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Research Paper

Molecular basis of glucose homeostasis in the Pacific oyster (*Crassostrea gigas*): insights from transcriptome and hexokinase phylogeny analysis*

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Abstract Glucose homeostasis is a fundamental physiological process in both vertebrates and invertebrates, yet its regulatory mechanisms in molluscs remain largely unexplored. This study investigates temporal hemolymph glucose dynamics and associated molecular responses in the Pacific oyster *Crassostrea gigas*. Annual monitoring revealed significant seasonal variation in hemolymph glucose concentrations, with post-spawning oysters exhibiting the lowest levels. A glucose injection experiment demonstrated rapid uptake kinetics, followed by a return to baseline, suggesting the presence of efficient metabolic regulation. Transcriptomic analysis on hepatopancreas tissue identified *cgHK2-2* as the dominant hexokinase isoform induced by hyperglycemia, while other *HK* genes (*cgHK2* and *cgHK2-like*) showed tissue-specific but non-inducible expression profiles in the investigated tissue. Furthermore, upregulation of *cgPPP1R3B* and *cgPCSK1* indicate possibly conserved glycogen metabolism and insulin-like signaling pathways. Phylogenetic analysis revealed divergent evolutionary trajectories of hexokinase in protostomes versus chordates, with oysters lacking a clear glucokinase orthologue. These findings highlight key molecular players in oyster glucose metabolism and suggest both conserved and lineage-specific regulatory strategies.

Keyword: glucose homeostasis; hexokinase; *PPP1R3B*; *PCSK1*; transcriptome analysis; seasonal variation

1 INTRODUCTION

Glucose homeostasis refers to the process by which organisms maintain hemolymph glucose concentrations within a relatively stable range through specific mechanisms, which is essential for the proper functioning of physiological processes (Häusl et al., 2019). The evolution of increasing systemic complexity led to the development of highly specialized endocrine systems and organs in vertebrates, such as the liver and pancreas, which play key roles in regulating glucose metabolism (Steinke et al., 2006; Pan et al., 2024). Rapid glucokinase (GCK) and insulin response plays vital role in vertebrates blood glucose homeostasis regulation (Sternisha and Miller, 2019). In contrast, invertebrates encompass a diverse array of species and represent one of the earliest animal groups on earth, having undergone independent evolution for hundreds of millions of years (Erwin, 2011). During this time, they have developed unique physiological characteristics and metabolic strategies to adapt to various ecological niches. In some invertebrates,

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such as *Drosophila*, hormones similar to insulin, known as insulin-like peptides (ILPs), have been shown to regulate hemolymph glucose homeostasis (Wu and Brown, 2006). Researches from both Deuterostomia and Protostomia have indicated the possible ancient origin of the hemolymph glucose regulation pathway (Chatterjee and Perrimon, 2021; Xu et al., 2021).

However, differences in genetic toolkits lead to fundamental variations in the mechanisms underlying hemolymph glucose homeostasis across animal clades. In vertebrates, the evolution of vertebrate hexokinase involved gene duplication, recombination, mutation, and functional diversification, giving rise to one smaller *GCK* gene containing a single hexokinase domain, and three larger hexokinase genes (*HK1–HK3*), each with two hexokinase domains (Irwin and Tan, 2014). Hexokinases catalyze the phosphorylation of glucose and other six-carbon sugars, with glucokinase primarily expressed in the pancreas, where it functions as a hemolymph glucose sensor and regulates glucose-stimulated insulin secretion (Sternisha and Miller, 2019). Through these pathways, the hemolymph glucose levels are maintained within a narrow physiological range.

Glucose serves as a universal fuel source, powering cellular activity from bacteria to mammals. It is believed that the hexokinases found in bacteria and eukaryotes evolved from a common ancestral gene. However, long-term evolutionary divergence has led to the specialization of glucose-regulating tissues and mechanisms. For example, in *Drosophila*, the open circulatory system leads to separate nutrient and oxygen delivery, with the neurons and fat body responsible for nutrient sensing and glycogen storage. Despite these differences, insulin-like secretion still plays a central role in *Drosophila* glucose homeostasis (Chatterjee and Perrimon, 2021), and hexokinase remains essential for responding to fluctuations in circulating glucose levels (Miyamoto and Amrein, 2019; Oh et al., 2019).

As the most species-rich phylum among marine animals and the clade Lophotrochozoa, molluscs remain largely understudied in terms of hemolymph glucose regulation. Studies suggest that hemolymph glucose levels in bivalves modulate carbohydrate accumulation, such as glycogen synthesis in muscle, and are closely linked to the energy requirements of reproduction, with increased glucose availability promoting glycogen storage and subsequent mobilization during gametogenesis (Bayne, 1976; Fearman et al., 2009). Additionally, the ability of oysters to regulate hemolymph glucose impacts their stress resistance and growth (Pérez-Velasco et al., 2022). Hemolymph glucose levels are also used as an indicator of molluscs' physiological responses to environmental stress (Fei et al., 2023).

