Report

Genomic basis of evolutionary adaptation in a warm-blooded fish

Xin Wang,[1,2,10] Meng Qu,[1,2,10] Yali Liu,[1,2,10] Ralf F. Schneider,[3] Yue Song,[4] Zelin Chen,[1,2] Hao Zhang,[1,2,5] Yanhong Zhang,[1,2] Haiyan Yu,[1] Suyu Zhang,[4] Dongxu Li,[5] Geng Qin,[1,2] Shaobo Ma,[1,2] Jia Zhong,[1,2] Jianping Yin,[1,2] Shuaishuai Liu,[1,2] Guangyi Fan,[4,6] Axel Meyer,[7,*] Dazhi Wang,[5,*] and Qiang Lin[1,2,8,9,*]

*Correspondence: axel.meyer@uni-konstanz.de (A.M.); dzwang@xmu.edu.cn (D.W.); linqiang@scsio.ac.cn (Q.L.)

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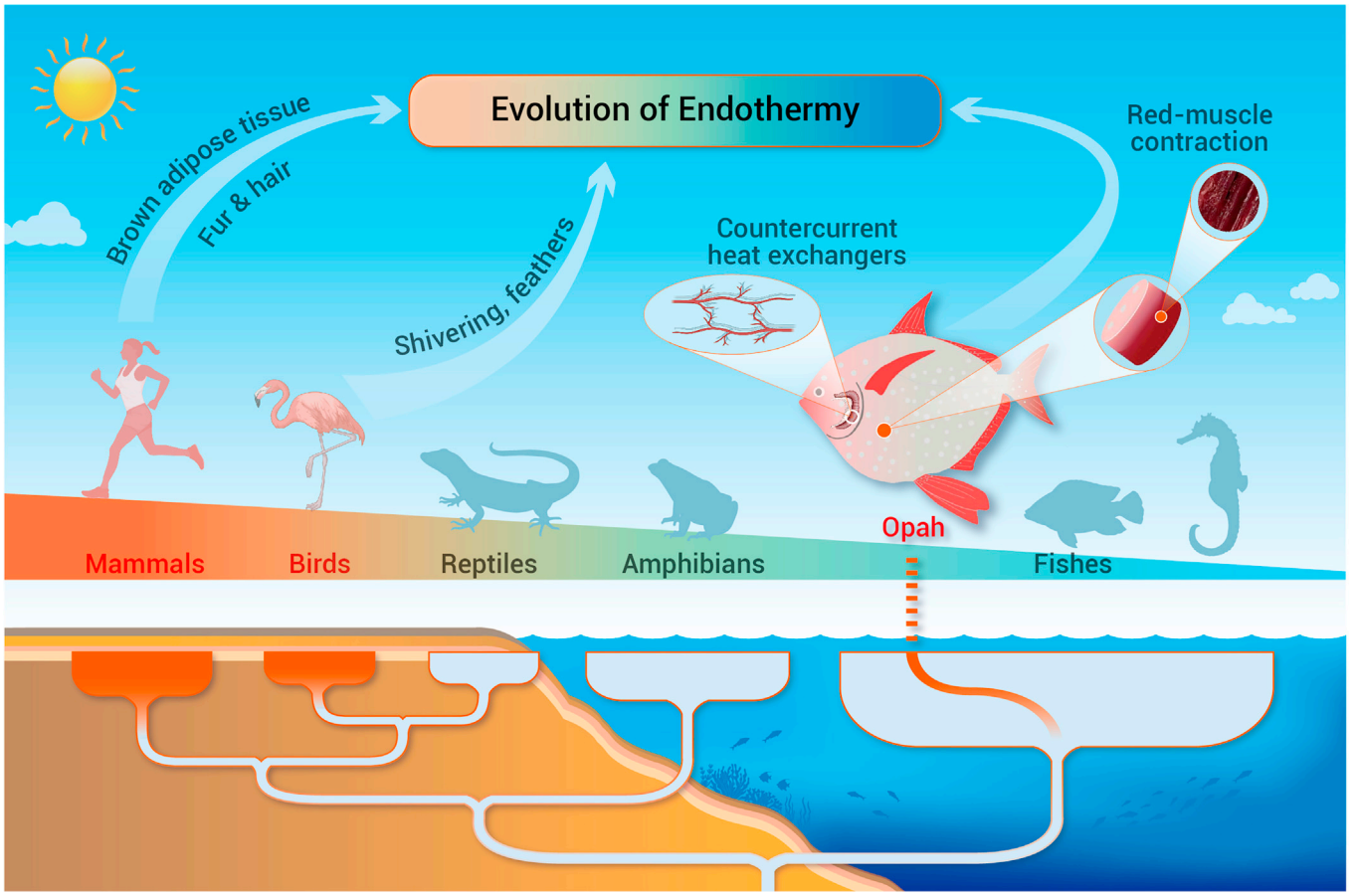
Graphical abstract



