal tract of tunicates but protease activity is weak (Yonge 1925). Although the absorption process and underlying mechanism are unclear, absorbed saccharides are stored in the hepatopancreas/pyloric gland (Ermak 1977; Gaill 1980; Gaill 1981).

## Genome-wide characterization of digestive system-related differentiation marker genes in protochordates

Among protochordates, *C. intestinalis*, *O. dioica* and *Branchiostoma floridae* genomes were sequenced in the 2000s and the comparative genomic research based on their genome information determined the phylogenetic relationships among lancelet, tunicates and vertebrates (Dehal et al. 2002; Delsuc et al. 2006; Putnam et al. 2008). In addition, genomic information provided by these genome research enable us to compare and evaluate specific genetic backgrounds that correlate to traits on a genome-wide scale (Satou et al. 2005; Holland et al. 2008).

### Phylogenetic position of protochordates revealed by genome research

Clarifying the phylogenetic relationship among vertebrates, tunicates and lancelets is necessary to trace the evolution of traits in protochordates. Traditionally, lancelets were considered to be phylogenetically closer to vertebrates than tunicates based on morphological similarity; however, genomic information and genome-wide molecular phylogenetic analyses in chordates demonstrate that tunicates are a sister group of vertebrates and lancelets are the sister group of vertebrates plus tunicates (Dehal et al. 2002; Delsuc et al. 2006; Putnam et al. 2008). After these initial studies, the genome projects of other lancelet (*Branchiostoma belcheri*, *Branchiostoma lanceolatum*), tunicate (*Halocynthia roretzi*), echinoderm (*Strongylocentrotus purpuratus*) and hemichordate (*Ptychodera flava* and *Saccoglossus kowalevskii*) species were implemented. Genome-wide molecular phylogenetic analyses reveal the phylogenetic position among deuterostomes, indicating that the phylogenetic basis for tracing trait evolution to vertebrates has been established.

### Annotation and cataloging of genes in the protochordate genome

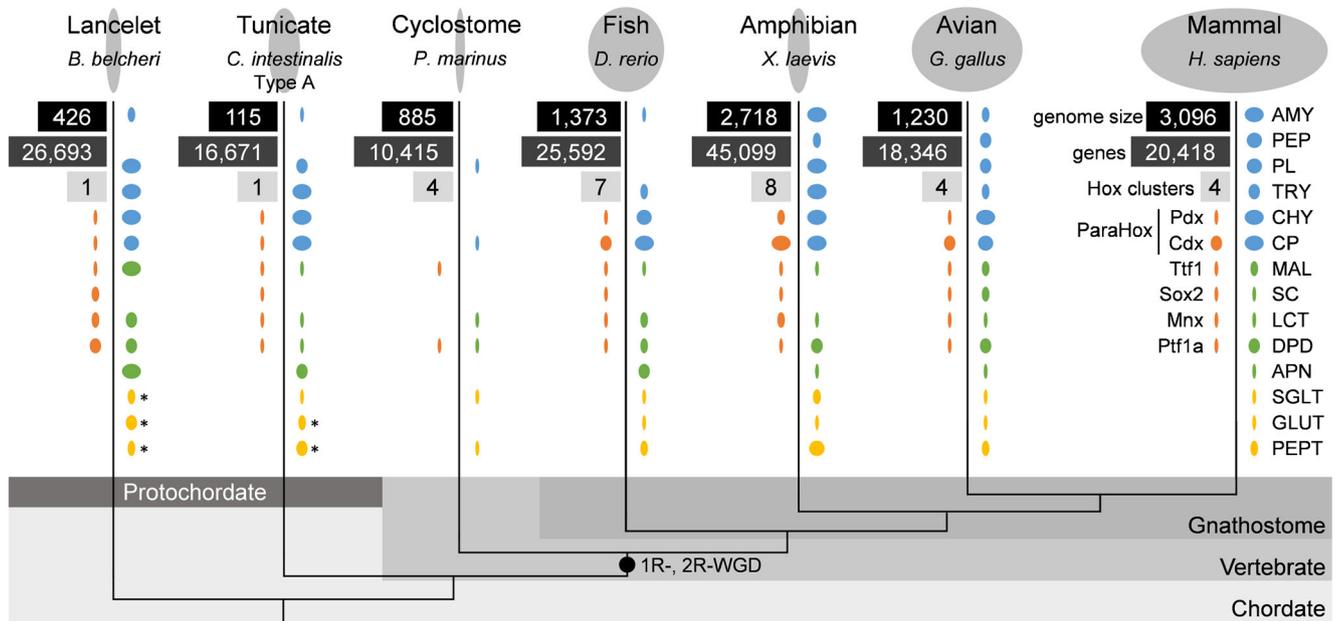
Genome sequencing studies not only reveal the entire genomic DNA sequence but also comprehensively assess the genes present in the genome. Genes within a given genome are modeled by prediction algorithms, annotated and listed as genetic catalogs. Currently, the genomes of 2 lancelet species

and 14 tunicate species are publicized with gene annotations (Satou et al. 2005; Putnam et al. 2008; Huang et al. 2014; Brozovic et al. 2018). The comprehensive evaluation of the entire genome facilitates the construction of objective and reliable evolutionary scenarios.

### Lancelet digestive system–related differentiation marker genes

The lancelet genome information is available in the genome databases of *B. floridae* (<https://genome.jgi.doe.gov/Braf11/Braf11.home.html>) and *B. belcheri* (<http://genome.bucm.edu.cn/lancelet/>) (Putnam et al. 2008; Huang et al. 2014). In lancelet genome databases, genes can be identified using BLAST and keyword searches. Additionally, many identified genes within the lancelet genome are registered to public databases (e.g., NCBI). According to the known digestive system–related genes contained in the NCBI, particularly digestive enzyme genes, brush-border membrane enzyme genes, transporter genes, innate immune-related genes and peptide hormone genes, lancelet genomes possess pancreatic exocrine digestive enzyme genes, including alpha-amylase, pancreatic lipase, trypsin, chymotrypsin and carboxypeptidase A/B,

despite the observation that lancelets do not possess a morphologically distinct pancreas (Fig. 2). The gene encoding the gastric exocrine digestive enzyme pepsin is not reported in the NCBI database and the lancelet genome databases (Fig. 2). In mammals, absorptive genes including the brush-border membrane enzyme genes maltase, sucrase, lactase, dipeptidase and aminopeptidase N and the intestinal transporter genes sodium glucose cotransporter (SGLT) 1, glucose transporter (GLUT) 5 and peptide transporter (PEPT) 1 and 2 have been investigated (Fig. 2). Lancelets also possess homologs of maltase, lactase, dipeptidase, aminopeptidase N, SGLT1, GLUT5 and PEPT1/2 (Fig. 2). For innate immune-related genes, intelectin genes are shared among lancelet and vertebrate genomes (Yan et al. 2013). Among genes encoding digestive system-related peptide hormone and neuropeptide, an insulin/insulin-like growth factor (IGF)-like gene (Guo et al. 2009; Lecroisey et al. 2015), calcitonin (CT)/CT gene-related peptide (CGRP) family peptide genes (Sekiguchi et al. 2016) and pituitary adenylate cyclase-activating polypeptide (PACAP)/glucagon-like peptide genes (On et al. 2015) have been characterized, while other homologs of digestive system-related peptides, hormones and neuropeptides have not been reported.