Oysters, as one of the most extensively studied molluscs, exhibit low tissue specialization and primarily rely on neuroendocrine cells to produce signaling molecules in response to internal and external stressors (Fabbri et al., 2024). Research suggests that ILPs in oysters may play a role in hemolymph glucose regulation (Xu et al., 2021). However, the precise mechanisms governing hemolymph glucose homeostasis in oysters remain unclear. Given that glycogen content is a key economic trait in oysters, understanding the regulatory mechanisms of hemolymph glucose homeostasis is of considerable significance.

In this study, glucose was administered via injection to induce hyperglycemia in oysters, and the temporal changes in hemolymph glucose levels were monitored. Hepatopancreas samples were then collected for transcriptomic analysis, followed by differential gene expression analysis to identify potential pathways involved in hemolymph glucose regulation. Additionally, the expression patterns of hexokinase genes were examined in samples collected over an annual growth cycle. This study provides valuable data to support further research on hemolymph glucose regulation and key biological processes in oysters, including growth, reproduction, stress response, and glycogen accumulation.

2 MATERIAL AND METHOD

2.1 Experimental animal and pretreatment

The experimental animals were two-year-old oysters from the same breeding group. These oysters were separated into individual monomers and intensively cultured in the aquaculture waters of Jiaonan, Qingdao, Shandong Province. Tissues including the adductor muscle, heart, gill, mantle, labial palps, and hepatopancreas were collected from three oysters and flash-frozen in liquid nitrogen for gene expression pattern analysis. Additionally, farm-cultured oysters were sampled annually at approximately one-month intervals to assess seasonal variations in hemolymph glucose levels and gene expression patterns (Miao et al., 2022; Qian et al., 2023), with nine oysters collected

at each time point. To facilitate continuous blood sampling, a small hole was drilled in the right shell above the pericardial cavity. Additionally, the small piece of shell on the adductor muscle side was carefully removed using an oyster knife to expose the muscle for intramuscular injection, ensuring that the mantle tissue remained intact. Following these procedures, the treated oysters were returned to seawater and allowed to recover for approximately 24 h.

2.2 Glucose injection

An experiment was conducted to investigate the kinetics of glucose in oyster hemolymph. Oysters (average shell height: 109.8±8.1 mm) were randomly divided into two treatment groups and intramuscularly injected with 200 μL of glucose solutions at different concentrations. Three individuals received 10-mg/mL glucose in PBS, while six received 30 mg/mL. One oyster injected with PBS served as a control. Approximately 20 μL of hemolymph was collected from the pericardial cavity at 21 time points: 20 and 40 s post-injection, every minute from 1 to 10 min, every 5 min from 10 to 30 min, and every 30 min from 30 to 180 min for hemolymph glucose analysis.

Based on the glucose response curve, injection and sampling protocols were optimized according to the animal size for transcriptomic analysis. Eighteen healthy oysters of similar size (average shell height: 83.9±6.4 mm) were anesthetized in 8% $MgCl_2$ seawater and randomly divided into two groups. The glucose group (G, n=9) received 100 μL of 30-mg/mL glucose in 0.22-μm filtered artificial seawater (ASW); the control group (M, n=9) received 100 μL of ASW. Hemolymph was collected from three individuals per group at 10, 30, and 150 min post-injection as biological replicates. Corresponding hepatopancreas tissues were harvested and flash-frozen in liquid nitrogen for RNA-seq.

To validate key gene expression patterns, a larger-scale experiment was conducted using 162 healthy oysters with an average shell height at 104.0±10.9 mm. Individuals were pretreated as described in Section 2.1 and randomly divided into G and M groups (n= 81 each). The G group received 200 μL of 30-mg/mL glucose in ASW, and the M group received an equal volume of ASW. Hemolymph samples were collected from 27 oysters per group at 10, 30, and 150 min.

2.3 Hemolymph glucose content determination

Hemolymph collected from the pericardial cavity was temporarily stored on ice and then centrifuged at 500×g for 5 min at 4 °C to remove hemocytes. The resulting supernatant was used to determine hemolymph glucose level using the Glucose Assay Kit (GAGO20, Sigma) following the manufacturer's instructions. Briefly, a standard curve was generated with diluted glucose standard solution at concentrations of 0, 0.002 5, 0.005, 0.007 5, 0.01, 0.02, 0.08 mg/mL. Then, 40 μL of the diluted hemolymph sample was added to a 96-well plate, mixed with 80 μL of assay reagent, and incubated at 37 °C for 30 min. The reaction was then stopped by adding 6-mol/L sulfuric acid, and absorbance was measured at 540 nm. Glucose concentration was calculated based on the standard curve and expressed in mg/mL.

2.4 RNA extraction, real-time quantitative PCR, and transcriptomic analysis

The total RNA of hepatopancreas was extracted using the TRIzol reagent (Invitrogen, USA). RNA purity and integrity were assessed using NanoDrop 2000 spectrophotometer and the RNA Nano 6000 Aaasy Kit of Agilent Bioanalyzer 2100 system. Real-time quantitative PCR (qPCR) was performed using the Rapid Taq Master Mix (Vazyme, P222, China) on a QuantStudio 6 Flex Real-Time PCR System (Thermo Fisher, USA). The *cgEF* gene was used as the internal reference. The primers used in this study are listed in Table 1.

