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RESEARCH REPORT

Identification and expression analysis of Na+/K+-ATPase and NKA-interacting protein in oyster

K Zhang[1], J Xue[2], W Li[3], C Gu[2], P Wang[2], Y Jia[1*], X Liu[1]

[1]National Engineering Research Center for Marine Aquaculture, Zhejiang Ocean University, Zhoushan, Zhejiang, China
[2]CGN (Shengsi) New Energy Holdings Co., Ltd., Zhoushan, Zhejiang, China
[3]Zhejiang Marine Ecology and Environment Monitoring Center, Zhoushan, Zhejiang, China

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Abstract

Oysters hold a key ecological position in intertidal ecosystems worldwide, and their distribution across various sea areas is largely influenced by the range of salinity. Sodium-potassium ATPase (NKA), a central ion pump involved in maintaining cellular osmotic balance, plays a pivotal role in oyster adaptation to salinity fluctuations. This study identified one NKA-α subunit and two distinct NKA-β subunits by utilizing genome assemblies and transcriptomics data from multiple oyster species. Furthermore, we discovered a conserved NKA-interacting protein (NKAIN) in oysters. Through transcriptome assembly, we identified five different splice variants of NKAIN in oysters. Sequence alignment and phylogenetic analysis confirmed the high conservation of NKA-α and -β subunits in oysters, albeit with significant distinction between the two -β subunits. Multi-population comparative transcriptomic analysis illuminated the regulatory roles of NKA and NKAIN in the adaptive responses to salinity stress. The findings shed light on the compositional details and osmotic regulation mechanisms of the oyster's NKA-NKAIN system, thereby enhancing our understanding of the comprehensive osmotic adaptation mechanisms in oysters. This knowledge is instrumental for future studies aiming to improve oyster resilience to environmental salinity changes.

Key Words: oyster; sodium-potassium ATPase; NKA-interacting protein; osmotic adaptation

Introduction

Oysters represent a diverse and globally widespread group of bivalve mollusks that inhabit nearly all estuarine and intertidal environments worldwide (Zhang *et al.,* 2012; Guo *et al.,* 2018). As quintessential filter feeders, they serve pivotal ecological functions such as cleansing seawater, curbing eutrophication, and sustaining the stability within marine ecosystems. Certain oyster species are considered keystones in shaping coastal and estuarine ecological dynamics (Beck *et al.,* 2011). For bivalves, salinity change is particularly problematic because it drives a reduction in activity and energy acquisition, while also increasing the energy demand for maintaining cell volume and avoiding osmotic shock (Berger and Kharazova, 1997). Salinity tolerance significantly influences the distribution range of certain oyster populations along coastal regions (Xiao *et al.,* 2018). For instance, the estuarine oyster (*Crassostrea ariakensis*) and the Hong Kong oyster (*Crassostrea hongkongensis*), known for their broad salinity adaptability, predominantly reside in estuarine zones. In contrast, the Pacific oyster (*Crassostrea gigas*) and the Portuguese oyster (*Crassostrea angulata*) are more commonly found in intertidal and subtidal habitats (Peng *et al.,* 2021). Kumamoto oysters (*Crassostrea sikamea*) are naturally distributed along rocky shores and hard structures in the low-to-mid intertidal zone, preferring a salinity range of approximately 20-25 PSU (practical salinity units) (Wang *et al.,* 2024). Unraveling the osmoregulatory mechanisms employed by oysters is vital for deepening our understanding of their environmental acclimatization and resilience.

Similar to numerous marine invertebrates, oysters possess a characteristic open circulatory system where blood and interstitial fluid are not distinct entities, thereby allowing their blood osmotic pressure to directly mirror fluctuations in environmental salinity (Jia and Liu, 2018). Ions

Corresponding author:
Yanglei Jia
National Engineering Research Center for Marine Aquaculture
Zhejiang Ocean University
Zhoushan, Zhejiang, China
E-mail: jiayanglei@zjou.edu.cn

Table 1 Selected genome assemblies for different oysters in this study

Species	Common names	Accession No.	Genome Size (Mb)	Scaffold N50 (Mb)
Ostrea denselamellosa	Flat oyster	GCA_024699665.1	635.89	13.95
Ostrea edulis	European flat oyster	GCA_032173915.1	946.04	94.82
Saccostrea echinata	Blacklip rock oyster	GCF_033153115.1	923.09	19.68
Saccostrea glomerata	Sydney rock oyster	GCA_003671525.1	788.10	0.80
Crassostrea angulata	Portuguese oyster	GCF_025612915.1	624.27	60.48
Crassostrea ariakensis	Estuarine oyster	GCA_020458035.1	663.15	66.34
Crassostrea gigas	Pacific oyster	GCF_902806645.1	647.89	58.46
Crassostrea hongkongensis	Hong Kong oyster	GCA_016163765.1	757.93	72.33
Crassostrea nippona	Iwagaki oyster	GCA_033439105.1	528.99	50.90
Crassostrea virginica	Eastern oyster	GCF_002022765.2	684.70	75.90