**Fig. 2** Current state of knowledge about chordate genomes and shared basis on developmental, digestive and absorptive genes. The size of the gray circle over each animal name reflects the number of species whose genomes are sequenced according to the NCBI (<https://www.ncbi.nlm.nih.gov/>) or Ensembl (<https://asia.ensembl.org/index.html>). The numbers in black boxes represent genome size. The numbers in dark gray boxes represent the number of genes. The numbers in pale gray boxes represent the number of Hox clusters. Representative genes were listed based on the public database: *H. sapiens* (NCBI), *G. gallus* (NCBI), *X. laevis* (NCBI), *D. rerio* (NCBI), *P. marinus* (Ensembl), *C. intestinalis* type A (NCBI and Ghost DB) and *B. belcheri* (NCBI and Lancelet DB). Colored circles

indicate the state of representative digestive system–related genes; each color shows developmental transcription factors (orange), exocrine digestive enzyme genes (blue), terminal enzyme genes (green), or transporter genes (yellow); the size of colored circle corresponds to the number of genes (1, 2, 3, 4, 5, or more). Asterisk indicates the gene registered only in Ghost DB or Lancelet DB. AMY, amylase; APN, aminopeptidase N; CP, carboxypeptidase A/B; CHY, chymotrypsin; DPD, dipeptidase; GLUT, glucose transporter 5; LCT, lactase; MAL, maltase; PEP, pepsin; PEPT, peptide transporter 1/2; PL, pancreatic lipase; SC, sucrase; SGLT, sodium glucose cotransporter 1; TRY, trypsin; WGD, whole-genome duplication

## Tunicate digestive system–related differentiation marker genes

Genomic information for the tunicate *C. intestinalis* is published in genome databases (<http://ghost.zool.kyoto-u.ac.jp/>, <https://www.aniseed.cnrs.fr/>) (Satou et al. 2005; Brozovic et al. 2018) and various comprehensive genome-wide searches have been performed in *C. intestinalis*. Recent genomic and morphological studies suggest that *C. intestinalis* includes two cryptic species, *C. intestinalis* Type A (*C. robusta*) and Type B (*C. intestinalis*) (Suzuki et al. 2005; Sato et al. 2012; Brunetti et al. 2015). In this review, we unify the names *C. intestinalis* Type A and Type B into *C. intestinalis* for convenience, as a distinct morphological or molecular difference has not been reported between their digestive systems.

Although tunicates have lost many genes through the rapid evolution of their genomes, many digestive enzyme genes, brush-border membrane enzyme genes and transporter genes are basically shared (Dehal et al. 2002; Nakayama and Ogasawara 2017). *C. intestinalis* possesses gene encoding pancreatic exocrine digestive enzymes, including alpha-amylase, pancreatic lipase, trypsin, chymotrypsin and carboxypeptidase A/B; however, an orthologous gene to the vertebrate gastric digestive enzyme gene, pepsin, has not been reported (Fig. 2) (Nakayama and Ogasawara 2017). With regard to absorptive genes, brush-border membrane enzyme gene encoding maltase, lactase, dipeptidase and aminopeptidase N and the transporter gene encoding SGLT1 are registered in the NCBI (Fig. 2). According to BLAST and keyword searches within the genome database, other absorptive transporter homologs of GLUT5 and PEPT1/2 exist in the *Ciona* genome (Hayashibe et al. 2017). Other tunicates *O. dioica* and *H. roretzi* possess homologs of vertebrate pancreatic exocrine digestive enzyme genes, brush-border membrane enzyme genes and transporter genes except pepsin genes. Regarding innate immune-related genes, gene encoding intelectin (Hayashibe et al. 2017), Toll-like receptor (TLR) (Sasaki et al. 2009) and variable region-containing chitin-binding proteins (VCBPs) (Liberti et al. 2014) are reported in *Ciona* genome. For peptide hormone and neuropeptide genes, homologs of insulin (Olinski et al. 2006), tachykinin (Satake et al. 2004), CT (Sekiguchi et al. 2009), galanin/galanin-like peptide (Kawada et al. 2011), neurotesin-like peptide (Kawada et al. 2011) and CCK/gastrin peptide (Johnsen and Rehfeld 1990; Sekiguchi et al. 2010) are identified in *C. intestinalis*. PACAP has been identified from *Chelyosoma productum* (McRory and Sherwood 1997); however, other peptide hormone genes (e.g. secretin, glucagon, somatostatin, ghrelin and pancreatic polypeptide) have not been reported in *C. intestinalis* and other tunicate genomes. These peptide hormone genes might have been lost in the tunicate lineage or acquired in an ancestral vertebrate.

## Expression of digestive system–related differentiation marker genes in protochordates

Gene function is analyzed using various methods at the gene, mRNA, or protein level. The temporal and spatial localization of gene transcripts are both particularly fundamental with regard to functional analyses. Temporal and spatial gene expression patterns are revealed by methods that are appropriate for the purpose, including RT-PCR, expressed sequence tag (EST) analysis, microarray, RNA-seq, or in situ hybridization (ISH).

## Sequence level evaluations of gene expression

Transcriptional profiles have been performed in lancelets and tunicates, mainly via RNA-seq and EST analyses. Although RNA-seq and EST analyses focusing on the digestive system are rare, comprehensive RNA-seq and EST analyses in developmental stages and major tissues including the alimentary canal have been reported.

In lancelets, several RNA-seq analyses have been reported in *Branchiostoma* species (*B. floridae*, *B. belcheri*, and *B. lanceolatum*) (Marlétaz et al. 2018). According to RNA-seq data published in the *B. belcheri* genome database (<http://genome.bucm.edu.cn/lancelet/>), pancreatic exocrine digestive enzyme genes and brush-border membrane enzyme genes are expressed. Also, an EST analysis of the alimentary canal in *B. belcheri* demonstrated that the transcripts of pancreatic exocrine digestive enzyme genes and innate immune-related molecules are detected in the hepatic cecum and midgut (He et al. 2018). Additionally, ESTs of lysosomal protease genes are abundant in the hepatic cecum and these may be related to intracellular digestion (He et al. 2018).