Table 1 Primers used for real-time qPCR analysis

Primer	Gene ID	Sequence
cgEF-qF	LOC105338957	5′-ATCACCAAGGCTGCACAGAAAG-3′
cgEF-qR		5′-TCCGACGTATTTCTTTGCGATT-3′
cgHK2-2-qF	LOC105317783	5′-CCATTGCAAGGAGAAAGGACAT-3′
cgHK2-2-qR		5′-TGTCGGCATAATTCCCAGCC-3′
cgHK2-qF	LOC105330845	5′-CCTGACGGTTCAGAAAAAGGG-3′
cgHK2-qR		5′-TGAAACCCAGAGGCAGCTTT-3′
cgHK2-like-qF	LOC105346675	5′-CCGACAGGTGGACAAACACT-3′
cgHK2-like-qR		5′-GCGATCCGACAGTCCTCATC-3′

Upon successful sample qualification, gene library construction was initiated following the Illumina library preparation protocol. After passing quality control, distinct libraries were pooled based on the target data output and sequenced using the Illumina NovaSeq platform. Cleaned sequencing reads were mapped to the oyster genome assembly version "cgigas_uk_roslin_v1" using HISAT2 (Kim et al., 2019). Gene expression levels were quantified in transcripts per million mapped reads (TPM) using StringTie2 (Kovaka et al., 2019). Statistical analyses and visualizations were conducted in R software (Ihaka and Gentleman, 1996), with various packages utilized for specific analyses: The "apeglm" package (Zhu et al., 2019) was used for logarithmic fold change (LFC) estimation using the adaptive Student's t prior shrinkage estimator. The DESeq2 package (Love et al., 2014) was applied to identify differentially expressed genes (DEGs) with a threshold of $|LFC| \geq 1.5$ and P value <0.01. The clusterProfiler package (Yu et al., 2012) was used for KEGG pathway enrichment analysis.

2.5 Phylogenetics analysis of hexokinase family

Hexokinase protein sequences from representative species of Chordata, Arthropoda, Echinodermata, Porifera, Annelida, Protozoa, Cnidaria, Ctenophora, Filasterea, Spermatophyta, and Mollusca (including oysters) were downloaded from the NCBI database. BLASTP searches were performed using human and *Drosophila* hexokinase sequences as queries against the genome data of the selected species to ensure comprehensive identification of hexokinase proteins. Multiple sequence alignment was conducted using MAFFT version 7 (Katoh and Standley, 2013) with the L-INS-i algorithm. Alignment trimming was carried out with trimAl, retaining the C-terminal hexokinase domain of vertebrate hexokinase proteins for phylogenetic analysis. A phylogenetic tree was constructed using MrBayes (Ronquist et al., 2012) with GTR+I+ Γ(gamma) evolutionary model. Four Markov chains were run for 3×10^6 generations to generate a posterior probability distribution. The resulting tree was visualized and edited using Chiplot (Xie et al., 2023).

3 RESULT

3.1 Kinetics of glucose in oyster hemolymph

A total of 13 sampling time points were examined to monitor the annual variation in oyster hemolymph glucose levels, with 30 individuals assessed at each time point. The average hemolymph glucose level was approximately 0.014 mg/mL, exhibiting significant seasonal fluctuations (Fig.1a). The highest levels were recorded in May, averaging 0.025 mg/mL in May 2020 and 0.028 mg/mL in May 2021. The lowest levels, around 0.006 mg/mL were observed in July, likely due to spawning. Additionally, many oysters in November displayed low hemolymph glucose levels, possibly due to limited food availability. Overall, average hemolymph glucose levels remained stable between 0.009 and 0.014 mg/mL from August to March of the following year. Oysters with hemolymph glucose levels exceeding 0.100 mg/mL in the natural environment were rarely observed in this study.

Following intramuscular injection, hemolymph glucose levels rose rapidly within 20 s, peaking between 1 and 10 minutes post-injection (mpi). Elevated glucose levels persisted for up to 2 h, before returning to normal at approximately 3 h post-injection (Fig.1b). Based on the glucose kinetics in oyster hemolymph, an injection concentration of 30 mg/mL was selected to artificially elevate hemolymph glucose levels for transcriptome analysis. Sampling time points were set at 10, 30, and 150 mpi. At each time point, hemolymph glucose levels remained elevated but showed a significant decreasing trend over time (Fig.1c).

3.2 Transcriptomic response of oysters to high hemolymph glucose levels

Transcriptomic analysis revealed that oysters exhibit a relatively weak transcriptional response to elevated hemolymph glucose levels, with substantial variation in gene expression observed between individuals. Only a limited number of DEGs were identified, suggesting that the glucose stimulus may not have been strong enough to provoke a widespread transcriptional response. In the first RNA-seq experiment, 233 DEGs (114 upregulated), 375 DEGs (214 upregulated), and 301 DEGs (137 upregulated) were detected at 10, 30, and 150 mpi, respectively, when glucose-treated samples were compared to controls (Supplementary Table S1). Similarly, in the second validation experiment, 294 DEGs (156 upregulated), 428 DEGs (198 upregulated), 384 DEGs (194 upregulated) were identified at the corresponding time points (Supplementary Table S2). To enhance robustness, the union set of DEGs from both experiments was used for subsequent analysis.