constitute the primary contributors to osmotic pressure both in the hemolymph and in seawater. Transcellular ion transport constitutes the principal means by which these organisms rapidly respond to osmotic challenges. This ion movement across cellular membranes is an active transport process, often facilitated by various types of transporter proteins. The Sodium potassium ATPase (Na+-K+-ATPase, or NKA) is a key class of ion transporter protein that expends one ATP molecule to extrude three sodium ions (Na+) from the cell while importing two potassium ions (K+), thereby establishing an ion concentration gradient (Skou, 1998). Notably, over half of the energy produced in animal cells is utilized by this NKA pump, underscoring its significant role in maintaining osmotic pressure equilibrium (Clausen *et al.,* 2017). Thus, the operation of NKA is fundamental to how oysters and other marine organisms adapt and regulate their internal environment in response to variations in external salinity.

The NKA complex comprises α and β subunits, with the α subunit serving as the central catalytic component, featuring dedicated binding sites for Na^+ and K^+ ions, as well as hosting the ATP hydrolysis catalytic domain. The β subunit, on the other hand, primarily functions as a regulatory element that modulates the conformational dynamics of the α subunit (Therien and Blostein, 2000). Across vertebrates, distinct types of α and β subunits exist in their genomes, and notable differences in their expression patterns arise among various tissues, leading to a multitude of unique α-β subunit combinations (Malik *et al.,* 1996; Guynn *et al.,* 2002). These specific combinations contribute to tissue-specific functionalities. Moreover, the functionality of the NKA-β subunit is intricately governed by interactions with NKA-interacting proteins (NKAIN). Researches have demonstrated that the transmembrane domains of NKAIN molecules can potentially form channel-like structures or engage in interactions with additional membrane proteins, thereby executing essential biological roles (Gorokhova *et al.,* 2007). These findings suggest that NKAIN proteins not only fine-tune the performance of the NKA pump but also participate in broader cellular signaling and functional regulation networks at the level of the cell membrane (Gorokhova *et al.,* 2007).

Until now, research focusing on NKA in mollusks has been relatively sparse. A study conducted on Pacific abalone has shed light on the phenomenon that under low-salinity stress conditions, both the expression levels and enzymatic activities of the NKA subunits experience a substantial decline. Moreover, it was discovered that their expression regulation is orchestrated by the cAMP signaling pathway (Jia and Liu, 2018). Nevertheless, the subunit composition profile of NKA in oysters and its specific role in osmotic pressure regulation remains uncharted territory. In the current investigation, we have endeavored to fill this knowledge gap by identifying the fundamental composition of NKA in oysters using genomic

Table 2 Selected transcriptomic data for different species in this study

Species	Tissue	Run No.	Bytes (Gb)	Application
C. gigas	Mantle-edge	SRR334212	1.74	Expression analysis
C. gigas	Digestive gland	SRR334213	1.92	Expression analysis
C. gigas	Female gonad	SRR334214	1.77	Expression analysis
C. gigas	Gill	SRR334215	2.05	Expression analysis
C. gigas	Mantle-inter	SRR334216	2.22	Expression analysis
C. gigas	Muscle	SRR334217	2.03	Expression analysis
C. gigas	Hemolymph	SRR334218	1.75	Expression analysis
C. gigas	Labial palp	SRR334219	2.73	Expression analysis
C. gigas	Male gonad	SRR334220	2.76	Expression analysis
C. hongkongensis	Gill	SRR7777763	2.02	Expression analysis
C. hongkongensis	Gill	SRR7777764	2.05	Expression analysis
C. hongkongensis	Gill	SRR7777765	2.03	Expression analysis
C. hongkongensis	Gill	SRR7777766	2.09	Expression analysis
C. hongkongensis	Gill	SRR7777767	2.20	Expression analysis
C. hongkongensis	Gill	SRR7777768	2.00	Expression analysis
C. sikamea	Mix	SRR6201763	3.53	Trinity assemble
C. ariakensis	Mix	SRR6201764	2.19	Trinity assemble
C. hongkongensis	Mix	SRR6201765	2.73	Trinity assemble
C. angulata	Mix	SRR6201766	2.93	Trinity assemble

assembly data. We have also probed into the existence of variants and explored the expression patterns through comprehensive transcriptomic analysis. These novel findings lay down a crucial groundwork for advancing our understanding of the inherent distribution patterns and ecological adaptation mechanisms of oysters in response to osmotic stressors.

Materials and methods

Materials

In the present study, we capitalized on available genome assemblies for a total of ten oysters representing three distinct genera, upon which pertinent analyses were carried out (Table 1). To complement this genomic data, transcriptomic sequences from four different naturally distributed oyster species were gathered and subjected to de novo assembly, aiming to identify potential alternative splicing events of the genes associated with NKA (Table 2). Furthermore, we augmented our dataset by collecting transcriptomic data from different tissue samples of the Pacific oyster and from natural gradient salinity zones in the habitat of the Hongkong oyster (Table 2). These data were then used to investigate the expression levels of the NKA subunits and NKAIN in oysters.