Public summary

- Endothermy has evolved multiple times in fishes (teleosts and chondrichthyans)
- Opah genome explaining genetic changes in heat production and the sensory and immune system
- Convergent evolution of genes in endothermic vertebrate lineages was investigated
- Analyses of the pectoral muscle of opah revealed numerous highly expressed genes for thermogenesis

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[1]CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China

[2]Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), Guangzhou 511458, China

[3]Marine Evolutionary Ecology, GEOMAR Helmholtz Centre for Ocean Research Kiel, 24105 Kiel, Germany

[4]BGI-Qingdao, Qingdao 266555, China

[5]State Key Laboratory of Marine Environmental Science/College of the Environment and Ecology, Xiamen University, Xiamen 361102, China

[6]BGI-Shenzhen, Shenzhen 518083, China

[7]Department of Biology, University of Konstanz, Konstanz 78464, Germany

[8]University of the Chinese Academy of Sciences, Beijing 100101, China

[9]Laboratory for Marine Fisheries Science and Food Production Processes, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao 266237, China

[10]These authors contributed equally

*Correspondence: axel.meyer@uni-konstanz.de (A.M.); dzwang@xmu.edu.cn (D.W.); linqiang@scsio.ac.cn (Q.L.)

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Few fishes have evolved elevated body temperatures compared with ambient temperatures, and only in opah (*Lampris* spp) is the entire body affected. To understand the molecular basis of endothermy, we analyzed the opah genome and identified 23 genes with convergent amino acid substitutions across fish, birds, and mammals, including *slc8b1*, which encodes the mitochondrial Na^+/Ca^{2+} exchanger and is essential for heart function and metabolic heat production. Among endothermic fishes, 44 convergent genes with suggestive metabolic functions were identified, such as *glrx3*, encoding a crucial protein for hemoglobin maturation. Numerous genes involved in the production and retention of metabolic heat were also found to be under positive selection. Analyses of opah's unique inner-heat-producing pectoral muscle layer (PMI), an evolutionary key innovation, revealed that many proteins were co-opted from dorsal swimming muscles for thermogenesis and oxidative phosphorylation. Thus, the opah genome provides valuable resources and opportunities to uncover the genetic basis of thermal adaptations in fish.

INTRODUCTION

Endothermy, the ability of an organism to metabolically produce heat to achieve a high, stable body temperature, is one of the most fascinating traits that has evolved repeatedly.[1,2] This mechanism provides some independence from environmental thermal fluctuations by permitting high activity levels even with low or variable temperatures.[3] Mammals and birds were long considered the only lineages of endothermic vertebrates, and whole-body endothermy is thought to have evolved independently in these two lineages.[1] Except for muscular thermogenesis, in placental mammals, metabolic heat is mainly produced by nonshivering thermogenesis in brown adipose tissue. Heat production in birds likely occurs through muscular thermogenesis, the production of heat through the uncoupling of calcium ions in the sarcoplasmic reticulum of muscle cells, activated by sarcolipin.[1] However, endothermy has also been discovered in other vertebrate clades, with different types of endothermy having evolved independently at least five times in fishes (teleosts and chondrichthyans).[4] The thermogenic strategies of teleosts and chondrichthyans are mainly attributed to contractions of the slow-twitch aerobic red muscles during constant swimming, a configuration often called "red muscle endothermy".[1,4] For example, tuna (Scombridae) and mackerel sharks (Lamnidae) can elevate their core body temperature, whereas the brain and eye regions can become warmer than ambient temperatures in billfishes (Istiophoridae). In contrast to such regional endothermy, opahs are the only known teleosts that can warm their entire body by circulating locally warmed-up blood and are thus the only whole-body endotherm species aside from mammals and birds.[4–6]