KEGG pathway enrichment analysis showed that the DEGs were mainly associated with sugar or fatty

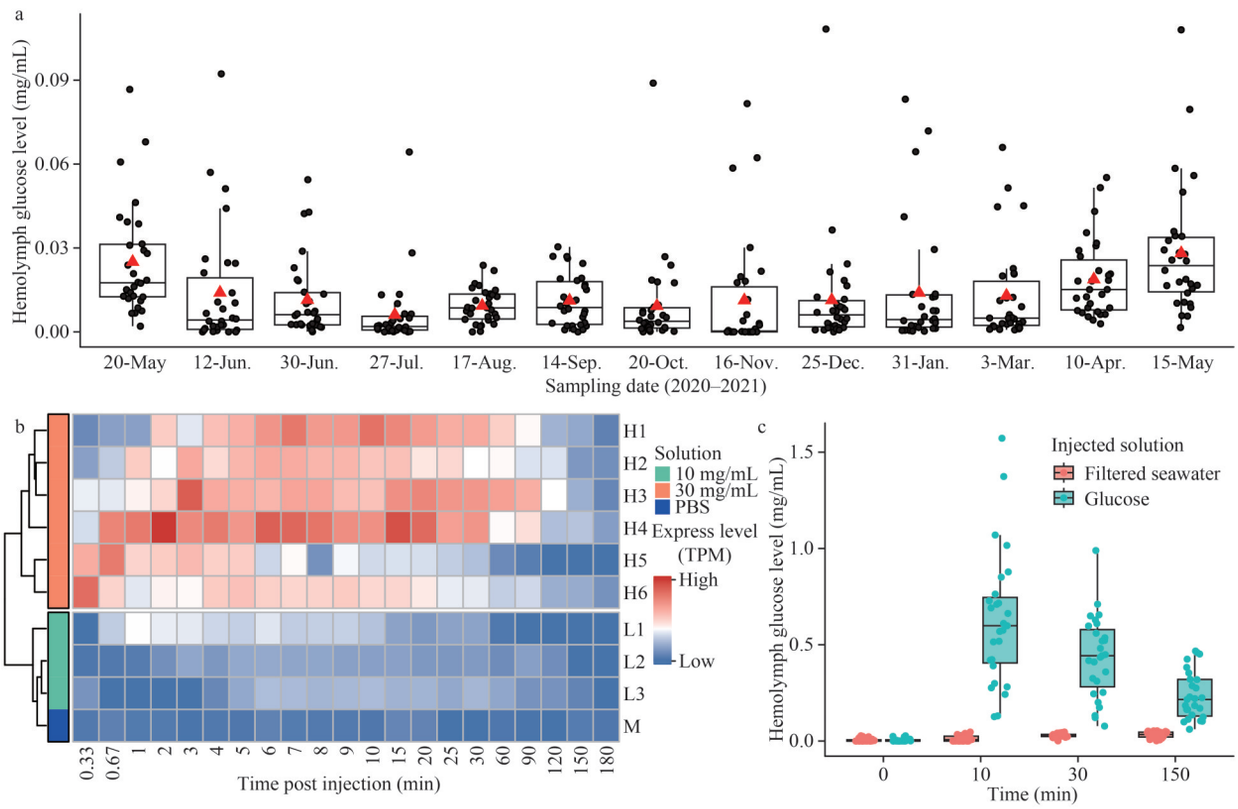

Fig.1 Variation of oyster hemolymph glucose concentration during seasonal farm culture and following artificial injection

a. the annual monitoring data of oyster hemolymph glucose concentration; data from 20 May to 20 Oct. were previously reported (Miao et al., 2022); b. changes in hemolymph glucose concentration in oysters injected with 30-mg/mL glucose solution (H1–6), 10-mg/mL glucose solution (L1–3) or PBS as a control (M); c. hemolymph glucose concentration in oysters used for RNA-seq analysis following injection with glucose solution or artificial seawater (ASW) as a control.

acid metabolism (Fig.2a), as well as stress and immune responses (Supplementary Table S3). At 150 mpi, the glycolysis/gluconeogenesis pathway was significantly enriched, within which a hexokinase gene (LOC105317783, designated as c*gHK2-2* in this study) showed significant differential expression (Fig.2b).

Two other genes potentially involved in the glucose response also exhibited significant differential expression in the glucose-treated groups. One gene encodes protein phosphatase 1 regulatory subunit 3B (*cgPPP1R3B*) (Fig.2c), a glycogen-targeting protein that binds to glycogen and regulates protein phosphatase 1 (*PP1*). *PP1*, in turn, promotes glycogen synthesis by dephosphorylating glycogen synthase and inhibits glycogen breakdown by preventing the dephosphorylation of glycogen phosphorylase. The other gene encodes an enzyme orthologous to proprotein convertase 1 (*cgPCSK1*) (Fig.2d), which plays a key role in the maturation of ILPs.