Methods

Identification of NKA subunits in oysters

In this study, the published abalone NKA-α and -β subunit sequences (MG767304 and MG767305) were utilized as query sequences for conducting a de novo genome-wide identification for the corresponding NKA subunits in different oysters using the local BLAST (v2.15.0) software with default parameters (Camacho *et al.,* 2009). The mouse NKAIN protein (ABN51166.1) was regarded as query to identify the corresponding orthologs in oysters. Simultaneously, the selected transcriptomic datasets were subjected to de novo assembly using

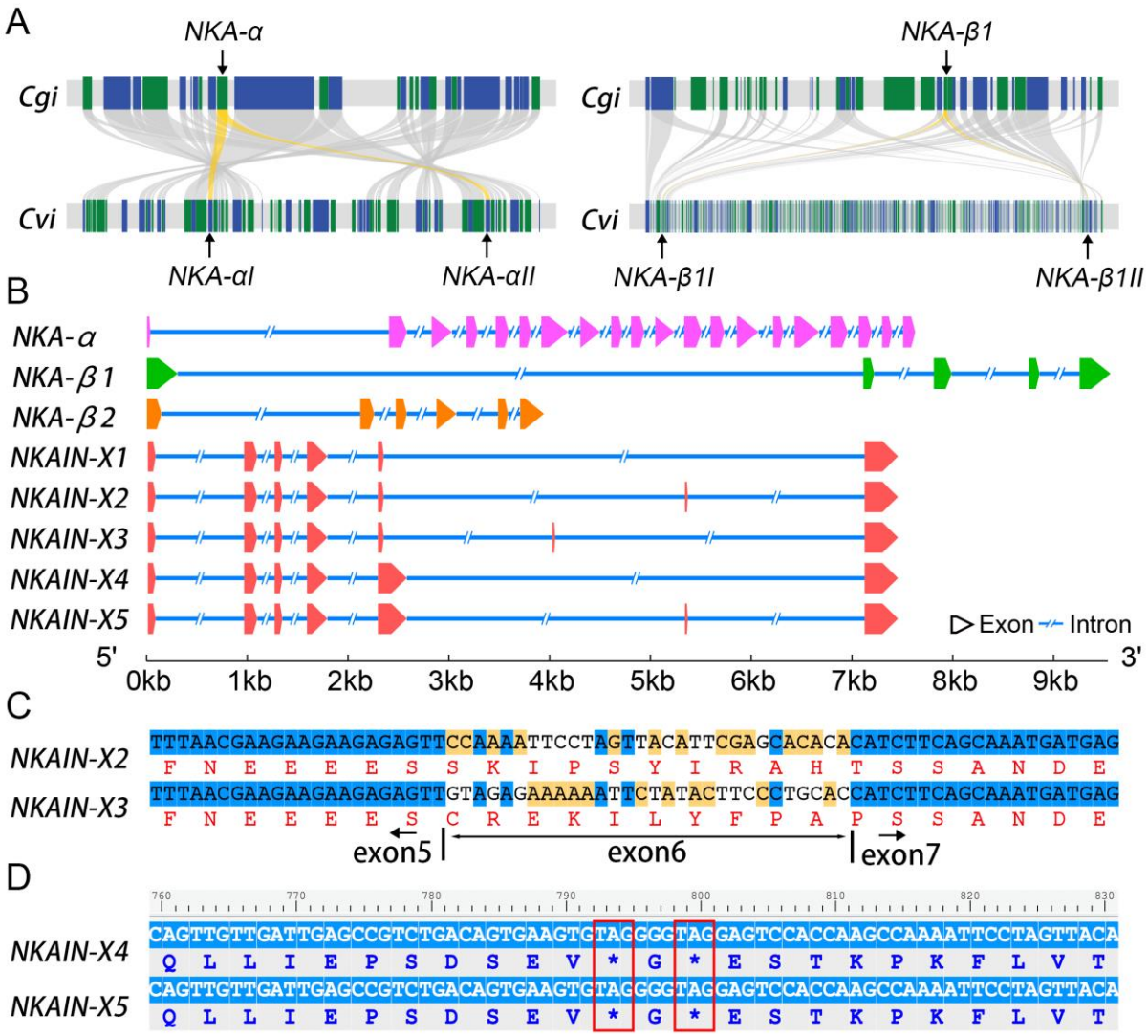

Fig. 1 Gene structure and variable splinters of NKA subunits and NKAIN in oysters. (A) Duplication of the NKA subunits in *C. virginica*. (B) Gene structure of the NKA subunits and NKAIN variants in oysters. (C) Comparison of the Sixth exon sequence between NKAIN-X2 and -X3 in oysters. (D) In-frame stop codons in the NKAIN-X4 and -X5 in oysters

the Trinity (v2.12) software (Grabherr *et al.,* 2011). This enabled the reconstruction of full-length transcripts from the short-read sequencing data. Upon successful assembly, the de novo assembled transcripts were screened to retrieve the possible alternative splicing coding sequences of each NKA subunit and NKAIN in oysters. This step aimed to identify any differential isoforms that could be generated through the alternative splicing process, which may play a role in the functional diversity and environmental adaptation of the NKA enzyme in oysters.

