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# Hepatic caecum of amphioxus and origin of vertebrate liver

Shicui Zhang [1, 2*], Zhaokang Shen [2], Haifeng Li [2]

[1] College of Life and Geographic Sciences, Key Laboratory of Biological Resources and Ecology of Pamirs Plateau in Xinjiang Uygur Autonomous Region, Kashi University, Kashi 844000, China

[2] Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao 266003, China

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**Abstract**

Liver is characteristic of all vertebrates. As a critical hub for many physiological processes including metabolism, innate immunity, protein synthesis and detoxification, its evolutionary origin was largely underappreciated in history, and only received due attention in recent decades. It has been suggested by morphological, ultrastructural and immunohistochemical studies that the hepatic caecum of amphioxus is homologous to the liver of vertebrate species. Molecular biology studies demonstrated that amphioxus hepatic caecum expresses plenty of vertebrate liver-specific genes. Our functional studies revealed significant similarities between amphioxus hepatic caecum and vertebrate liver. We also found that the functions of hepatic caecum are subjected to the regulation of pituitary hormones just as the liver does. These provide solid evidences supporting the notion that the hepatic caecum is the homologue of liver, which may represent the first stage in chordate evolution, laying a foundation for the subsequent formation of the liver as we know it in vertebrates. Further studies on the specification and morphogenesis of hepatic caecum in amphioxus will shed more lights on the origin and evolution of vertebrate liver.

**Key words:** protochordate, amphioxus, hepatic caecum, liver, evolution

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## 1 Introduction

The term liver is derived from Greek *liparós*, fat. Liver, the largest organ in our human body, is located asymmetrically in the right upper abdomen, separated from the chest cavity by the hemidiaphragm. It was said that the study of liver anatomy, especially with the specific examination of the sheep liver, dates back to the earliest period of Babylonian history about 3 000 BC (Jastrow, 1907). The liver was the single organ which was used for fortunetelling among the Babylonians, Greeks and Romans as it was regarded as the site of the soul, the vital organ and the central place of all mental and emotional activities (much later the organ heart started to serve this activity in these civilizations) (Riva et al., 2011). Now we know that the liver is a vascular glandular organ and performs many functions, including nutrient metabolism, immunoreaction, synthesis of blood-clotting proteins and complement factors, and detoxification of xenobiotic compounds (Trefts et al., 2017).

The liver is anatomically composed of several types of cells such as hepatocytes, cholangiocytes (biliary epithelial cells), stellate cells, Kupffer cells and sinusoidal endothelial cells (Abdel-Misih and Bloomston, 2010). The principal cell type of liver is the hepatocyte, which consists of the most part of the organ volume, and executes many of the functions generally associated with the liver. Hepatocytes are organized into a typically hexagonal shape around the central vein, forming the functional structural unit of the liver, the lobule. At the vertices of the hexagon are the portal triads making up of the hepatic artery (which supplies oxygenated blood to the liver), hepatic portal vein (which carries blood from the digestive organ to the liver), and bile ducts. Circulatory units within the lobule (hepatocyte chords) is different from a common capillary bed as the endothelial cells of the liver do not form tight junctions. This generates a sinusoidal network, the large-bore fenestrated capillaries of the liver, which minimizes barriers between hepatocytes and the blood traversing the sinusoid. Oxygenated blood in the hepatic artery combines with nutrient-rich blood from the portal circulation in the sinusoid, and then flows over the cells of the lobule and drains into the central vein. This ensures the blood composition exiting the lobule having different properties from the blood entering the lobule. As blood flows across the lobule, cells utilize oxygen and process nutrients, while forming metabolites and waste products. Along the length of the sinusoid, blood is deoxygenated, and metabolic byproducts are secreted from cells.

All vertebrates possess a definitive liver. It has been extensively studied anatomically, histologically, biochemically, physiologically and developmentally (Abdel-Misih and Bloomston, 2010). However, the study on the evolutionary origin of liver was largely underappreciated in history, and only received due attention in recent decades.