Opah is a large mesopelagic circumglobal species with the unique ability to produce large quantities of metabolic heat in its dark red, aerobic pectoral musculature, putatively an evolutionary key innovation that induces forward thrust during continuous swimming by powering pectoral fin oscillation, and this is insulated from the cold surrounding waters by a thick layer of fatty connective tissue.[7,8] Furthermore, to reduce the cooling of warm blood in the gills, where most heat is lost, opah has evolved a crucial second key change, a counter-current retia mirabilia system in its gills.[7] The convoluted alternating arteries with cold-oxygenated and warm-deoxygenated blood form the basis of the heat exchange system. With whole-body endothermy, the opah has the capacity for enhanced physiological functions in their cold, deep, and nutrient-rich habitats, below the ocean thermocline. Its adaptation to whole-body endothermy facilitates enhanced physiological functions, such as increased muscle power, increased capacity for more sustained performance, enhanced temporal resolution and neural conductance for the eye and brain, and an increased rate of food digestion and assimilation.[7] Moreover, as endothermy is thought to represent an optimum level in the trade-off between metabolism and fitness, the case of the opah can inform us of how endothermy affects organism evolution and survivorship.[9,10]

The ability of the opah to maintain body regions at a warmer temperature than the ambient water challenges the general concept of "cold-bloodedness" in fish.[3] However, unlike mammals and birds, the genetic characteristics associated with the occurrence of endothermy in fish have rarely been investigated. In many respects, the features of the entirely endothermic opah provide an unparalleled model to explore the molecular basis of endothermy as an adaptive trait in the ocean environment. In this study, we generated a chromosome-level genome of the Smalleye Pacific Opah (*Lampris incognitus*) (Figure 1A). We then conducted comparative genomic, transcriptomic, and proteomic analyses to identify the molecular adaptations accompanying endothermy. In addition, we explored the genetic patterns that are shared during the convergent evolution of endothermy among endothermic fishes, mammals, and birds.

RESULTS

Genome features and long terminal repeat (LTR) retrotransposons

PacBio sequencing technology was used to generate a high-quality genome of a male opah. We generated 183.23 Gb of PacBio clean data with approximately 136-fold coverage. These data were assembled into a 1,367.47 Mb genome, which approximated the size estimated by *k*-mer distribution (1,374.37 Mb, Figure S1), with a contig N50 size of 3.819 Mb and a scaffold N50 size of 21.58 Mb (Table S1). Hi-C reads were used to scaffold the contig-level assemblies, resulting in 22 chromosome-level scaffolds, which comprised the opah genome (Figures S2 and S3, Tables S2 and S3). In total, 24,658 genes were predicted (Tables S4–S6) using a combination of de novo gene prediction programs and homology-based methods, along with RNA-seq data. The evaluation of the genome for completeness based on Benchmarking Universal Single-Copy Orthologs (BUSCO; database: vertebrata_odb9) and the Core Eukaryotic Genes Mapping Approach (CEGMA) resulted in values of 94.08% and 98.91%, respectively (Tables S7 and S8). Genome annotation completeness was also evaluated using BUSCO, and the results showed that our gene set contained 95.78% complete and 2.28% fragmented ortholog genes (Table S9), showing that our gene annotation was highly complete. In total, 167 unique gene families were identified in the opah genome compared with those in zebrafish, fugu, and spotted gar (Figure S4).