Phylogenetic analysis of the NKA subunits in oysters

The NKA-α and -β subunits, along with the NKAIN open reading frames (ORFs), were initially aligned using the MATTF (v7.520) software with the default parameters (Katoh and Toh 2008). The resulting alignments for each orthologs were visually represented using the DNAMAN X (v10.3.516) software. Subsequently, the identity matrices for these orthologs were computed based on the alignment results and graphically displayed in the form of heatmaps via the heatmap plugin integrated within the TBtools toolkit (v2.080) (Chen *et al.,* 2023). Additionally, to evaluate the evolutionary selection pressures acting on these orthologs, the Ka/Ks ratio calculation was performed using the KaKs Calculator plugin available in TBtools. This analysis provides insights into their functional conservation and adaptive evolution in oysters.

Gene structure and alternative splicing analysis

Following the identification of the open reading frame (ORF) sequences of each NKA related orthologs in oysters, the splice junctions were

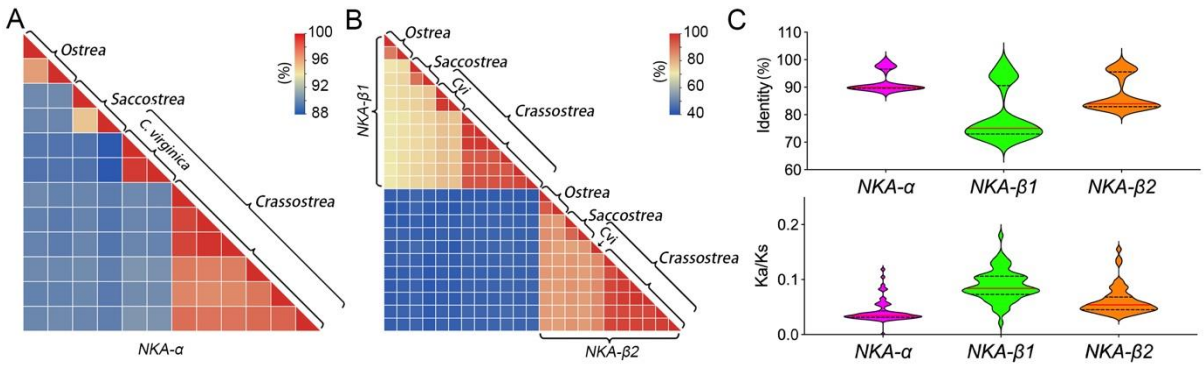

Fig. 2 Phylogenetic analysis of the NKA-α and -β subunits. (A) Similarities between the NKA-α subunit in oysters. (B) Similarities between the NKA-β subunits in oysters. (C) Conservation and selection presure of the NKA subunits in oysters

meticulously determined using the Magic-BLAST (v1.7.2) alignment tool (Boratyn *et al.,* 2019). With the obtained splice junction information, the exon-intron structures of each ortholog were systematically visualized using the Gene Structure Display Server (GSDS) 2.0 web-based platform (Hu *et al.,* 2014).

Synteny analysis of the NKA subunits in oysters

Initially, the genomic distribution of the recognized orthologs was mapped and visually represented based on the available annotation information. To discern the collinearity relationships among these orthologs across different oyster species, the conserved syntenic blocks were detected using the JCVI comparative genomics pipeline (v1.1.15) (Wang *et al.,* 2012). Furthermore, to delve deeper into the micro-synteny relationships of individual orthologs, multiple collinear regions were analyzed and illustrated using the "Find Gene Block Evolutionary Path by Gene Pairs" plugin within the TBtools software.

Protein structure analysis

Building upon the amino acid sequences of the identified NKA subunits, structural predictions were made using the trRosetta online server, which harnesses the power of deep learning algorithms to predict tertiary structures with high accuracy (Du *et al.,* 2021). The resultant models were then rendered and analyzed using PyMol software (v3.0.0), enabling a detailed visualization of the three-dimensional architecture of these subunits (Delano, 2002).

Expression analysis

The transcriptomic sequencing reads were aligned to their respective genome databases using the Hisat2 aligner software (v2.2.1) (Kim *et al.,* 2019). Following the alignment process, the number of reads that successfully matched to each gene region was quantified. Subsequently, the matched reads were processed to calculate the FPKM values, which is a widely adopted method for normalizing gene expression data across different samples. This normalization procedure was executed using the StringTie2 software (v2.2.0), thus providing a standardized measure of gene expression levels that takes into account both the length of the gene and the total number of reads in each sample (Kovaka *et al.,* 2019). Statistical significance of differentially expressed genes (DEGs) was analyzed using the DEseq methods based on a negative binomial distribution. The threshold settings were as follows: a fold change > 2.00 and an adjusted p-value < 0.05 (Love *et al.,* 2014).