Genome sequencing has revealed that amphioxus or lancelet (cephalochordate), instead of ascidian (urochordate), has a closer relationship with the common ancestor of chordates (Delsuc et al., 2006; Putnam et al., 2008). Hence, amphioxus is an ideal model organism for the study of the origin and evolution of vertebrates including their tissues and organs. We have studied the developmental and evolutionary biology of amphioxus for

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*Corresponding author, E-mail: sczhang@ouc.edu.cn

over 30 years (Zhang, 2020). Here we summarize the progress on the study of amphioxus hepatic caecum, with an emphasis of the evolutionary origin of vertebrate liver.

## 2 Topological, morphological and developmental homologies

The germ layers endoderm, ectoderm and mesoderm are the three major embryonic cell types formed during gastrulation. The liver arises from the midventral endoderm of foregut first as a thickening of the endodermal epithelium and then as a hollow diverticulum, called the liver bud. The bud grows cephalad, while the cells proliferate and migrate into the surrounding septum transversum mesenchyme (Ober and Lemaigre, 2018; Zaret, 2000, 2016). The freshly formed hepatocytes surround spaces within the septum transversum mesenchyme, and the entire domain is delineated or encapsulated as the liver bud develops rapidly into an organ (Fig. 1a).

The thickening and budding of definitive endoderm involve the action of a series of extracellular growth factor signals in right timing. The earliest signals for initiating liver bud outgrowth from the foregut endoderm are fibroblast growth factor (FGF), and bone morphogenic proteins (BMPs). Both FGF and BMPs are from the overlying cardiac mesoderm and septum transversum derived from mesoderm (Fig. 1b). Other extracellular signals include at least transforming growth factor $\beta$ (TGF-$\beta$), Wnt and NOTCH, that are supported by expression and activity of transcription factors in the FoxA and GATA families within the endodermal epithelium (Zaret, 2000, 2016). The members of the families such as FoxA1 and GATA4 function as pioneer factors, interacting with their DNA-binding motifs within compact chromatin to alter nucleosome localization. This alteration of chromatin conformation produces a status of transcriptional competence for these and other downstream transcription factors, which eventually results in the creation and maintenance of a gene expression program important for differentiation and mature function (Trefts et al., 2017). It is during differentiation that the liver cells start to express plenty of genes that encode proteins required for hepatic specialized functions. For example, the key gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1, 6-bis-phosphatase (F6Pase) and glucose-6-phosphatase (G6Pase) are expressed predominantly or solely in hepatocytes, enabling the liver to perform gluconeogenesis. Other enzymes or proteins that are typical of or enriched in liver include aminotransferases (alanine aminotransferase and tyrosine aminotransferase), catabolite enzymes (phenylalanine hydroxylase, tryptophane oxygenase, serine dehydratase and urea cycle enzymes), serum proteins ($\alpha$-fetoprotein, albumin, insulin-like growth factor and blood-clotting proteins) and xenobiotic biotransforming enzymes (alcohol dehydrogenase, glutathione transferase and cytochromes P450), etc.

Several organs in invertebrate species have been suggested to be a functional equivalence of the vertebrate liver. For example, the fat body of insects, which in general consists of a mass of whitish cells (Snodgrass, 1935; Roma et al., 2005), plays a critical role in the dynamics of energy storage and mobilization, most biosynthetic activity and neutralization of non-utilized substances (Arrese and Soulages, 2010; Chapman, 1998; Keeley, 1985). However, the fat body is originated from the mesodermal walls of coelomic cavities during embryogenesis (Gillott, 1995), differing from the endodermal origin of vertebrate liver, thus it is not the homologue of the liver. Likewise, the hepatopancreas, a paired and tubular gland which is related with the mid-intestine of bivalves, gastropods and crustaceans (Rőszer, 2014; van Weel, 1974), performs some functions of the vertebrate liver including absorption of nutrients, glycogen and lipid storage and detoxification and heavy metal deposition (Goddard and Martin, 1966; Sumner, 1965; Ahearn et al., 2004) as well as the vertebrate pancreas including secretion of digestive enzymes like amylase, proteases, and lipases (Mansour-Bek, 1954; Vonk, 1960; van Weel, 1961, 1970; Arvy, 1969), but evolutionarily it is not the true homologue of the vertebrate liver or pancreas (Rőszer, 2014; van Weel, 1974). The hepatopancreas, initially called mid-intestinal gland (von Siebold, 1848), develops during embryogenesis from the symmetric outgrowth of the right and left walls of primitive gut, differing from the fact that the vertebrate liver arises as a ventral out-pocketing of the gut. Moreover, the tubules of hepatopancreas are ramified to primary and secondary branches, and its microscopic structure is fundamentally different from that of a true liver (van Weel, 1974). By sharp contrast, the hepatic cecum (i.e., liver-like sack) of amphioxus has been proposed to be the precursor of the liver since its first discovery by Müller (1844). The hepatic caecum is an out-pocketing of the mid-gut, which protrudes forward and extends along the right side of the posterior part of the pharynx. In Xiamen amphioxus (*Branchiostoma belcheri*), the hepatic caecum buds off at about 37 d post fertilization, and fully forms in about 42 d larvae (personal communication, Wang Yiquan in Xiamen University). Mounting data tend to bolster the notion of the homology of amphioxus hepatic caecum and vertebrate liver.