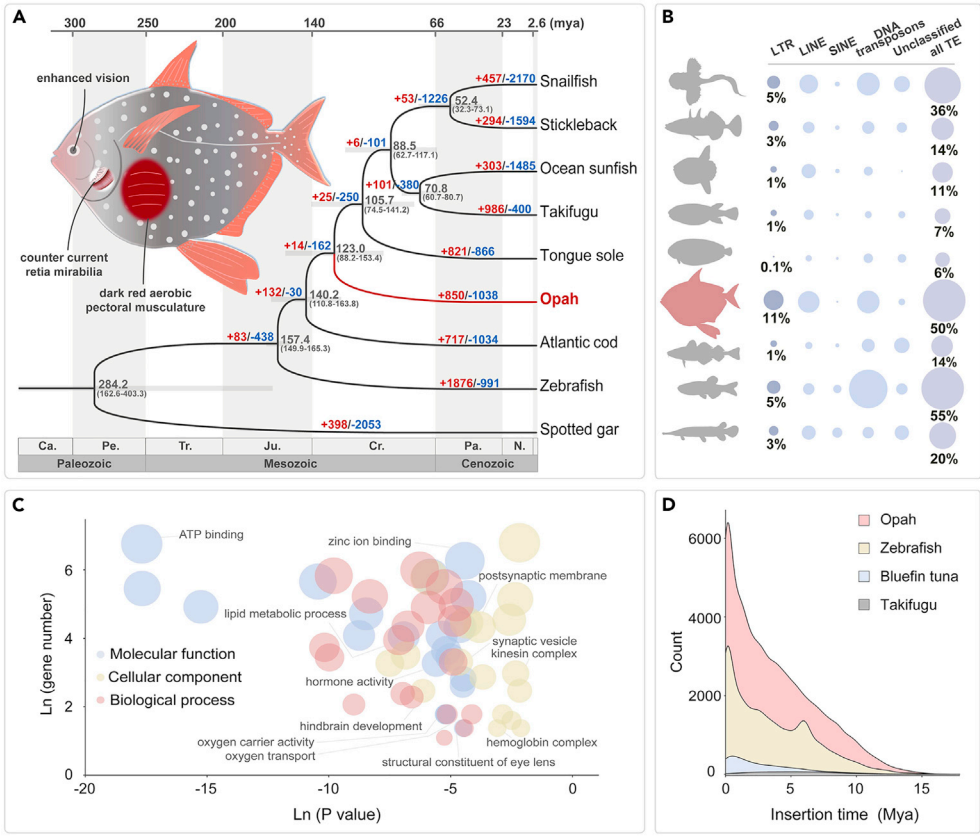

Figure 1. Evolutionary history of opah (A) Evolutionary key innovations and specialized physiological features involved in the endothermy of the Smalleye Pacific Opah, *Lampris incognitus*, and gene family expansion (red)/contraction (blue) in opah and other teleosts. The bootstrap value of all nodes is 100. (B) Percentages of transposable elements (TEs) in the different teleost genomes studied. LTR, long terminal repeats; LINE, long interspersed element; SINE, short interspersed element. (C) Enriched GO functional categories that were closest to the LTR retrotransposons (2 kb up and downstream). (D) Expansion of LTR retrotransposon in opah, zebrafish, bluefin tuna, and takifugu.

A phylogenomic analysis assigned the opah to a basal position within the advanced spiny-rayed Teleostei clade (Acanthomorpha) (Figure S5), which is in agreement with recent studies.[11,12] The opah genome was found to contain a relatively high percentage of transposable elements (TEs) and other interspersed repeats (taken together, ~50%). Specifically, the genome contained the highest percentage of the LTR retrotransposon superfamily (11%) reported in any teleost to date (Figure 1B, Tables S10, and S11). Enriched genes in spatial proximity to the LTR retrotransposons (2 kb up and downstream) were investigated to discover the evolutionary role of the extremely high LTR content in the opah genome. Among these genes, many gene ontology terms related to metabolism and the sensory system were significantly overrepresented, including the lipid metabolic process (GO:0006629, P = 4.2e-04), ATP binding (GO:0005524, P = 1.9e-08), glycerol transport (GO:0015793, P = 7.3e-04), oxygen transport (GO:0015671, P = 2.7e-03), and structural constituent of the eye lens (GO:0005212, P = 5.1e-03) (Figure 1C, Table S12). The expansion of LTRs during the evolution of the opah might thus have affected gene expression and translocation,[13] likely contributing to the endothermic ability of the opah and its adaptations to the deep-sea environment. In opah, the LTR retrotransposons were inferred to have started expanding approximately 15 million years ago (Figure 1D), which might have coevolved with (and potentially facilitated) whole-body endothermy.