Results

Identification of the NKA subunits and NKAIN

Based on the chosen genome assemblies, we embarked on a genome-wide search for the NKA subunits in oysters. The study uncovered the presence of one NKA-α subunit and two distinct NKA-β subunits, namely NKA-β1 and NKA-β2. Remarkably, the NKA-α and -β1 subunits exhibited duplication specifically in the eastern oyster genome (Fig. 1A). Comparative genomic investigations disclosed that this expansion resulted from an intrachromosomal large-scale segmental duplication event. In addition to the NKA subunits, we also identified one ortholog of NKAIN in oysters.

Proceeding with the analysis, we used the de novo assembled transcriptomes to determine the potential alternative splicing events in the NKA subunits and NKAIN. Our findings showed that there is only one splice form for both the NKA-α and -β subunits in oysters (Fig. 1B). Gene structure analysis highlighted significant differentiation between the two NKA-β subunits in the oyster genome. Moving to NKAIN, we detected a total of five splice variants derived from the transcriptome assemblies (Fig. 1B). The first four exons at the 5' end and the final exon at the 3' end appeared to be highly conserved across all five NKAIN variants. Of particular interest, NKAIN-X1, -X2, and -X3 primarily differed in their sixth exon (Fig. 1C). Notably, the alternative splicing in the fifth exon in NKAIN-X4 and -X5 extended the coding sequence compared to the other three variants. However, the presence of

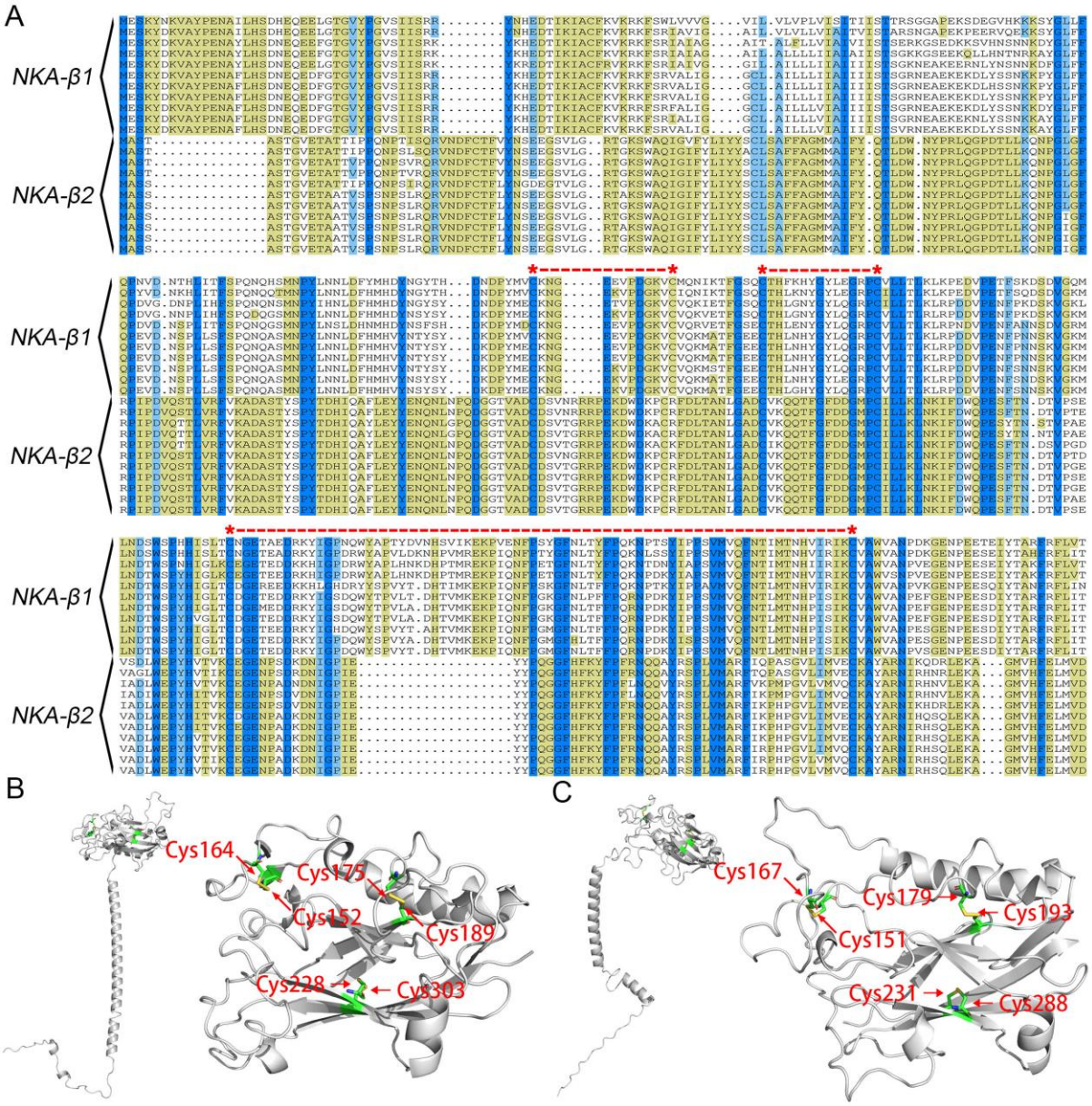

Fig. 3 Comparisons between the two NKA-β subunits in Ostreoidea. (A) Multiple alignments of the two NKA-β subunits in Ostreoidea. (B) Protein structure of NKA-β1 in Pacific oyster (ma-7npaf). (C) Protein structure of NKA-β2 in Pacific oyster (ma-kuu5v)

in-frame stop codons within the fifth exon in these two variants led to truncated protein sequences, suggesting a potential impact on the functionality and regulation of the NKAIN protein in oysters (Fig. 1D).