3.3 Phylogenetic analysis and expression pattern of oyster hexokinase genes

As hexokinase is a key enzyme involved in glucose metabolism regulation and was identified among the DEGs, a phylogenetic analysis was conducted to further explore its potential function through ortholog identification. The results suggest that hexokinases (HKs) have followed distinct evolutionary trajectories in Chordata and Protostomia. The overall HKs phylogeny reflects the species phylogenetic tree, with a notable exception: the deuterostome sea urchin (*Strongylocentrotus purpuratus*), whose HKs occupy a basal position relative to both the Chordata and Protostomia clades.

Within Chordata, HKs form a monophyletic group that diverges into several specialized subtypes. One such subtype, *GCK*, has evolved specifically in vertebrates to function as a glucose sensor in specialized cells, regulating insulin secretion. The ascidian, which retains a notochord during its larval stage but lose it in adulthood,

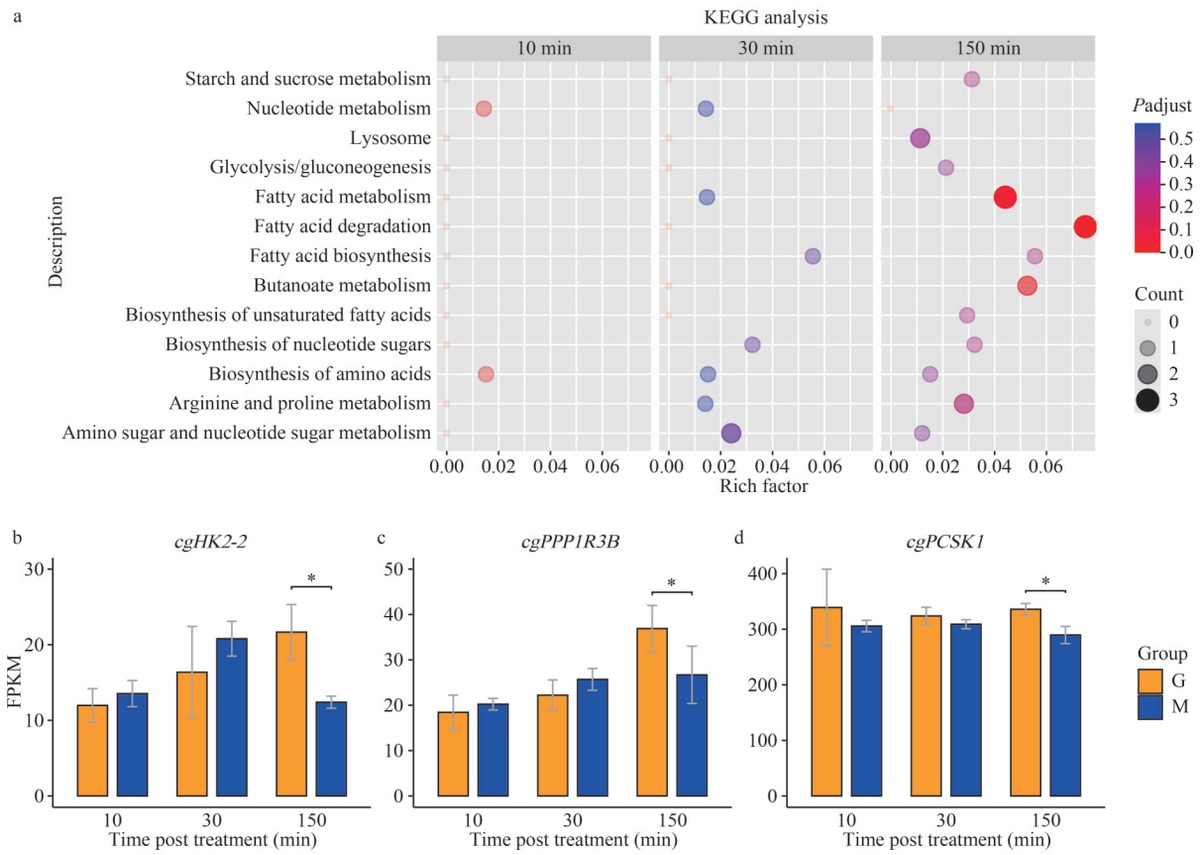

Fig.2 The KEGG pathway enrichment analysis and expression patterns of *cgHK2-2*, *cgPPP1R3B*, and *cgPCSK1*

a. KEGG pathway enrichment analysis of the DEGs between G and M comparison groups at 10, 30, and 150 mpi; b–d. expression levels of *cgHK2-2*, *cgPPP1R3B*, and *cgPCSK1*. Asterisks indicate statistically significant differences between comparison groups (P<0.05).

possess HKs that are phylogenetically closer to other chordate orthologs than those of amphioxus.

In contrast, HKs from oysters and other molluscs, as well as from arthropods and annelids, form a distinct evolutionary clade separate from that of Chordata. This Protostomia cluster further divided into two major groups (designated Group 1 and Group 2 in Fig.3), reflecting divergent molecular evolution within protostome lineages. Two oyster hexokinases (*cgHK2* and *cgHK2-like*) were grouped into Group 1, while the differentially expressed *cgHK2-2* identified in the RNA-seq analysis is clustered in Group 2.

In the annual monitoring of relative expression levels, the three oyster HKs exhibited distinct seasonal expression patterns (Fig.4a–c). Notably, all isoforms showed reduced transcriptional activity following the reproductive period (August), suggesting a shift in metabolic priorities post-spawning. During non-reproductive stages, the HK genes displayed oscillatory expression profiles, indicating their dynamic involvement in regulating carbohydrate metabolism throughout the year.