The hepatic caecum is also called digestive caecum, hepatic diverticulum, digestive diverticulum or midgut diverticulum. The side walls of hepatic caecum are much thicker than the top and bottom walls, making hepatic caecum a quite flat organ from the cross section (Fig. 2). Its inner wall is composed of regular arranged cells that can be clearly defined as two types: columnar epithelial cell and granular cell (Welsch, 1975). The former cell has a nucleus located in the bottom of the cell and surrounded by

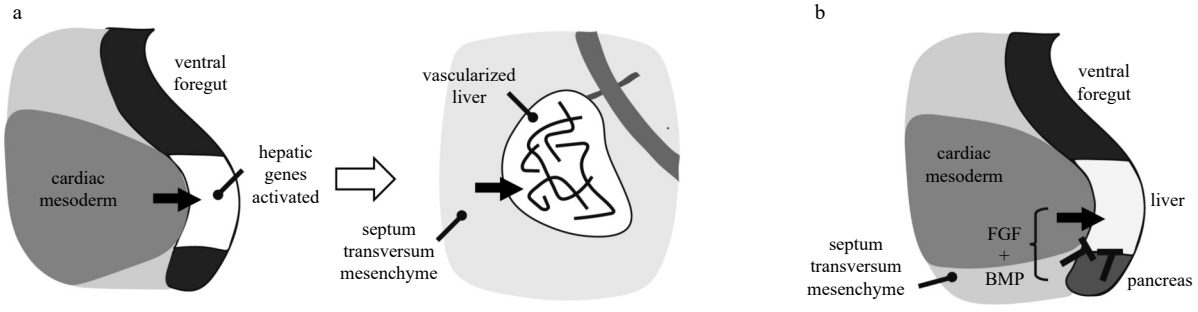


**Fig. 1.** Early development of liver. a. Phases of organogenesis. b. Combinatorial FGF and BMP signaling induces the liver fate while repressing the pancreas fate within the endoderm. Relevant components are denoted; arrows indicate critical signaling interactions.

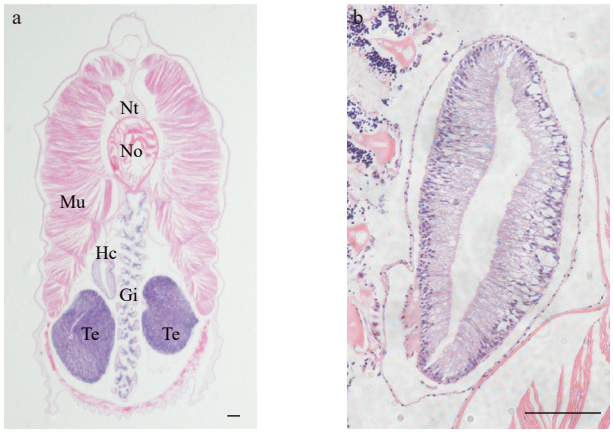


**Fig. 2.** Histological sections of amphioxus hepatic caecum. a. A cross section stained with H and E, showing the relative position of hepatic caecum. b. A magnified cross section showing the hepatic caecum. The nucleus of columnar epithelial cells is located at the base of the cell and surrounded by fat particles. The nucleus of granular cells is located at a higher position of the cell, close to its top surface. Gi, gill; Hc, hepatic caecum; Mu, muscle; No, notochord; Nt, neural tube; Te, testis. Scale bars represent 100 μm.