In tunicates, RNA-seq analyses are reported mainly in *C. intestinalis*, *H. roretzi* and *O. dioica*. In representative species, gene expression determined by RNA-seq at each developmental stage is shown in the genome database Aniseed (<https://www.aniseed.cnrs.fr/>). EST analyses have been performed in *C. intestinalis* at each developmental stage and in adult tissue and the data sets are presented in each gene page in the Ghost DB (<http://ghost.zool.kyoto-u.ac.jp/>). This EST data in adult tissues identified the expression of genes encoding pancreatic exocrine digestive enzymes and absorptive proteins in the alimentary canal (Hayashibe et al. 2017; Nakayama and Ogasawara 2017). Additionally, ESTs of several innate immune-related genes are present in the alimentary canal. Transcriptional profiling by RNA-seq and EST analysis indicate that lancelet and tunicate digestive and absorptive genes are functional within the alimentary canal.

## Special evaluations of transcripts at the tissue or individual level

To analyze the localization of a specific transcript within a tissue, ISH analysis is effective for revealing the localization of specific nucleic acid sequences (e.g., RNA transcripts) in a section of tissue or an entire tissue. In protochordates, gene expression knowledge based on ISH analysis has been accumulated, including expression patterns of digestive system-related differentiation marker genes.

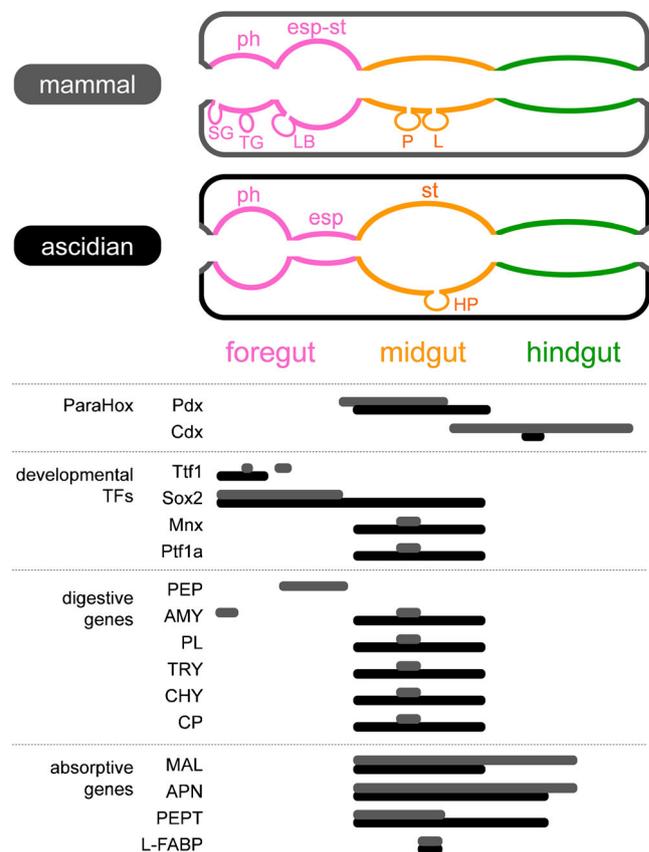
Expression of lancelet digestive system-related genes has been analyzed mainly in *Branchiostoma* species; however, the number of analyzed genes is small. Thus, our understanding of functional regionalization within the lancelet alimentary canal is incomplete. The gene encoding the pancreatic exocrine digestive enzyme, trypsin, is expressed in the hepatic cecum and midgut (Feng and Zhang 2012). Another pancreatic exocrine digestive enzyme gene, the gene encoding chymotrypsin-like protease, is also expressed in the hepatic cecum and midgut (Sun et al. 2010). Lysosomal protease-encoding genes are also expressed in the hepatic cecum epithelium, which may be necessary for intracellular digestion (He et al. 2018). The expression patterns of brush-border membrane enzyme genes and transporter genes remain unclear. Regarding other absorptive genes, the gene encoding fatty acid binding protein (FABP), which is involved in lipid absorption, is expressed widely in the hepatic cecum, midgut and hindgut (Orito et al. 2015). Regarding innate immune-related genes, intelectin genes are expressed in the hepatic cecum and gut epithelium (Yan et al. 2012). Several genes encoding the endocrine peptide hormone homologs have been characterized. An insulin/IGF-like gene is detected in the alimentary canal in both embryo and adult in *B. belcheri* and *Branchiostoma lanceolatum* (Guo et al. 2009; Lecroisey et al. 2015). It is reported that CT/CGRP family peptide genes are expressed in the midgut in *B. floridae*, suggesting that the CT/CGRP family peptide possesses a function in the alimentary canal like a vertebrate CT/CGRP family member, amylin (Sekiguchi et al. 2016). Also, the expressions of vertebrate liver-related genes such as *plasminogen*, *antithrombin*, *glutathione-S-transferase* and *G6Pase* have been detected in the hepatic cecum (Liang et al. 2006; Fan et al. 2007; Liu and Zhang 2009; Wang et al. 2015).

Tunicate digestive system-related differentiation marker gene expressions have been reported. In *C. intestinalis*, the regionality of gene expression within the alimentary canal and accessory organs has been particularly revealing. Digestive genes, including genes encoding pancreatic exocrine digestive enzymes, are expressed specifically in the stomach (Nakayama and Ogasawara 2017) (Fig. 3). Regarding absorptive genes, genes encoding the brush-border membrane enzyme maltase and aminopeptidase N, are expressed in the stomach and intestine and a transporter gene, PEPT1/2, is expressed in the stomach and intestine (Hayashibe et al. 2017). Other absorptive genes, such as

FABP, are expressed widely from the esophagus to the intestine. In particular, the gene homologous to a liver-type of FABP (L-FABP) gene of vertebrates is expressed strongly in the pyloric gland (Fig. 3) (Orito et al. 2015). Genes encoding the endocrine peptide hormone homologs insulin (Thompson and Di Gregorio 2015), tachykinin (Satake et al. 2004) and CT (Sekiguchi et al. 2009) are expressed in the alimentary canal.

## Developmental patterning of the protochordate alimentary canal

The morphology of the digestive system is determined by developmental processes based on the specific expression patterns of transcription factors. Fundamental developmental transcription factors are shared among triploblastic animals



**Fig. 3** Comparison of developmental, digestive and absorptive gene expression patterns between mammals and ascidians. A schematic diagram of the alimentary canal and accessory organs of mammals and ascidians is shown with three regions: foregut (pink), midgut (orange) and hindgut (green). Black bars indicate mammalian gene expression domains and gray bars indicate ascidian gene expression domains. AMY, amylase; APN, aminopeptidase N; CP, carboxypeptidase A/B; CHY, chymotrypsin; esp, esophagus; HP, hepatopancreas/pyloric gland; L, liver; LB, lung/bladder; L-FABP, liver-specific type of fatty acid binding protein; MAL, maltase; P, pancreas; PEP, pepsin; PEPT, peptide transporter 1/2; ph, pharynx; PL, pancreatic lipase; SG, salivary gland; st, stomach; TG, thyroid gland; TRY, trypsin

and their expression patterns are similar (Annunziata et al. 2014; Boyle et al. 2014). Among the developmental transcription factors in the alimentary canal and accessory organs, Hox, ParaHox, Ttf1, Sox2, Ptf1a and Mnx1 are well known and their expression patterns have been analyzed in vertebrates (Grapin-Botton and Melton 2000; Murtaugh 2007).