Tissue-specific expression analysis further revealed differential distribution among the three HK isoforms (Fig. 4d–f). All exhibited notably high transcript levels in the labial palps, while expression in the transparent muscle was consistently low. Among them, *cgHK2-like* showed a pronounced preference for the mantle tissue, with significantly higher expression compared to other tissues examined, highlighting potential tissue-specific functions beyond general glucose metabolism.

4 DISCUSSION

4.1 Hemolymph glucose dynamics of oyster

The transient glucose profile observed after glucose injection, peaking around 10 min, reflects rapid glucose uptake kinetics akin to vertebrates. Despite the absence of well-defined insulin-like peptide (ILP) regulation in oysters, the quick return to baseline glucose levels by 150 min suggests the existence of robust homeostatic mechanisms. These may involve hexokinase-mediated phosphorylation

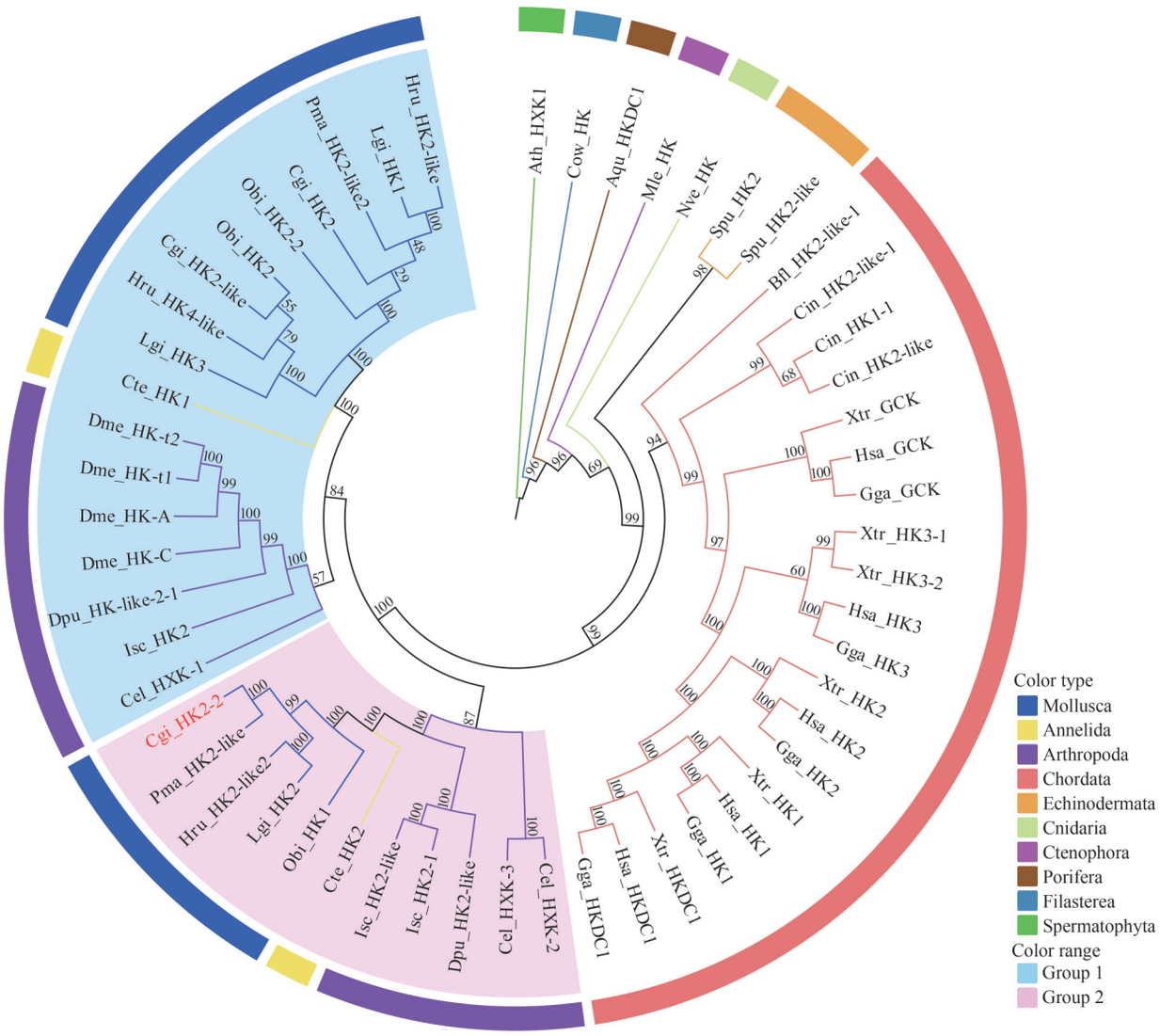

Fig.3 Phylogenetic tree of hexokinase

All names are abbreviated according to the scientific name of the species: Cow: *Capsaspora owczarzaki*; Mle: *Mnemiopsis leidyi*; Aqu: *Amphimedon queenslandica*; Nve: *Nematostella vectensis*; Spu: *Strongylocentrotus purpuratus*; Isc: *Ixodes scapularis*; Dpu: *Daphnia pulex*; Dme: *Drosophila melanogaster*; Bfl: *Branchiostoma floridae*; Cin: *Ciona intestinalis*; Xtr: *Xenopus tropicalis*; Cel: *Caenorhabditis elegans*; Cte: *Capitella teleta*; Lgi: *Lottia gigantea*; Pma: *Pecten maximus*; Cgi: *Crassostrea gigas*; Obi: *Octopus bimaculoides*; Hru: *Haliotis rufescens*; Hsa: *Homo sapiens*; Gga: *Gallus gallus*; with outgroup Ath: *Arabidopsis thaliana*. The NCBI accession numbers of all protein sequences used in the phylogenetic analysis are listed in Supplementary Table S4.