fat particles. Above the nucleus are irregular shaped vesicles, lysosomes and particles rich in glycogen. Occasionally lipid droplets can be observed within the cytoplasm. Mitochondria present closely beneath the upper surface of plasma membrane. Rough endoplasmic reticulum, a symbol of potential of protein synthesis in cell, is dense in this type of cell. The latter cell is more granular, and has a nucleus located at a higher position of the cell and with larger nucleoli. Mitochondria are located at the bottom of the cell instead of the top, being distinct from those of columnar epithelial cell. The cytoplasm of granular cell is abundant in mucus particles. The columnar epithelial cell is mainly in the lateral wall of hepatic caecum, while the granular cell in the top and bottom wall, yet both types of cell are found throughout the entire inner wall. These two types of cell are believed to participate in extracellular secretion. The inner wall of hepatic caecum contains ciliated bands, that promote the flow of the secreted contents via the swing of the cilia and lead them to the midgut (Welsch, 1975).

Amphioxus hepatic caecum has a hemal system which is vascularized not only by an arterial vessel, but also by a peculiar intestinal vein (Beklemishev, 1969; Rähr, 1979; Subbotin, 2018). Venous blood from the postcapillary network of the caudal intestine is gathered into an unpaired sub-intestine vein, which breaks into a capillary network again and brings blood to the caecum. The capillaries in the hepatic caecum are again gathered into a single vein (Fig. 3). This unique intestinal vein/caecum arrangement in amphioxus bears an apparent resemblance to the portal vein/liver system in vertebrates.

In the original thesis of Alexander Kovalevsky in 1865 on the development of *Amphioxus Lanceolatus*, published in Russian, he described that “developing diverticulum stretches from the gut” (Subbotin, 2019). Now we know that during larval development, the hepatic caecum of amphioxus branches off the gut in a position comparable to that of the vertebrate liver. Moreover, the cells of hepatic caecum begin to express genes encoding proteins needed for the unique specialized functions of the liver during differentiation. For example, the liver-specific homologous genes of liver fatty acid binding protein (L-FABP) and intestinal FABP (I-FABP) in amphioxus are initially expressed in the posterior two thirds of the primitive gut of 2 d larvae, including the mid-gut where the hepatic caecum emerges later (Wang et al., 2008). Similarly, the homologue of mammalian liver-specific gene encoding phosphatidylcholine transfer protein also exhibits a tissue-specific expression pattern in amphioxus, with high level in the region of the primitive gut where the hepatic caecum will form