Genome-wide signatures of convergent evolution in endothermic lineages

A whole-body endothermic phenotype evolved independently in mammals, birds, and opahs, and we analyzed these three lineages to detect molecular signals accompanying the convergent evolution of this phenotype (Figure 2A). After selecting five endothermic species (opah, turkey, chicken, mouse, and human) as foreground branches, 204 genes were found to have a convergent signature for positive selection, which were enriched in terms related to primary metabolic processes (GO: 0044238, P = 2.3e-05), organic substance metabolic processes (GO: 0071704, P = 8.0e-05), and carbohydrate derivative biosynthetic processes (GO: 1901137, P = 1.7e-03) (Table S13).

Genes with convergent amino acid substitutions were further screened among the selected mammals, birds, and opah (Table S14). Notably, *slc8b1* (mitochondrial sodium/potassium/calcium exchanger 6) showed signs of convergence, and it encodes the mitochondrial Na^+/Ca^{2+} exchanger (NCLX)[14] and is essential for heart function and metabolic heat production. A substitution site (M460I) was found to be located in the Na_Ca_ex domain (Figure S6A, Table S14), which is conserved among ectothermic teleosts, chondrichthyans, and reptiles. Because our orthologous gene sets comprised only two mammals and two birds, we manually expanded the scope of this comparison with 64 species, including 10 species of mammals, birds, and reptiles, five amphibians, and 29 fishes (Table S15). We found that the convergent amino acid change (M460I) was restricted to the whole-body endothermic mammals, birds, and opah and thus not found in the regional endothermic fishes, bluefin tuna, and great white shark (Figure S6A), implying potentially different mechanisms for evolving whole-body and regional endothermy. Additionally, another 22 convergent genes were detected among four of the five endothermic species (including opah; Figure 2A, Table S14).

Despite these similarities, teleosts and chondrichthyans generally generate heat mainly with their swim musculature, which differs from the heat production strategy of mammals and birds.[15,16] To account for this discrepancy, we performed another convergent evolution analysis comprising only fishes, namely the opah, Pacific bluefin tuna, and the great white shark (Figure 2B). Firstly, positive selection analyses were conducted with three different datasets by using either the opah, the Pacific bluefin tuna, or the great white shark as the foreground branch, revealing the genetic landscape of 36 genes with adaptive mutations that occurred during the evolution of endothermic fishes (Table S16). Among them, the *opa1* gene is crucial for the maintenance of mtDNA,[17] and *pyroxd2* plays important roles in regulating mitochondrial functions.[18] Then, we set all three endothermic fishes as foreground branches simultaneously. A total of 112 genes were found to have convergent signatures of positive selection, which were enriched in gene ontology terms related to a citrate lyase complex (GO: 0009346, P = 4.4e-03), energy homeostasis (GO: 0097009, P = 5.5e-03), and mitochondrial DNA metabolic process (GO: 0032042, P = 5.5e-03) (Table S17). In addition, we calculated the Ka/Ks ratio for each gene and found that four GO terms related to heat production and metabolism had significantly higher Ka/Ks ratios in the three endothermic fishes (Figure 2C, Table S18).

Among the endothermic fishes, 78 convergent amino acid replacements in 44 genes were identified (Table S19). One particular convergent amino acid substitution was identified in all three endothermic fish species at the V198A site of *glutaredoxin 3* (*glrx3*) (Table S19), a gene that plays a crucial role in Fe/S protein biogenesis and is indispensable for hemoglobin maturation.[19] The convergent site V198A was found to be located in the GRX domain, which has thiol oxidoreductase activity that is responsible for the reduction in protein disulfides or glutathione-protein mixed disulfides and is involved in cellular redox regulation. Convergent amino acid substitutions might affect the activity of GRX, which could in turn influence the function of Glrx3. We manually analyzed 45 bony fishes and six chondrichthyans and found that this convergent amino acid change (V198A) only existed in the three endothermic fishes (Figure S6B). This substitution is located in the GRX domain, which is conserved among teleosts, chondrichthyans, and lamprey, indicating the ancient and conserved role of *glrx3* in binding Fe/S clusters. In addition, three convergently changed sites (R8L, R159Q, and S201A) in the citrate lyase