followed by glycogen storage, a hypothesis supported by the observed transcriptomic responses and prior biochemical data (Liu et al., 2019; Zhang et al., 2024).

The annual survey revealed significant seasonal fluctuations in hemolymph glucose concentrations. Post-spawning oysters (e.g., July and August) consistently exhibited the lowest glucose levels, likely reflecting energy depletion and metabolic reallocation toward reproduction. In contrast, moderate glucose concentrations were maintained from autumn through winter, supporting the hypothesis of glycogen accumulation as an adaptive strategy for colder months (Lee et al., 2018). Notably, glucose levels in wild oysters rarely exceeded 0.100 mg/mL, even under natural fluctuations, indicating a tightly regulated upper threshold for glycemia.

4.2 Role of hexokinases in oyster glucose regulation

Hexokinases (HKs) are central to glucose metabolism, acting as gatekeepers for glycolysis. Among the three oyster HK isoforms identified in this

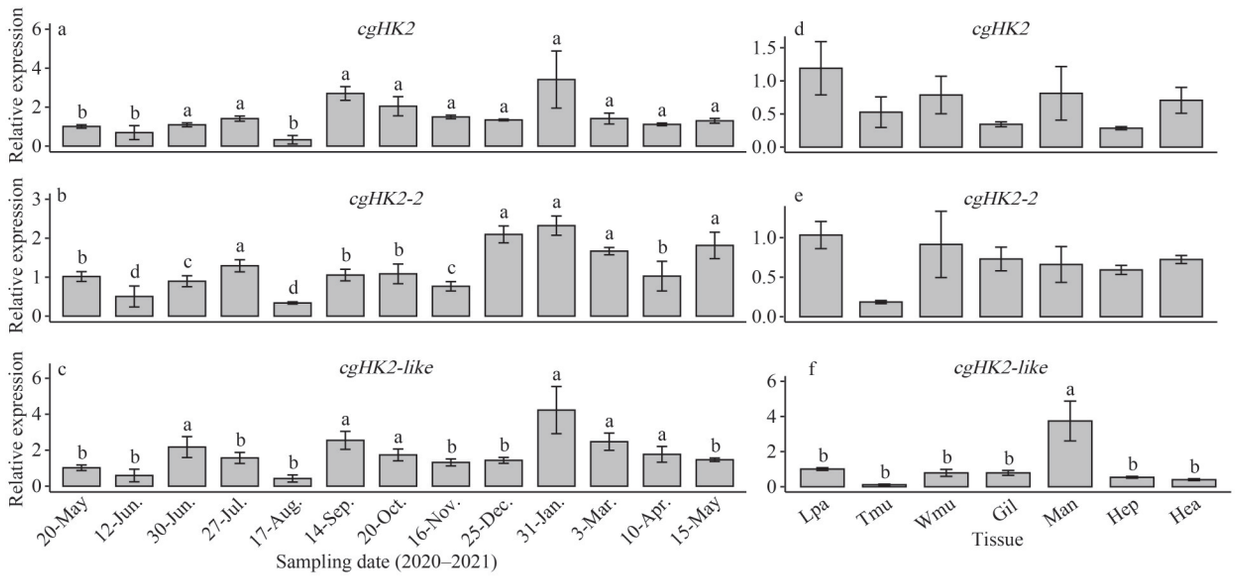

Fig.4 qPCR analysis of HK gene expression across annual sampling and various adult oyster tissues

a–c. annual expression profiles of HK genes in hepatopancreas samples of cultured oysters collected from May 2020 to May 2021; d–f. tissue-specific expression levels of HK genes in seven adult oyster tissues: Lpa: labial palp; Tmu: transparent muscle; Wmu: white muscle; Gil: gill; Man: mantle; Hep: hepatopancreas; Hea: heart. Different letters above the bars indicate statistically significant differences (P<0.05).

study, *cgHK2-2* displayed pronounced transcriptional induction following glucose injection, suggesting it plays a primary role in acute glycemic regulation. This behavior is reminiscent of vertebrate *HK2*, which is also insulin-inducible and associated with enhanced glycolytic flux during hyperglycemia (Shimobayashi et al., 2023).