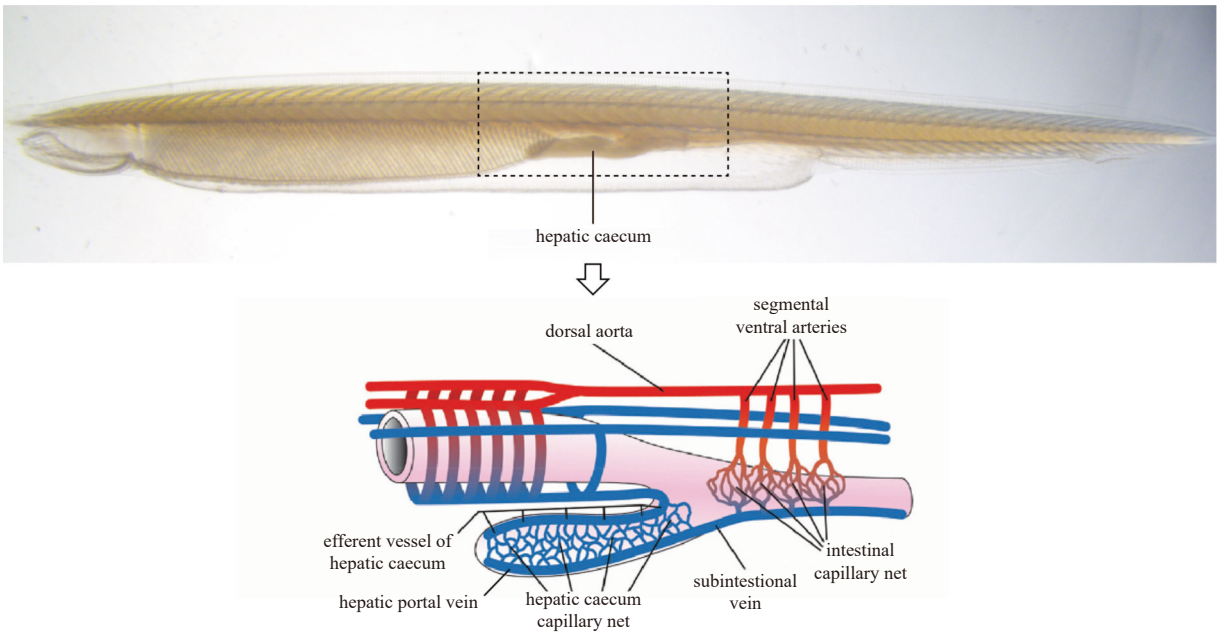


**Fig. 3.** Vascularization of amphioxus hepatic caecum. Venous blood from the postcapillary network of the caudal intestine is collected into an unpaired sub-intestine vein, which breaks into a capillary network again and brings blood to the caecum. The capillaries in the hepatic caecum are again collected into a single vein. This intestinal vein/caecum arrangement in amphioxus bears an apparent resemblance to the portal vein/liver system in vertebrates. The drawing in the figure showing the capillary network in amphioxus hepatic caecum is from Subbotin (2019).

later. Additional support for the homology of amphioxus hepatic caecum and vertebrate liver is that the hepatic caecum expresses plenty of vertebrate liver-specific genes such as those coding for glutathione-S-transferase (Fan et al., 2007), alanine aminotransferase (ALT) (Jing and Zhang, 2011), fibrinogen-related protein (Fan et al., 2008), plasminogen-like protein (Liu and Zhang, 2009), transferrin-like protein (Liu et al., 2009), C/EBP$\alpha/\beta$ (Wang et al., 2009), IGF-like factor (Guo et al., 2009), G6pase (Wang et al., 2015), complement system factors (Gao et al., 2014; He et al., 2008) and cytochrome P450 (Mizuta and Kubokawa, 2007). However, the molecular mechanisms that regulate the specification and morphogenesis of the hepatic caecum during development remains largely unknown, and needs further study.

## 3 Functional homologies

Vertebrate liver performs multiple functions such as metabolism, immunoreaction, blood-clotting protein and complement factor synthesis, and detoxification. Like vertebrate liver, amphioxus hepatic caecum is involved in metabolic activities, including the maintenance of the balance of carbohydrate metabolism (Fig. 4). In vertebrates, glucose glucokinase (GCK) catalyzes hexose phosphorylation and consumes ATP to convert glucose to glucose-6-phosphate, which is then hydrolyzed to phosphogroup and free glucose by G6Pase. In amphioxus, the enzymes resembling GCK and G6Pase have been identified. They are both mainly distributed in the hepatic caecum, and may regulate carbohydrate metabolism as doing in the liver (Li et al., 2014; Wang et al., 2015). For example, GCK activity in the hepatic caecum is significantly decreased by fasting, whereas it is markedly increased by feeding. In vertebrates, G6Pase gene is subject to regulation by growth hormone (GH) (Leung and Woo, 2010). We also show that treatment with GH induces a similar expression pattern of G6Pase gene in both zebrafish and amphioxus. In addition, G6Pase activity in amphioxus hepatic caecum is clearly regulated by feeding and fasting as observed in vertebrate liver (Wang et al., 2015). Collectively, these suggest that the hepatic caecum, like vertebrate liver, plays a role in the mediation of glucose homeostasis in amphioxus.

The metabolic activities of hepatic caecum are not restricted to carbohydrate metabolism as several proteins involved in lipid/protein metabolism are synthesized within this organ. It is known that FABPs play an important role in lipid metabolism, and phosphatidylcholine transfer protein (PCTP) participates in the coordination and coupling of phospholipid metabolism with vesicle trafficking. Both FABPs and PCTP are predominantly distributed in amphioxus hepatic caecum (Orito et al., 2015; Tian et al., 2007; Wang et al., 2008a). ALT catalyzes the transfer of an amino group from alanine to α-ketoglutarate in the alanine cycle