Phylogenetic analysis of the NKA subunits and NKAIN

To further understand the evolutionary relationship and conservation of the NKA subunits among oyster species, multiple sequence alignments were conducted using the identified protein sequences. The results showed that the NKA subunits were highly conserved across oyster species, with the NKA-α subunit exhibiting approximately 90% conservation rate (Fig. 2). Typically, the orthologs within the same genus were found to be more closely conserved compared to those across different genera. However, a noteworthy exception was observed in the eastern oyster, where the NKA-α and both NKA-β subunits (NKA-β1 and -β2) displayed notably lower conservation levels when compared to other *Crassostrea* species (Fig. 2A and 2B). These data suggested that the eastern oyster was independently ancient speciated from the Crassostrea.

It should be noted that the two NKA-β subunits showed considerable divergence when compared with each other, which was consistent with the gene

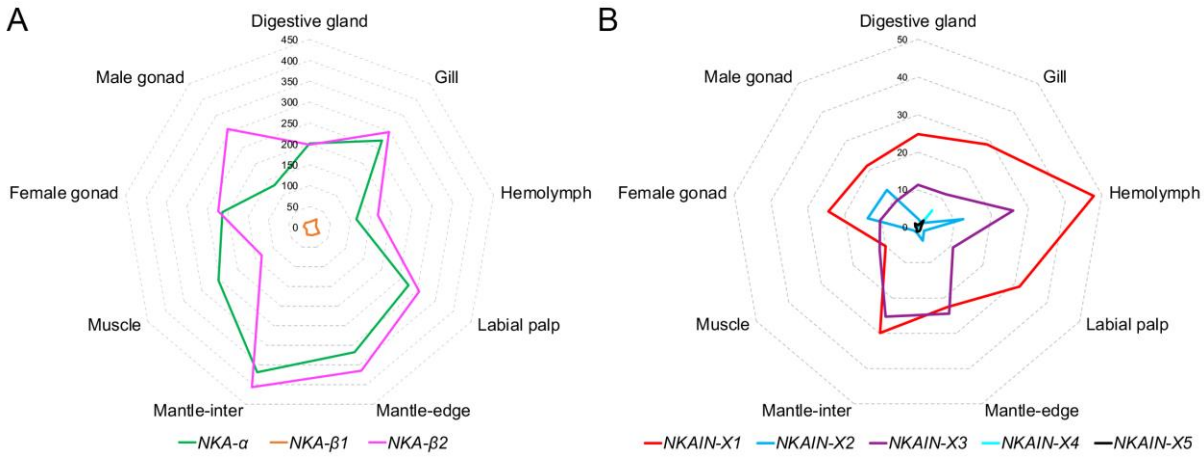

Fig. 4 Expression level of the NKA subunits and NKAIN in different tissues. (A) Expression level of the NKA subunits in different tissues. (B) Expression level of the NKAIN variants in different tissues

structure analysis (Fig. 2B). These findings point towards independent evolutionary origins and diversification of these two β subunit orthologs within oysters. Further analysis employing the Ka/Ks ratio indicated that the NKA subunits in oysters have undergone strong purifying selection, suggesting that these genes have evolved primarily under constraints to maintain their original function (Fig. 2C).

Structure analysis of the NKA-β subunits

Indeed, despite the differences observed in the gene structure and sequence similarity between the two NKA-β subunits in oysters, their protein structures exhibit significant conservation (Fig. 3). Both orthologs feature a single transmembrane domain, followed by an extracellular globular domain. This shared structural characteristic implies a common functional role in their interaction with the NKA complex. Of particular interest is the finding that three cysteine bridges within the extracellular globular domain are consistently present in both NKA-β subunits (Fig. 3B and 3C). The conservation of these cysteine residues across the two subunits underscores their likely importance in stabilizing the protein structure and possibly mediating interactions with other molecules. Cysteine bridges, also known as disulfide bonds, are critical for maintaining protein conformation and function (Fig. 3B and 3C). Therefore, the presence of these conserved cysteines in both NKA-β subunits strongly suggests their functional significance in the overall functioning of the sodium-potassium ATPase complex in oysters.