Conversely, *cgHK2* and *cgHK2-like* were not differentially expressed in response to glucose but showed strong tissue-specific expression patterns. *cgHK2* was enriched in muscle tissue, hinting at an insulin-responsive role similar to vertebrate *HK2* in skeletal muscle. *cgHK2-like* showed preferential expression in the mantle, a metabolically active tissue involved in shell formation (Zhang et al., 2012) and reproductive energy management (Li et al., 2009). Their downregulation post-reproduction suggests a role in long-term energy conversion, potentially from carbohydrates to lipids, rather than acute glucose clearance. The distinct expression profiles and regulatory patterns among these isoforms suggest functional divergence, possibly shaped by tissue specialization and evolutionary constraints. These findings reinforce the need to study HK isoforms contextually, as their roles may extend beyond simple glycolysis into broader physiological and developmental functions.

4.3 Evolutionary insight from HK phylogeny

Phylogenetic analysis revealed a clear evolutionary separation of HKs between Chordata and Protostomia. Oyster HKs cluster with arthropods and annelids rather than chordates, forming two distinct subgroups that likely reflect ancient gene duplication events and subsequent divergence. Interestingly, the sea urchin (*Strongylocentrotus purpuratus*), a deuterostome, displayed a basal phylogenetic position relative to both chordate and protostome HKs, highlighting possible lineage-specific retention or loss.

Unlike vertebrates, oysters lack a clear GCK orthologue—an enzyme crucial for glucose sensing in pancreatic β-cells (Matschinsky and Wilson, 2019; Abu Aqel et al., 2024). Structural analyses suggest key substrate-binding residues diverge significantly between vertebrate GCK and HKs (Velho et al., 1997). Whether any molluscan HK can functionally mimic GCK remains an open question. Notably, *Drosophila* has evolved a testis-specific HK variant with similar residue substitutions, offering a possible invertebrate orthologue of GCK-like specialization (Duvernell and Eanes, 2000).

4.4 PPP1R3B and glycogen regulation

Glycogenesis plays a vital role in maintaining glucose homeostasis by storing excess glucose as glycogen (Petersen et al., 2017). The oyster *cgPPP1R3B*, encoding the regulatory subunit of protein phosphatase 1 involved in glycogenesis, was significantly upregulated following glucose

elevation. This mirrors mammalian systems where PPP1R3B regulates glycogen synthase activity, enhancing glycogen storage under hyperglycemic conditions. In human, a variant linked to the expression regulation of *PPP1R3B* has been associated with glycemic traits through genome-wide association studies (GWAS) (Manning et al., 2012), while liver-specific overexpression of *PPP1R3B* significantly increased hepatic glycogen storage in mice (Mehta et al., 2017). In oysters, the key role of *cgPPP1R3B* in glycogenesis has also been confirmed by both GWAS and biochemical assays (Liu et al., 2019). The consistent findings across species reveals a specific regulatory mechanism and further underscores the gene's critical function in glucose metabolism in oysters. Its induction in oysters not only confirms the role of glycogenesis in their glucose clearance but also supports the model of parallel evolution in metabolic regulation across bilaterians.

4.5 PCSK1 and implication for ILP signaling

Although no ILPs were identified as differentially expressed in this study, we detected a gene orthologous to *PCSK1*, a proprotein convertase involved in ILP maturation in other species. This gene has been shown to be regulated by cgPDX, a transcription factor implicated in insulin gene expression in oysters (Xu et al., 2021). The presence and transcriptional activity of *cgPCSK1* suggest a latent ILP signaling pathway that could be further explored in future studies, potentially uncovering a molluscan insulin regulation system.

Collectively, our findings highlight a complex, multi-layered regulatory framework for glucose homeostasis in oysters. The interplay between acute glycemic response (mediated by *cgHK2-2*), long-term energy storage (via *cgPPP1R3B*), and potential ILP-related signaling pathways (*cgPCSK1*) underscores a sophisticated metabolic control system, despite the evolutionary distance from vertebrates. The divergence of HK isoforms, coupled with their distinct expression profiles and evolutionary trajectories, suggests functional specialization shaped by the unique life history and physiology of molluscs. These insights contribute to a growing understanding of invertebrate glucose regulation and may offer comparative perspectives on metabolic evolution.

5 CONCLUSION

This study revealed critical components of glucose homeostasis in the Pacific oyster, integrating physiological measurements with transcriptomic and phylogenetic analyses. Seasonal and experimentally induced fluctuations in hemolymph glucose highlighted the dynamic regulatory responses that was orchestrated through specific gene networks. The transcriptional activation of *cgHK2-2* and *cgPPP1R3B* confirmed their central roles in glucose metabolism, while tissue-specific expression of other HK isoforms pointed to metabolic specialization. Phylogenetic and structural divergence from vertebrate HKs further emphasized the unique regulatory landscape in molluscs. These findings laid the groundwork for future research into invertebrate evolution of metabolic regulation.

6 DATA AVAILABILITY STATEMENT

The raw sequencing data have been deposited in the Sequence Read Archive (SRA) under BioProject accession number PRJNA1249974. The primary data supporting the findings of this study are included in the article and Supplementary Tables S1–S4. Additional datasets used and analyzed during the study are available from the corresponding author upon reasonable request.

7 ACKNOWLEDGMENT

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S4) is available in the online version of this article at https://doi.org/10.1007/s00343-025-5118-9.