### Partial understanding of developmental expression in the lancelet alimentary canal

In lancelets, the expression of several developmental transcription factors has been reported by ISH analysis but most expression patterns have been analyzed in early developmental stages. Therefore, the expression patterns in late developmental stages when the alimentary canal is formed are less understood. With regard to Hox genes that pattern the alimentary canal along the anterior-posterior axis, the lancelet genome contains 15 Hox genes and their expression patterns have been reported in early developmental stages (Holland and Holland 1996; Schubert et al. 2005; Pascual-Anaya et al. 2012). Only *Hox1* and *Hox14* are expressed in the endoderm. *Hox1* is expressed in the pharynx and *Hox14* is expressed in the hindgut. Endodermal expression of other Hox genes has not yet been reported in lancelets. Analyses of later developmental stages are required to confirm if other Hox genes are expressed in the endoderm. Regarding ParaHox genes (*Gsx*, *Pdx* and *Cdx*), which pattern the midgut and hindgut along the anterior-posterior axis in mice (Gamer and Wright 1993; Jonsson et al. 1994), *Pdx* is expressed in the midgut in early larvae (Brooke et al. 1998). Another ParaHox gene, *Cdx*, is expressed in the hindgut in early larvae. *Gsx*, however, is not expressed in the alimentary canal, which is consistent with the expression pattern observed for vertebrate *Gsx* (Grapin-Botton and Melton 2000).

A developmental transcription factor in the alimentary canal, *SoxB1c*, is expressed in the pharyngeal endoderm and is homologous to *Sox2*, which specifies the esophagus and stomach in vertebrates (Meulemans and Bronner-Fraser 2007). *Ttf1*, which is an essential factor in the thyroid and lung of vertebrates, is expressed in the endostyle (Ogasawara 2000). The expression of other representative developmental transcription factors (e.g., *Ptf1a* and *Mnx*) known in vertebrates has not yet been reported. Although additional expression analyses of lancelet developmental transcription factors are necessary, the current data appear to show that expression patterns of lancelet ParaHox genes (*Pdx* and *Cdx*), *Sox2* and *Ttf1* are essentially shared with vertebrates.

### Shared developmental patterning in the alimentary canal of tunicates

The genome of the tunicate *C. intestinalis* contains 9 Hox genes (*Hox1*, *Hox2*, *Hox3*, *Hox4*, *Hox5*, *Hox6*, *Hox10*, *Hox12* and

*Hox13*) with a broken Hox cluster and these 9 Hox genes are all expressed in the alimentary canal with the anterior-posterior ordered expression pattern in juveniles (Ikuta et al. 2004; Nakayama et al. 2016) (Fig. 3). In ParaHox genes, *Pdx* and *Cdx* are expressed in the stomach and intestine of juveniles, respectively, except for *Gsx* (Fig. 3) (Nakayama et al. 2016).

In functional analyses of *Ciona* Hox genes, knockout of *Hox10* results in the absence of the posterior intestine and knockout of *Hox12* results in abnormal formation of the posterior intestine (Kawai et al. 2015; Sasakura and Hozumi 2018). Developmentally, the posterior intestine expressing *Hox10* and *Hox12* in the juvenile is derived from the endodermal strand in the larva (Nakazawa et al. 2013). Therefore, *Hox10* and *Hox12* are thought to be related to the endodermal strand cell migration and/or intestinal formation (Kawai et al. 2015). Also, other Hox genes and ParaHox genes (*Pdx* and *Cdx*) may play developmental roles in the alimentary canal in tunicates.

Other developmental transcription factor genes, *SoxB1*, *Ttf1*, *Ptf1a* and *Mnx*, are expressed in the *Ciona* alimentary canal with similar expression patterns to those of vertebrates. *SoxB1*, which is homologous to the vertebrate gene *Sox2*, is expressed from the pharynx to the stomach (Nakayama and Ogasawara 2017). *Ttf1* is expressed in the endostyle (Ogasawara et al. 1999). *Ptf1a* and *Mnx*, which specify the pancreas in vertebrates, are also expressed in the stomach with the positional relationship along the dorsal-ventral axis as the vertebrate pancreas (Nakayama and Ogasawara 2017). Additionally, the expression domain of *Ptf1a*, which upregulates the transcription of exocrine digestive enzyme genes in the vertebrate pancreas, overlaps with the expression of pancreatic exocrine digestive enzyme genes in the stomach. In *C. intestinalis*, these gene expression patterns of the transcription factors suggest that part of the gene regulatory network (GRN) is shared between the tunicate stomach and vertebrate pancreas (Fig. 3). Comparing the expression patterns of developmental transcription factors among chordates increases our understanding of morphological divergence in the alimentary canal and accessory organs.

### Future perspectives

Finally, we discuss some topics in the evolution of the digestive system in chordates based on the knowledge about the system in protochordates, as described above.

### Origin of the vertebrate liver

The liver is a characteristic organ in vertebrate digestive systems. The origin and evolution of the vertebrate liver has been discussed in studies on protochordates. The tunicate hepatopancreas/pyloric gland has been regarded as a structure homologous to the liver based on histological and histochemical

studies (Willey 1893; Ermak 1977). Recent molecular analyses on *C. intestinalis* also showed that L-FABP gene is expressed in the pyloric gland, suggesting homology between the hepatopancreas/pyloric gland and the liver (Orito et al. 2015). Furthermore, morphologically both the hepatopancreas/pyloric gland and the liver show a branching structure, indicating their shared developmental mechanism. However, the developmental mechanism of the hepatopancreas/pyloric gland is unclear and so, further studies to reveal the developmental process and the underlying molecular mechanism need to be conducted.

The lancelet hepatic cecum has also been regarded as a homolog of the liver (Müller 1844). Several homologs of vertebrate liver-related genes are expressed in the hepatic cecum (Liang et al. 2006; Fan et al. 2007; Liu and Zhang 2009; Wang et al. 2015) and this finding may contribute to the notion that there is homology between the hepatic cecum and the liver. However, other investigations have revealed that the hepatic cecum expresses extracellular and intracellular digestion-related genes and that it is functionally different from the liver (Sun et al. 2010; Feng and Zhang 2012; He et al. 2018). These inconsistent findings suggest two different evolutionary scenarios for the origin of the liver. The first scenario is that the lancelet hepatic cecum is not a homologous structure of the vertebrate liver and that the ancestral organ of the liver was acquired as a common ancestor of vertebrates or outgroups (vertebrates and tunicates). The second scenario is that although the hepatic cecum is a homologous structure of the liver, their functions have been diverged through the evolutionary process of chordates. Therefore, to elucidate the evolutionary origin of the liver, functional as well as developmental investigations are essential. In conclusion, the hepatopancreas/pyloric gland in tunicates is thought to be homologous to the liver in vertebrates. However, the evolutionary relationship between the lancelet hepatic cecum and vertebrate liver is controversial.