Expression of the NKA subunits and NKAIN in oysters

Using the Pacific oyster as a model organism, we examined the expression patterns of the NKA subunits and NKAIN variants across different tissues based on transcriptomic data. The results revealed that both the NKA-α subunit and the two NKA-β subunits show conservative expression across various oyster tissues (Fig. 4A). However, it was noticeable that the expression level of NKA-β1 is significantly lower than that of the other subunits. This suggests that while NKA-β2 might be the primary structural subunit contributing to the NKA complex in oysters, NKA-β1 could potentially play a more regulatory role. Turning to the NKAIN variants, we found that NKAIN-X1 and NKAIN-X3 were the predominant isoforms expressed in oyster tissues (Fig. 4B). Additionally, NKAIN-X2 displayed a significantly high expression level in the gonads and hemolymph, indicating its possible specialized function in these tissues.

To investigate the responsiveness of NKA subunits and NKAIN variants to salinity adaptation, we chose the Hong Kong oysters inhabiting Zhenhai Bay in south China, an area characterized by naturally occurring salinity gradients along the coastline (Fig. 5A). Upon analyzing the expression patterns, it was observed that the NKA-α and -β2 subunit consistently maintained a high expression level across various populations, regardless of salinity conditions (Fig. 5B). On the other hand, the expression level of the NKA-β1 subunit, which was hypothesized to act as a regulatory subunit, exhibited a significant increase in populations dwelling in high salinity areas (Fig. 5B). Parallel to the NKA subunits, the expression levels of NKAIN variants were also assessed. Results showed that NKAIN-X1 was the most abundantly expressed isoform, and its expression level notably increased in response to hyper-salinity adaptation (Fig. 5C).

Discussion

NKA was a vital ion channel that facilitates the active transport of Na+ and K+ ions across cell membranes, playing a pivotal role in maintaining intracellular osmotic pressure balance (Fambrough *et al.,* 1994). NKA operates as a heterodimeric P-type 2C ATPase composed of a catalytic α subunit and an auxiliary β subunit, which is indispensable for proper plasma membrane localization (Rajasekaran

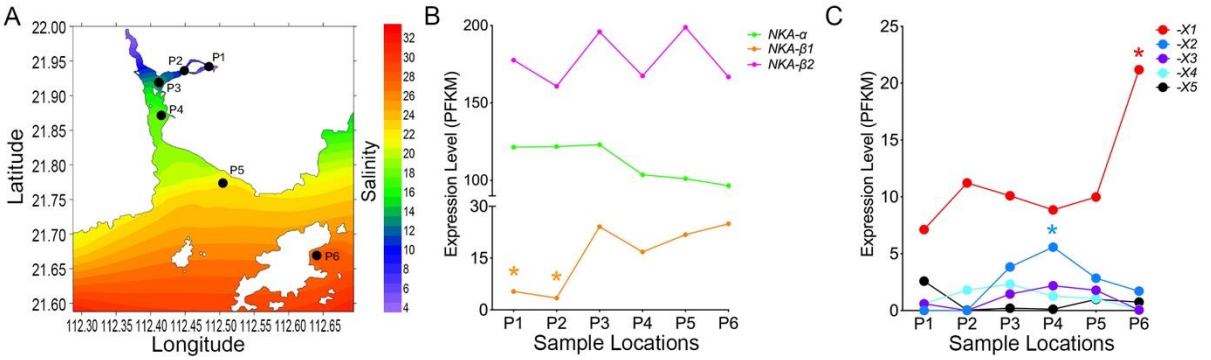

Fig. 5 Expression of the NKA subunits and NKAIN in response to different salinities. (A) Salinity of the related oyster polulations along the coast of the bay. (B) Expression level of the NKA subunits in different oyster populations. (C) Expression level of the NKAIN variants in different oyster populations. (*P<0.05)

et al., 2004). In vertebrates, multiple NKA isoforms exist that assemble into distinct isozymes, each displaying subtly different kinetic properties to meet the diverse ion-transport requirements of various cell types (Guynn *et al.,* 2002). Previous studies revealed that most invertebrates possess a single copy of the NKA-α subunits, while the number of introns shows high variability (Thabet *et al.,* 2016). However, the current study reveals that oysters possess only a single NKA-α subunit. In this regard, the repertoire of NKA isozymes in oysters appears significantly restricted compared to species belonging to other taxonomic orders. This suggests that oysters may have evolved a unique mechanism to regulate their ion homeostasis, perhaps relying on other factors such as post-translational modifications or interacting partners like NKAIN to achieve the necessary functional specificity and flexibility.

While the NKA-β subunit lacks direct ion-binding sites and does not exhibit catalytic activity, it plays a crucial regulatory role in controlling the overall enzymatic function of the NKA complex (Hasler *et al.,* 1998). In the present investigation, we have identified two distinct types of NKA-β subunits in oysters. Surprisingly, these two orthologous subunits exhibited remarkable differences in their gene structure and sequence similarities, hinting at significant functional divergence between them. It is noteworthy that both NKA-β subunit are consistently present across various oyster species. This evidence supports the hypothesis that the duplication and subsequent expansion of the two NKA-β subunits predates the speciation events that led to the modern diversity of oysters. The conservation of these two subunits throughout oyster evolution points to their fundamental importance in the regulation and adaptation of the NKA complex in these marine bivalves.