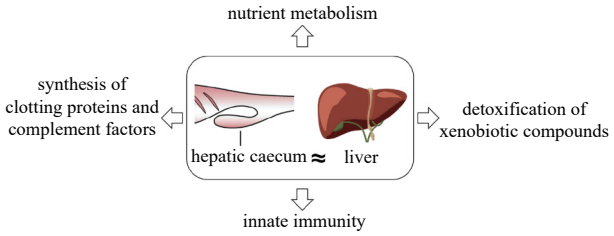


**Fig. 4.** Functional similarities between amphioxus hepatic caecum and vertebrate liver. The hepatic caecum and the liver execute functions in common at least in metabolism, immunoreaction, blood-clotting protein and complement factor synthesis, and detoxification.

to form pyruvate and glutamate, and cathepsins L and B are associated with multiple biological processes including intracellular protein catabolism and turnover. Notably, ALT and cathepsins L and B are also mainly localized in amphioxus hepatic caecum (Jing and Zhang, 2011; Wang et al., 2008b). All these implicate that the hepatic caecum is closely related with lipid/protein metabolism.

Emerging evidences indicate that mammalian liver is an immune-relevant organ which plays a vital role in innate immunity (Gao et al., 2008), especially in acute phase response (APR). APR is the systemic physiological response which occurs immediately after the onset of infection, trauma, inflammatory processes and some malignant conditions. One of the most striking consequences of APR is the fluctuation in concentrations of various plasma proteins, collectively known as the acute phase proteins (APPs). APPs can protect the body via inhibiting microbial growth, restricting tissue damage and restoring metabolic homeostasis. In vertebrate hepatocytes, the synthesis of APPs is regulated by liver-specific transcription factors including hepatocyte nuclear factor 4 (HNF-4), CAAT/enhancer binding protein (C/EBP) and signal transducer and activator of transcription (STAT), that together form a complex regulatory network to ensure the rapid and effective occurrence of APR and to promote its rapid return to normal status (Cereghini, 1996; Gao et al., 2008). We have shown that in amphioxus, the concentrations of the APPs antithrombin (AT), ALT, factor B-like protein Bf/C2, transferrin-like factor and transthyretin (TTR) in the humoral fluids exhibit a pattern of change similar to that found in vertebrates following lipopolysaccharide (LPS) treatment (Liang and Zhang, 2006; Lun et al., 2006; Zhang et al., 2009). To systematically study the regulation of APR in amphioxus, we have first taken 129 liver-specific genes of zebrafish as the template, and identified 58 hepatic cecum-specific genes (equivalent to zebrafish liver-specific genes) in amphioxus by analyses of combining global genome survey with qRT-PCR. Totally, 52 out of the 58 hepatic cecum-specific genes respond to lipopolysaccharide treatment, displaying closely similar expression profiles in both the species zebrafish and amphioxus. Searching for and comparing the binding sites of the three transcription factors HNF-4, C/EBP and STAT, in the promoter regions of 52 APR-related genes reveals the presence of 2 or more transcription factors in most orthologous genes, suggesting that HNF-4, C/EBP and STAT form a similar APR regulatory network in both the species amphioxus and zebrafish (Wang and Zhang, 2011). The similarities in the liver/hepatic cecum-specific gene expression, APR and regulatory networks between amphioxus and zebrafish definitely support the notion that amphioxus hepatic cecum is the "pre-hepatic" structure homologous to vertebrate liver, which acts as an immunological organ and plays a vital role in APR (Fig. 4).

The mammalian complement system is part of our innate immune response, and comprises more than 30 different proteins and membrane-associated proteins, that are primarily synthesized in the liver (Sarma and Ward, 2011). Multiple copies of a large number of complement-related genes, including C1q-like, ficolin-like, 2 mannan-binding lectin-associated serine protease (MASP), 2 complement component 3 (C3), 3 Bf/C2, 5 C6-like and 427 CCP (complement control protein)-containing genes, have been identified in amphioxus genome. In this respect, the complement system of amphioxus appears more diverse and complex than the mammalian one (Huang et al., 2008). The mammalian complement system includes three activation pathways, i.e., the classical, alternative and lectin pathways. All the three pathways converge on the cleavage and activation of C3 by C3-