### Evolution of digestion in chordates

Many animals carry out the digestion of food by extracellular digestion only. In vertebrates, digestive enzymes for extracellular digestion are secreted by the stomach or pancreas. Homologs of the vertebrate pancreatic digestive enzyme genes (e.g.,  $\alpha$ -amylase, pancreatic lipase, trypsin, chymotrypsin and carboxypeptidase A/B) are found in the genomes of tunicates and lancelets and are expressed in their digestive systems (Feng and Zhang 2012; Nakayama and Ogasawara 2017). Gene expression profiles indicate that protochordates share the molecular basis of extracellular digestion with vertebrates. Furthermore, in sea urchins, homologs of vertebrate pancreatic digestive enzyme genes are expressed in the alimentary canal, indicating that the molecular basis of extracellular digestion is shared among deuterostomes (Perillo et al. 2016). In contrast, no orthologs of pepsin genes, which are known as vertebrate gastric digestive enzyme

genes, are found in protochordate genomes. Pepsin genes might have been acquired in the vertebrate lineage with the vertebrate stomach. In this context, the tunicate stomach may not be a structure homologous to the vertebrate stomach.

In addition to extracellular digestion, intracellular digestion is observed in protochordates (Burighel and Cloney 1997; Cima and Brena 2002; He et al. 2018). Recent analysis in lancelets demonstrates that intracellular digestion is observed mainly in the hepatic cecum and that cathepsins are involved in the underlying molecular mechanism (He et al. 2018). In chordates, cathepsins are known as lysosomal proteases, which are crucial enzymes in lysosomal digestion. Mechanisms underlying intracellular digestion in protochordates may thus be similar to those of underlying lysosomal digestion and therefore, intracellular digestion might have arisen in an ancestral chordate by evolution of the lysosomal digestion system. However, intracellular digestion might have been lost in an ancestral vertebrate with the development of extracellular digestion systems (e.g., stomach and pancreas). The scenario regarding protochordate intracellular digestion is still speculative and more investigations focusing on the molecular mechanisms involved in several protochordate species are required.

### Conclusion

In this review, we focus on the chordate digestive system in triploblastic animals and summarize the current knowledge of protochordate (lancelets and tunicates) digestive systems based on the digestive system-related differentiation marker genes and developmental transcription factor homologs revealed by genome-wide studies. A recent increase in genome sequencing has enabled the efficient identification of digestive system-related genes in chordates, including protochordates and vertebrates and aids in the understanding of the molecular basis of nutrition processing and morphological diversity of the alimentary canal and accessory organs. Currently, knowledge of the expression of each digestive system-related gene, especially digestive/absorptive genes and developmental transcription factors, is accumulating. Further studies are required to elucidate the GRN structure constituted by these developmental transcription factors and digestive/absorptive genes and to reveal the underlying developmental mechanism in the protochordate digestive system. Peripheral functions of nutrition processing (immune system, mucus system and endocrine system) are also crucial for understanding the evolution of the digestive system. Additionally, to understand the morphological diversity and evolution of the alimentary canal and accessory organs, ecological knowledge such as habitat, food habit and the lifestyle of each animal group will be considered along with the molecular information.

**Acknowledgements** We would like to thank Editage ([www.editage.jp](http://www.editage.jp)) for English language editing.

**Funding information** This work is supported by JSPS KAKENHI Grant Number JP17J06306 and the cooperative research program of the Institute of Nature and Environmental Technology, Kanazawa University Accept No. 18040.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants performed by any of the authors.