Salinity changes significantly affect the activity of the NKA, primarily by influencing the osmotic pressure balance within organisms (Jia and Liu, 2018). Despite extensive research on the role of NKA in osmotic regulation, much of this work has centered around teleost fish and crustaceans. Given that the NKA-α subunit possesses the catalytic function, it has been a prime focus in numerous studies across different taxa. For instance, previous research has shown that in shrimp, a decrease in salinity leads to a rapid increase in the transcript level of the NKA-α subunit gene, reflecting a compensatory response to maintain osmotic homeostasis (Pan *et al.,* 2006). Conversely, in teleost fish, higher salinity typically stimulates an increase in NKA activity in the gills. Transcript level analyses reveal a more complex response: while high salinity decreases the expression of the NKA-α1a isoform, it increases the expression of the NKA-α1b isoform; interestingly, the remaining three NKA-α isoforms remain unchanged under salinity stress (Richards *et al.,* 2003). When comparing these findings with oysters, it becomes apparent that the NKA-α subunit in oysters exhibits a less pronounced sensitivity to both hypo-salinity and hyper-salinity conditions. This suggests that oysters may employ distinct regulatory mechanisms to cope with salinity fluctuations, potentially involving other subunits or accessory proteins such as the NKA-β subunits and NKAIN variants that were discussed earlier.

Indeed, research on the β subunit of NKA has been relatively sparse compared to the α subunit due to its non-catalytic nature. A prior study on Pacific abalone demonstrated that the expression level of the NKA-β subunit is positively associated with changes in salinity, suggesting its involvement in osmoregulatory processes (Jia and Liu, 2018). In contrast, our current study highlights the differing adaptability of the duplicated NKA-β subunits in oysters to hypo-salinity and hyper-salinity conditions. As previously mentioned, we categorized the two NKA-β subunits as a structural subunit and a regulatory subunit based on their expression patterns. Notably, the regulatory subunit displayed a marked sensitivity to hyper-salinity adaptation compared to its structural counterpart. This differential response to salinity challenges between the two NKA-β subunits in oysters opens up new avenues for investigating the nuanced roles they play in response to osmotic shock and how these distinct subunits may contribute to the overall stability and efficiency of the NKA complex under varying environmental conditions. The finding that

the regulatory subunit responds more acutely to hyper-salinity could indicate its critical role in modulating the activity of the NKA complex in response to high salinity stress.

NKAIN, a recently discovered protein, has been found to interact with the NKA-β subunit, adding another layer of complexity to the regulation and function of the NKA complex (Gorokhova *et al.,* 2007). Strikingly, the seven-exon splice forms of NKAIN variants in oysters display remarkable conservation in their exon-intron boundaries when compared to vertebrates, indicating a high degree of evolutionary conservation across phyla. The strong evolutionary conservation of the transmembrane domains in NKAIN and its ability to induce Na+-specific conductance suggests that NKAIN might serve as a component of channel structures or influence the function of other membrane proteins. In vertebrates, NKAIN variants predominantly express in neuronal tissues and exhibit distinct yet overlapping expression patterns, pointing to their potential roles in neural functions (Gorokhova *et al.,* 2007). In contrast, the variants in oysters appear to localize mainly in the hemolymph, suggesting a probable response to changes in medium salinity or some other secondary adjustment mechanism in response to osmotic stress. Importantly, the expression of NKAIN-X1 is significantly enhanced under hyper-salinity conditions, which parallels the expression pattern of NKA-β1, the regulatory subunit in oysters that shows sensitivity to high salinity. These findings imply a close functional relationship between NKAIN-X1 and NKA-β1 in the osmotic adaptation of oysters to changing salinity levels, underscoring the intricate regulatory network that governs ion transport in these marine bivalves.

An intriguing finding was the slight expression of the NKAIN-X4 and NKAIN-X5 variants, which harbor in-frame stop codons, in several tissues. Although the expression levels were minimal, this observation raises questions about the potential regulatory or functional roles these truncated variants might play in the context of oyster physiology in response to osmotic shock. Further studies are needed to elucidate the exact biological implications of these findings.

In conclusion, the present study has identified one NKA-α subunit and two NKA-β subunits in oysters. Furthermore, a conserved NKAIN protein with five distinct splice variants that interact with the NKA-β subunit has also been discovered in the oyster genome. Based on the expression levels and their response patterns to salinity challenges, the two duplicated NKA-β subunits were classified as having distinct roles, with one acting as a structural subunit and the other as a regulatory subunit. The NKAIN variants were found to exhibit differential expression patterns across various tissues. Notably, both the regulatory NKA-β subunit and the NKAIN variants demonstrated a significant sensitivity to osmotic perturbations, indicating their essential roles in response to osmotic shock in oysters. Overall, these findings provide important insights into the molecular complexity and adaptability of the NKA system in oysters and highlight the dynamic interplay between NKA subunits and NKAIN in coping with environmental osmotic stress.

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