convertase, which then triggers a cascade of further cleavage and activation events. It has been shown that the complement system of amphioxus can be activated through alternative (C3 autohydrolysis) pathway, lectin (ficolin-MASPs activating C3) pathway and classical-like (C1q-MASPs activating C3) pathway (Gao et al., 2014, 2017; Huang et al., 2011; Li et al., 2008). Some of the key molecules involved in the complement activation pathways including C1q-like protein, ficolin, MASP1/3, C3, Bf/C2 and properdin have been identified in amphioxus (Endo et al., 2003; Gao et al., 2013, 2014, 2017; He et al., 2008; Huang et al., 2011; Suzuki et al., 2002). All the molecules except properdin are predominantly produced in the hepatic caecum, agreeing well with the fact that the liver is the main synthesis site of complement factors in the vertebrate. The properdin gene is ubiquitously expressed, which is also similar to the expression profile of mammalian properdin gene (Cortes et al., 2013). All these indicate that amphioxus hepatic cecum, like mammalian liver, plays a central role in the regulation of the complement system (Fig. 4).

Blood coagulation, or clotting, is extremely critical for the survival of all vertebrates by blocking the leakage of blood from the sites of injury and preventing infection by microbial invaders. Clotting follows the same fundamental pattern in vertebrates, with the culminating event of the thrombin-catalyzed conversion of fibrinogen into an insoluble fibrin (Doolittle and Surgenor, 1962; Doolittle, 1990). Fibrin clots are ultimately dissolved in due course to restore vascular potency (Longstaff and Thelwell, 2005). The enzymes involved in this process comprise the fibrinolytic system (Collen and Lijnen, 1991) which consists of plasminogen (Plg), plasminogen activators (PAs), plasminogen inhibitors (PAIs) and plasmin inhibitors (PIs). The central molecule in the fibrinolytic system is the glycoprotein Plg, which circulates in plasma as a proenzyme (Rijken and Sakharov, 2001). Following limited proteolysis by a PA, Plg is converted into the active enzyme plasmin. In addition, antithrombin (AT), a major inhibitor of the clotting serine proteinases (Frost et al., 2002; Olson and Björk, 1994), plays a critical role in the maintenance of normal haemostasis (Abildgaard, 1979). Both Plg and AT are primarily produced by the liver. We have shown the existence of Plg-like homologue in amphioxus (Liang and Zhang, 2006; Liu and Zhang, 2009). The recombinant amphioxus Plg-like protein is readily activated by human urokinase PA, and exhibits Plg-like activity. Amphioxus Plg-like protein can also auto-activate itself at neutral and alkaline pH at 4ºC without the addition of urokinase PA, and the auto-activation is accelerated by addition of human urokinase PA. We have also shown the presence of AT-like homologue in amphioxus (Chao et al., 2012; Liang et al., 2006). The recombinant amphioxus AT-like protein exhibits thrombin-inhibiting activity, which can be promoted by heparin. Mammalian AT inactivates the coagulation protease thrombin by forming stable equimolar AT/target enzyme complex (Danielsson and Björk, 1982, 1983). Amphioxus AT-like protein is also capable of interacting with bovine thrombin in the presence of heparin by forming AT-like protein-thrombin complex, implicating that amphioxus AT-like protein, resembling mammalian AT, utilizes a similar mechanism to bind to thrombin. Importantly, both Plg-like protein and AT-like protein are produced in the hepatic caecum in amphioxus, consistent with the synthesis of Plg and AT in vertebrate liver (Fig. 4). Taken together, these suggest that a primitive clotting system already emerged in amphioxus.