## References

- Annunziata R, Perillo M, Andrikou C, Cole AG, Martinez P, Amone MI (2014) Pattern and process during sea urchin gut morphogenesis: the regulatory landscape. *Genesis* 52:251–268
- Barrington EJW (1937) VI-The digestive system of *Amphioxus (Branchiostoma) lanceolatus*. *Proc Biol Sci* 228:553
- Boyle MJ, Yamaguchi E, Seaver EC (2014) Molecular conservation of metazoan gut formation: evidence from expression of endomesoderm genes in *Capitella teleta* (Annelida). *Evodevo* 5:39
- Brena C, Cima F, Burighel P (2003) Alimentary tract of Kowalevskiidae (Appendicularia, Tunicata) and evolutionary implications. *J Morphol* 258:225–238
- Brooke NM, Garcia-Fernández J, Holland PW (1998) The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. *Nature* 392:920–922
- Brozovic M, Dantec C, Dardailon J, Dauga D, Faure E, Gineste M, Louis A, Naville M, Nitta KR, Piette J, Reeves W, Scornavacca C, Simion P, Vincentelli R, Bellec M, Aicha SB, Fagotto M, Guérout-Bellone M, Haeussler M, Jacox E, Lowe EK, Mendez M, Roberge A, Stolfi A, Yokomori R, Brown CT, Cambillau C, Christiaen L, Delsuc F, Douzery E, Dumollard R, Kusakabe T, Nakai K, Nishida H, Satou Y, Swalla B, Veeman M, Volf JN, Lemaire P (2018) ANISEED 2017: extending the integrated ascidian database to the exploration and evolutionary comparison of genome-scale datasets. *Nucleic Acids Res* 46:718725
- Brunetti R, Gissi C, Pennati R, Caicci F, Gasparini F, Manni L (2015) Morphological evidence that the molecularly determined *Ciona intestinalis* type A and type B are different species: *Ciona robusta* and *Ciona intestinalis*. *J Zool Syst Evol Res* 53:186–193
- Burighel P, Brena C, Martinucci G, Cima F (2005) Gut ultrastructure of the appendicularian *Oikopleura dioica* (Tunicata). *Invertebr Biol* 120:278–293
- Burighel P, Cloney RA (1997) Urochordata. In: Harrison FW, Ruppert EE (eds) *Microscopic anatomy of invertebrates*, Volume 15 Hemichordata, Chaetognatha, and the invertebrate chordates. Wiley-Liss, NewYork p. 221–347
- Cima F, Brena C (2002) Multifarious activities of gut epithelium in an appendicularian (*Oikopleura dioica*: Tunicata). *Mar Biol* 141:479–490
- Dehal P, Satou Y, Campbell RK, Chapman J, Degnan B, De Tomaso A, Davidson B, Di Gregorio A, Gelpke M, Goodstein DM, Harafuji N, Hastings KE, Ho I, Hotta K, Huang W, Kawashima T, Lemaire P, Martinez D, Meinertzhagen IA, Necula S, Nonaka M, Putnam N, Rash S, Saiga H, Satake M, Terry A, Yamada L, Wang HG, Awazu S, Azumi K, Boore J, Branno M, Chin-Bow S, DeSantis R, Doyle S, Francino P, Keys DN, Haga S, Hayashi H, Hino K, Imai KS, Inaba K, Kano S, Kobayashi K, Kobayashi M, Lee BI, Makabe KW, Manohar C, Matassi G, Medina M, Mochizuki Y, Mount S, Morishita T, Miura S, Nakayama A, Nishizaka S, Nomoto H, Ohta F, Oishi K, Rigoutsos I, Sano M, Sasaki A, Sasakura Y, Shoguchi E, Shin-i T, Spagnuolo A, Stainier D, Suzuki MM, Tassy O, Takatori N, Tokuoka M, Yagi K, Yoshizaki F, Wada S, Zhang C, Hyatt PD, Larimer F, Detter C, Doggett N, Glavina T, Hawkins T, Richardson P, Lucas S, Kohara Y, Levine M, Satoh N, Rokhsar DS (2002) The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298:2157–2167
- Delsuc F, Brinkmann H, Chourrout D, Philippe H (2006) Tunicates and not cephalochordates are the closest living relatives of vertebrates. *Nature* 439:965–968
- Delsuc F, Philippe H, Tsagkogeorga G, Simion P, Tilak MK, Turon X, López-Legentil S, Piette J, Lemaire P, Douzery EJP (2018) A phylogenomic framework and timescale for comparative studies of tunicates. *BMC Biol* 16:39
- Ermak TH (1977) Glycogen deposits in the pyloric gland of the ascidian *Styela clava* (Urochordata). *Cell Tissue Res* 176:47–55
- Fan C, Zhang S, Liu Z, Li L, Luan J, Saren G (2007) Identification and expression of a novel class of glutathione-S-transferase from amphioxus *Branchiostoma belcheri* with implications to the origin of vertebrate liver. *Int J Biochem Cell Biol* 39:450–461
- Feng W, Zhang S (2012) A trypsin homolog in amphioxus: expression, enzymatic activity and evolution. *Mol Biol Rep* 39:1745–1753
- Gaill F (1980) Glycogen and degeneration in the pyloric gland of *Dendrodoa grossularia* (Ascidacea, Tunicata). *Cell Tissue Res* 208:197–206
- Gaill F (1981) Functions of digestive diverticula in marine invertebrates. I. Ascidiaceans fed with labelled glucose; its absorption and storage in the pyloric gland. *Cell Tissue Res* 219:185–195
- Gamer LW, Wright CV (1993) Murine Cdx-4 bears striking similarities to the *Drosophila* caudal gene in its homeodomain sequence and early expression pattern. *Mech Dev* 43:71–81
- Grapin-Botton A, Melton DA (2000) Endoderm development: from patterning to organogenesis. *Trends Genet* 16:124–130
- Guo B, Zhang S, Wang S, Liang Y (2009) Expression, mitogenic activity and regulation by growth hormone of growth hormone/insulin-like growth factor in *Branchiostoma belcheri*. *Cell Tissue Res* 338:67–77
- Hayashibe M, Nakayama S, Ogasawara M (2017) Shared hemocyte- and intestine-dominant expression profiles of intelectin genes in ascidian *Ciona intestinalis*: insight into the evolution of the innate immune system in chordates. *Cell Tissue Res* 370:129–142
- He C, Han T, Liao X, Zhou Y, Wang X, Guan R, Tian T, Li Y, Bi C, Lu N, He Z, Hu B, Zhou Q, Hu Y, Lu Z, Chen JY (2018) Phagocytic intracellular digestion in amphioxus (*Branchiostoma*). *Proc Biol Sci* 285:20180438
- Hirano T, Nishida H (2000) Developmental fates of larval tissues after metamorphosis in the ascidian, *Halocynthia roretzi*. II. Origin of endodermal tissues of the juvenile. *Dev Genes Evol* 210:55–63
- Holland LZ, Holland ND (1996) Expression of *AmphiHox-1* and *AmphiPax-1* in amphioxus embryos treated with retinoic acid: insights into evolution and patterning of the chordate nerve cord and pharynx. *Development* 122:1829–1838
- Holland LZ, Albalat R, Azumi K, Benito-Gutiérrez E, Blow MJ, Bronner-Fraser M, Brunet F, Butts T, Candiani S, Dishaw LJ, Ferrier DE, Garcia-Fernández J, Gibson-Brown JJ, Gissi C, Godzik A, Hallböök F, Hirose D, Hosomichi K, Ikuta T, Inoko H, Kasahara M, Kasamatsu J, Kawashima T, Kimura A, Kobayashi M, Kozmik Z, Kubokawa K, Laudet V, Litman GW, McHardy AC, Meulemans D, Nonaka M, Olinski RP, Pancer Z, Pennacchio LA, Pestarino M, Rast JP, Rigoutsos I, Robinson-Rechavi M, Roch G, Saiga H, Sasakura Y, Satake M, Satou Y, Schubert M, Sherwood N, Shiina T, Takatori N, Tello J, Vopalensky P, Wada S, Xu A, Ye Y, Yoshida K, Yoshizaki F, Yu JK, Zhang Q, Zmasek CM, de Jong PJ,

- Osoegawa K, Putnam NH, Rokhsar DS, Satoh N, Holland PW (2008) The amphioxus genome illuminates vertebrate origins and cephalochordate biology. *Genome Res* 18:1100–1011
- Huang S, Chen Z, Yan X, Yu T, Huang G, Yan Q, Pontarotti PA, Zhao H, Li J, Yang P, Wang R, Li R, Tao X, Deng T, Wang Y, Li G, Zhang Q, Zhou S, You L, Yuan S, Fu Y, Wu F, Dong M, Chen S, Xu A (2014) Decelerated genome evolution in modern vertebrates revealed by analysis of multiple lancelet genomes. *Nat Commun* 5:5896
- Ikuta T, Yoshida N, Satoh N, Saiga H (2004) *Ciona intestinalis* Hox gene cluster: its dispersed structure and residual colinear expression in development. *Proc Natl Acad Sci U S A* 101:15118–15123
- Johnsen AH, Rehfeld JF (1990) Cionin: a disulfotyrosyl hybrid of cholecystokinin and gastrin from the neural ganglion of the protochordate *Ciona intestinalis*. *J Biol Chem* 265:3054–3058
- Jonsson J, Carlsson L, Edlund T, Edlund H (1994) Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 371:606–609
- Kaji T, Hoshino Y, Henmi Y, Yasui K (2013) Longitudinal observation of Japanese lancelet, *Branchiostoma japonicum*, metamorphosis. Dataset Pap Biol 2013:6
- Kawada T, Ogasawara M, Sekiguchi T, Aoyama M, Hotta K, Oka K, Satake H (2011) Peptidomic analysis of the central nervous system of the protochordate, *Ciona intestinalis*: homologs and prototypes of vertebrate peptides and novel peptides. *Endocrinology* 152:2416–2427
- Kawai N, Ogura Y, Ikuta T, Saiga H, Hamada M, Sakuma T, Yamamoto T, Satoh N, Sasakura Y (2015) Hox10-regulated endodermal cell migration is essential for development of the ascidian intestine. *Dev Biol* 403:43–56
- Kocot KM, Tassia MG, Halanych KM, Swalla BJ (2018) Phylogenomics offers resolution of major tunicate relationships. *Mol Phylogenet Evol* 121:166–173
- Koyama H (2011) The postbranchial digestive tract of the ascidian, *Polyandrocarpa misakiensis* (Tunicata: Ascidiacea). 1. Oesophagus. *Zool Sci* 28:118–125
- Koyama H, Taneda Y, Ishii T (2012) The postbranchial digestive tract of the ascidian, *Polyandrocarpa misakiensis* (Tunicata: Ascidiacea). 2. Stomach. *Zool Sci* 29:97–110
- Lecroisey C, Le Pétilion Y, Escriva H, Lammert E, Laudet V (2015) Identification, evolution and expression of an insulin-like peptide in the cephalochordate *Branchiostoma lanceolatum*. *PLoS One* 10:e0119461
- Lescano MN, Fuentes VL, Sahade R, Tatián M (2011) Identification of gut contents and microscopical observations of the gut epithelium of the macrophagous ascidian *Cibacapsa gulosa* Monniot and Monniot 1983 (Phlebobranchia, Octacnemidae). *Polar Biol* 34:23–30
- Liang Y, Zhang S, Lun L, Han L (2006) Presence and localization of antithrombin and its regulation after acute lipopolysaccharide exposure in amphioxus, with implications for the origin of vertebrate liver. *Cell Tissue Res* 323:537–541
- Liberti A, Melillo D, Zucchetti I, Natale L, Dishaw LJ, Litman GW, De Santis R, Pinto MR (2014) Expression of *Ciona intestinalis* variable region-containing chitin-binding proteins during development of the gastrointestinal tract and their role in host-microbe interactions. *PLoS One* 9:e94984
- Liu M, Zhang S (2009) A kringle-containing protease with plasminogen-like activity in the basal chordate *Branchiostoma belcheri*. *Biosci Rep* 29:385–395
- Marlétaz F, Firbas PN, Maeso I, Tena JJ, Bogdanovic O, Perry M, Wyatt CDR, de la Calle-Mustienes E, Bertrand S, Burguera D, Acemel RD, van Heeringen SJ, Naranjo S, Herrera-Ubeda C, Skvortsova K, Jimenez-Gancedo S, Aldea D, Marquez Y, Buono L, Kozmikova I, Permayer J, Louis A, Albuixech-Crespo B, Le Petillon Y, Leon A, Subirana L, Balwierz PJ, Duckett PE, Farahani E, Aury JM, Manganot S, Wincker P, Albalat R, Benito-Gutiérrez È, Cañestro C, Castro F, D'Aniello S, Ferrier DEK, Huang S, Laudet V, Marais GAB, Pontarotti P, Schubert M, Seitz H, Somorjai I, Takahashi T, Mirabeau O, Xu A, Yu JK, Carninci P, Martínez-Morales JR, Crollius HR, Kozmik Z, Weirauch MT, Garcia-Fernández J, Lister R, Lenhard B, Holland PWH, Escriva H, Gómez-Skarmeta JL, Irimia M (2018) Amphioxus functional genomics and the origins of vertebrate gene regulation. *Nature* 564:64–70
- McRory J, Sherwood NM (1997) Two protochordate genes encode pituitary adenylate cyclase-activating polypeptide and related family members. *Endocrinology* 138:2380–2390
- Meulemans D, Bronner-Fraser M (2007) The Amphioxus SoxB family: implications for the evolution of vertebrate placodes. *Int J Biol Sci* 3:356–364
- Müller J (1844) Ueber den Bau und die Lebenserscheinungen des Branchiostoma lubricum Costa, *Amphioxus lanceolatus* Yarrell Abh K Preuss Akad Wiss Berl 79–116
- Murtaugh LC (2007) Pancreas and beta-cell development: from the actual to the possible. *Development* 134:427–438
- Nakashima K, Kimura S, Ogawa Y, Watanabe S, Soma S, Kaneko T, Yamada L, Sawada H, Tung CH, Lu TM, Yu JK, Villar-Briones A, Kikuchi S, Satoh N (2018) Chitin-based barrier immunity and its loss predated mucus-colonization by indigenous gut microbiota. *Nat Commun* 9:3402
- Nakayama S, Ogasawara M (2017) Compartmentalized expression patterns of pancreatic- and gastric-related genes in the alimentary canal of the ascidian *Ciona intestinalis*: evolutionary insights into the functional regionality of the gastrointestinal tract in Olfactores. *Cell Tissue Res* 370:113–128
- Nakayama S, Satou K, Orito W, Ogasawara M (2016) Ordered expression pattern of Hox and ParaHox genes along the alimentary canal in the ascidian juvenile. *Cell Tissue Res* 365:65–75
- Nakazawa K, Yamazawa T, Moriyama Y, Ogura Y, Kawai N, Sasakura Y, Saiga H (2013) Formation of the digestive tract in *Ciona intestinalis* includes two distinct morphogenic processes between its anterior and posterior parts. *Dev Dyn* 242:1172–1183
- Nielsen SE, Bone Q, Bond P, Harper G (2007) On particle filtration by amphioxus (*Branchiostoma lanceolatum*). *J Mar Biol Assoc UK* 87:983–989
- Ogasawara M (2000) Overlapping expression of amphioxus homologs of the thyroid transcription factor-1 gene and thyroid peroxidase gene in the endostyle: insight into evolution of the thyroid gland. *Dev Genes Evol* 210:231–242
- Ogasawara M, Di Lauro R, Satoh N (1999) Ascidian homologs of mammalian thyroid transcription factor-1 gene are expressed in the endostyle. *Zool Sci* 16:559–565
- Olinski RP, Dahlberg C, Thorndyke M, Hallböök F (2006) Three insulin-relaxin-like genes in *Ciona intestinalis*. *Peptides* 27:2535–2546
- On JS, Duan C, Chow BK, Lee LT (2015) Functional pairing of class B1 ligand-GPCR in cephalochordate provides evidence of the origin of PTH and PACAP/glucagon receptor family. *Mol Biol Evol* 32:2048–2059
- Onuma TA, Isobe M, Nishida H (2017) Internal and external morphology of adults of the appendicularian, *Oikopleura dioica*: an SEM study. *Cell Tissue Res* 367:213–227
- Orito W, Ohhira F, Ogasawara M (2015) Gene expression profiles of FABP genes in protochordates, *Ciona intestinalis* and *Branchiostoma belcheri*. *Cell Tissue Res* 362:331–345
- Pascual-Anaya J, Adachi N, Alvarez S, Kuratani S, D'Aniello S, Garcia-Fernández J (2012) Broken colinearity of the amphioxus Hox cluster. *EvoDevo* 3:28
- Perillo M, Wang YJ, Leach SD, Arnone MI (2016) A pancreatic exocrine-like cell regulatory circuit operating in the upper stomach of the sea urchin *Strongylocentrotus purpuratus* larva. *BMC Evol Biol* 16:117
- Petersen JK (2006) Ascidian suspension feeding. *J Exp Mar Biol Ecol* 342:127–137