Evidences have shown that amphioxus hepatic caecum also plays roles of detoxification and chemical defense. Early experiments demonstrated that the pigments entered into the blood or tissues of amphioxus could not pass through the guts and were sent to hepatic caecum through blood circulation, where they accumulated on the side walls, transformed into small spherical particles, and eventually degraded, suggesting occurrence of detoxification in hepatic caecum (Barrington, 1937; Rähr, 1979). This is apparently confirmed by the observation of Bhattacharya et al. (2008). They exposed both rosy barb (*Puntius conchonius*) and amphioxus (*Branchiostoma japonicum*) to carbon tetrachloride ($CCl_4$), an organic compound which is able to cause damage to liver, and found that in semi-lethal dose, $CCl_4$ induces serious damages to the liver of rosy barb as well as the hepatic caecum of amphioxus, suggesting that the hepatic caecum is homologous to the liver in rosy barb in respect to toxic damages of $CCl_4$. Additionally, the genes involved in detoxification or chemical defense, such as those encoding glutathione S-transferase (GST) and cytochrome P450 (CYP450), are highly expressed in the hepatic caecum of amphioxus. GST performs many functions including the catalysis of tripeptide glutathione (GSH) conjugation with diverse electrophilic substrates and metabolism of toxic substances. The primary synthesis site of GST in vertebrates is liver (Eaton and Bammler, 1999). We found that GST is mainly distributed in hepatic caecum of amphioxus (Fan et al., 2007). The CYP450 genes encode the most diverse class of enzyme superfamily members in nature, that are often abbreviated as CYPs. CYPs catalyze oxidative transformation and are involved in the metabolism of toxic substances such as drugs and chemical compounds. Genome-wide scanning revealed that amphioxus contains 16 families of CYPs, known as CYP1~CYP4, CYP7, CYP8, CYPII, CYPI7, CYPI9, CYP20, CYP24, CYP26, CYP27, CYP39, CYP46 and CYP51 families documented in vertebrates. Among CYP families, CYP1–CYP4 are strongly associated with chemical defense. In amphioxus exposed to tetrachlorodibenzo-p-dioxin, the expression of CYP1, CYP3 and CYP4 genes was significantly elevated in hepatic caecum, implying the involvement of hepatic caecum in chemical defense (Zhang, 2020).

In vertebrates, the function of liver is subjected to the regulation of pituitary hormones including GH and thyroid stimulating hormone (TSH). GH generally binds to the GH receptor on the hepatocyte membrane, stimulating the release of IGF, which regulates the proliferation, differentiation and apoptosis of both bone and muscle cells. Such a system is known as GH/IGF signaling pathway. TSH stimulates the synthesis and secretion of the thyroid hormones (THs) $T_4$ and $T_3$ via binding to the specific membrane TSH receptor (TSHR). A major target of THs is the liver, within which THs enter hepatocytes and combine with the thyroid hormone receptors (THRs), activating the TH/THR signaling pathway that acts directly on the target genes and regulates their transcription. Both GH/IGF signaling and TH/THR signaling are rather conserved and exist in all vertebrates. Our studies have proven the presence of the regulatory systems resembling GH/IGF and TH/THR pathways in amphioxus (Li et al., 2014, 2017; Liu and Zhang, 2011; Wang et al., 2009, 2018). In amphioxus, the Hatschek's pit, an organ equivalent to pituitary gland, can generate GH-like and TSH-like hormones that both play functions similar to vertebrate GH and TSH, regulating the activities of hepatic caecum in amphioxus (for review, see Zhang and Ji, 2022).

## 4 Conclusions and perspectives

Our understanding of the hepatic caecum of amphioxus has made great progress in the past two decades. Previously, both morphological and ultrastructural as well as immunohistochemical investigations suggested the homology of amphioxus hepatic

caecum and vertebrate liver. Molecular biology studies have revealed that the hepatic caecum expresses plenty of liver-specific genes including those encoding fatty acid binding protein, phosphatidylcholine transfer protein, glutathione-S-transferase, aminotransferase, vitellogenin and cytochrome P450. Our functional examinations have shown that the hepatic caecum, like the liver, performs multiple functions including metabolism, immunoreaction, blood-clotting protein and complement factor synthesis, and detoxification. Moreover, the function of hepatic caecum is subjected to the regulation of GH-like and TSH-like hormones generated by the Hatschek's pit of amphioxus, an organ equivalent to vertebrate pituitary gland. All these provide solid evidences bolstering the notion that the hepatic caecum is the liver homologue, though the mechanisms that regulate the specification and morphogenesis of the hepatic caecum remains to be examined.

The topography of vertebrate liver is characterized by the left smaller lobe and the right lobe, that are separated by the falciform ligament. The hepatic caecum of amphioxus arises as a ventral outpouching of the primitive gut and grows cephalad beneath the pharynx, but remains a hollow sac with a ciliated lining throughout life. It is highly likely that the hepatic caecum of amphioxus represents the first stage during chordate evolution in the development of liver as we know it in vertebrates. How the general architecture of the two-lobe liver emerged evolutionarily remains open and needs further study.

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