- Piette J, Lemaire P (2015) Thaliaceans, the neglected pelagic relatives of ascidians: a developmental and evolutionary enigma. *Q Rev Biol* 90:117–145
- Putnam NH, Butts T, Ferrier DE, Furlong RF, Hellsten U, Kawashima T, Robinson-Rechavi M, Shoguchi E, Terry A, Yu JK, Benito-Gutiérrez EL, Dubchak I, Garcia-Fernández J, Gibson-Brown JJ, Grigoriev IV, Horton AC, de Jong PJ, Jurka J, Kapitonov VV, Kohara Y, Kuroki Y, Lindquist E, Lucas S, Osoegawa K, Pennacchio LA, Salamov AA, Satou Y, Sauka-Spengler T, Schmutz J, Shin-I T, Toyoda A, Bronner-Fraser M, Fujiyama A, Holland LZ, Holland PW, Satoh N, Rokhsar DS (2008) The amphioxus genome and the evolution of the chordate karyotype. *Nature* 453:1064–1071
- Reece BJ, Urry AL, Cain LM, Wasserman AS, Minorsky VP, Jackson BR (2011) *Campbell Biology*, Global edn. Pearson, London
- Riisgård HU, Svane I (1999) Filter feeding in lancelets (Amphioxus), *Branchiostoma lanceolatum*. *Invertebr Biol* 118:423–432
- Ruppert EE (1997) Cephalochordata. In: Harrison FW, Ruppert EE (eds) *Microscopic anatomy of invertebrates*, 15 Hemichordata, Chaetognatha, and the invertebrate chordates. Wiley-Liss, New York p. 349–504
- Ruppert EE, Fox SR, Barnes DR (2003) *Invertebrate zoology*. Brooks/cole, Pacific Grove
- Sasaki A, Miyamoto Y, Satou Y, Satoh N, Ogasawara M (2003) Novel endostyle-specific genes in the ascidian *Ciona intestinalis*. *Zool Sci* 20:1025–1030
- Sasaki N, Ogasawara M, Sekiguchi T, Kusumoto S, Satake H (2009) Toll-like receptors of the ascidian *Ciona intestinalis*: prototypes with hybrid functionalities of vertebrate toll-like receptors. *J Biol Chem* 284:27336–27343
- Sasakura Y, Hozumi A (2018) Formation of adult organs through metamorphosis in ascidians. *Wiley Interdiscip Rev Dev Biol* 7
- Satake H, Ogasawara M, Kawada T, Masuda K, Aoyama M, Minakata H, Chiba T, Metoki H, Satou Y, Satoh N (2004) Tachykinin and tachykinin receptor of an ascidian, *Ciona intestinalis*: evolutionary origin of the vertebrate tachykinin family. *J Biol Chem* 279:53798–53805
- Sato A, Satoh N, Bishop JDD (2012) Field identification of ‘types’ A and B of the ascidian *Ciona intestinalis* in a region of sympatry. *Mar Biol* 159:1611–1619
- Satoh N (2009) An advanced filter-feeder hypothesis for urochordate evolution. *Zool Sci* 26:97–111
- Satou Y, Kawashima T, Shoguchi E, Nakayama A, Satoh N (2005) An integrated database of the ascidian, *Ciona intestinalis*: towards functional genomics. *Zool Sci* 22:837–843
- Schmidt-Rhaesa A (2007) *The evolution of organ systems*. Oxford University Press, Oxford, New York
- Schubert M, Yu JK, Holland ND, Escriva H, Laudet V, Holland LZ (2005) Retinoic acid signaling acts via Hox1 to establish the posterior limit of the pharynx in the chordate amphioxus. *Development* 132:61–73
- Sekiguchi T, Kuwasako K, Ogasawara M, Takahashi H, Matsubara S, Osugi T, Muramatsu I, Sasayama Y, Suzuki N, Satake H (2016) Evidence for conservation of the calcitonin superfamily and activity-regulating mechanisms in the basal chordate *Branchiostoma floridae*: insights into the molecular and functional evolution in chordates. *J Biol Chem* 291:2345–2356
- Sekiguchi T, Ogasawara M, Satake H (2010) Molecular and functional characterization of cionin receptors in the ascidian, *Ciona intestinalis*: the evolutionary origin of the vertebrate cholecystokinin/gastrin family. *J Endocrinol* 213:99–106
- Sekiguchi T, Suzuki N, Fujiwara N, Aoyama M, Kawada T, Sugase K, Murata Y, Sasayama Y, Ogasawara M, Satake H (2009) Calcitonin in a protochordate, *Ciona intestinalis*—the prototype of the vertebrate calcitonin/calcitonin gene-related peptide superfamily. *FEBS J* 276:4437–4447
- Sun T, Zhang S, Ji G (2010) Identification and expression of an elastase homologue in *Branchiostoma belcheri* with implications to the origin of vertebrate pancreas. *Mol Biol Rep* 37:3303–3309
- Suzuki MM, Nishikawa T, Bird A (2005) Genomic approaches reveal unexpected genetic divergence within *Ciona intestinalis*. *J Mol Evol* 61:627–635
- Thompson JM, Di Gregorio A (2015) Insulin-like genes in ascidians: findings in *Ciona* and hypotheses on the evolutionary origins of the pancreas. *Genesis* 53:82–104
- van Weel PB (1937) Die Ernährungsbiologie von *Amphioxus lanceolatus*. *Publ Staz Zool Napoli* 6:221–272
- Yan J, Wang J, Zhao Y, Zhang J, Bai C, Zhang C, Zhang C, Li K, Zhang H, Du X, Feng L (2012) Identification of an amphioxus intelectin homolog that preferably agglutinates gram-positive over gram-negative bacteria likely due to different binding capacity to LPS and PGN. *Fish Shellfish Immunol* 33:11–20
- Yan J, Xu L, Zhang Y, Zhang C, Zhang C, Zhao F, Feng L (2013) Comparative genomic and phylogenetic analyses of the intelectin gene family: implications for their origin and evolution. *Dev Comp Immunol* 41:189–199
- Yonge CM (1925) Studies on the comparative physiology of digestion III. Secretion, digestion, and assimilation in the gut of *Ciona intestinalis*. *Br J Exp Biol* 11:373–388
- Wang Y, Wang H, Li M, Gao Z, Zhang S (2015) Identification, expression and regulation of amphioxus G6Pase gene with an emphasis on origin of liver. *Gen Comp Endocrinol* 214:9–16
- Willey A (1893) Studies on the Protochordata II. The development of the neuro-hypophysial system in *Ciona intestinalis* and *Clavelina lepadiformis*, with an account of the origin of the sense-organs in *Ascidia mentula*. *Q J Microsc Sci* 35:295–316

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.