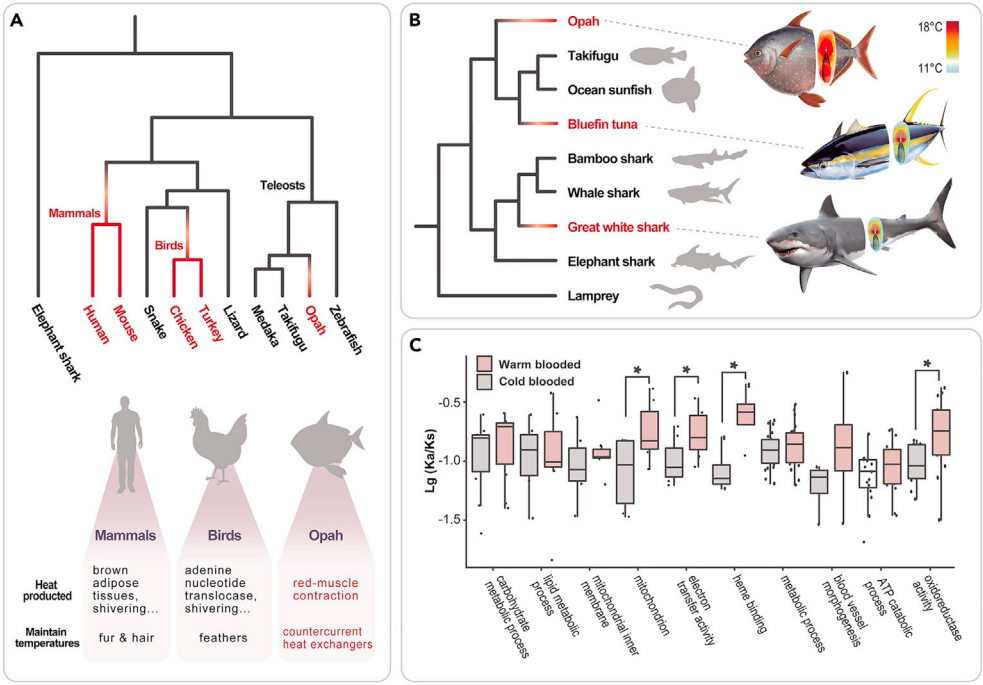

Figure 2. Genomic convergence analysis of endothermy in fishes (A) Phylogeny of independently evolved endothermic vertebrates. (B) The phylogeny consists of red muscle endothermic fishes (opah, Pacific bluefin tuna, and great white shark) and outgroups. (C) Comparison of the Ka/Ks ratios of genes involved in 10 GO terms related to heat production and metabolism between the endothermic foreground branch and ectothermic background branch of panel (B).

subunit β -like protein (Clybl) were found to be shared between opah and bluefin tuna (Table S19). Clybl plays important roles in energy metabolism, as it encodes a malate/β-methylmalate synthase, which can convert glyoxylate and acetyl-CoA to malate or glyoxylate and propionyl-CoA to β-methylmalate.[20] Additionally, we performed convergent analysis for background (nonendothermic species), and no convergent genes were found in either nonendothermic vertebrates (zebrafish, takifugu, medaka, lizard, snake, and elephant shark) or nonendothermic fish (takifugu, ocean sunfish, bamboo shark, whale shark, elephant shark, and lamprey). In summary, our genome scans provide evidence of convergent selective pressures and genetic responses shared by these endothermic fish species.

Evolutionary basis of whole-body endothermy

We performed comparative genomic analyses with other fish genomes to uncover opah-specific expanded/contracted gene families and positively selected genes (PSGs). We identified a total of 850 (36 gene families with $P < 0.05$) and 1,038 (38 gene families with $P < 0.05$) gene families to be expanded or contracted in opah, respectively (Figure 1A, Tables S20 and S21), and identified 233 genes with signatures of positive selection (Table S22). A notably large number of expanded gene families was enriched for GO terms related to the composition of muscle fibers (e.g., actin cytoskeleton GO: 0015629, myofibril GO: 0030016) and the contractile machinery of skeletal muscle (e.g., actin filament organization

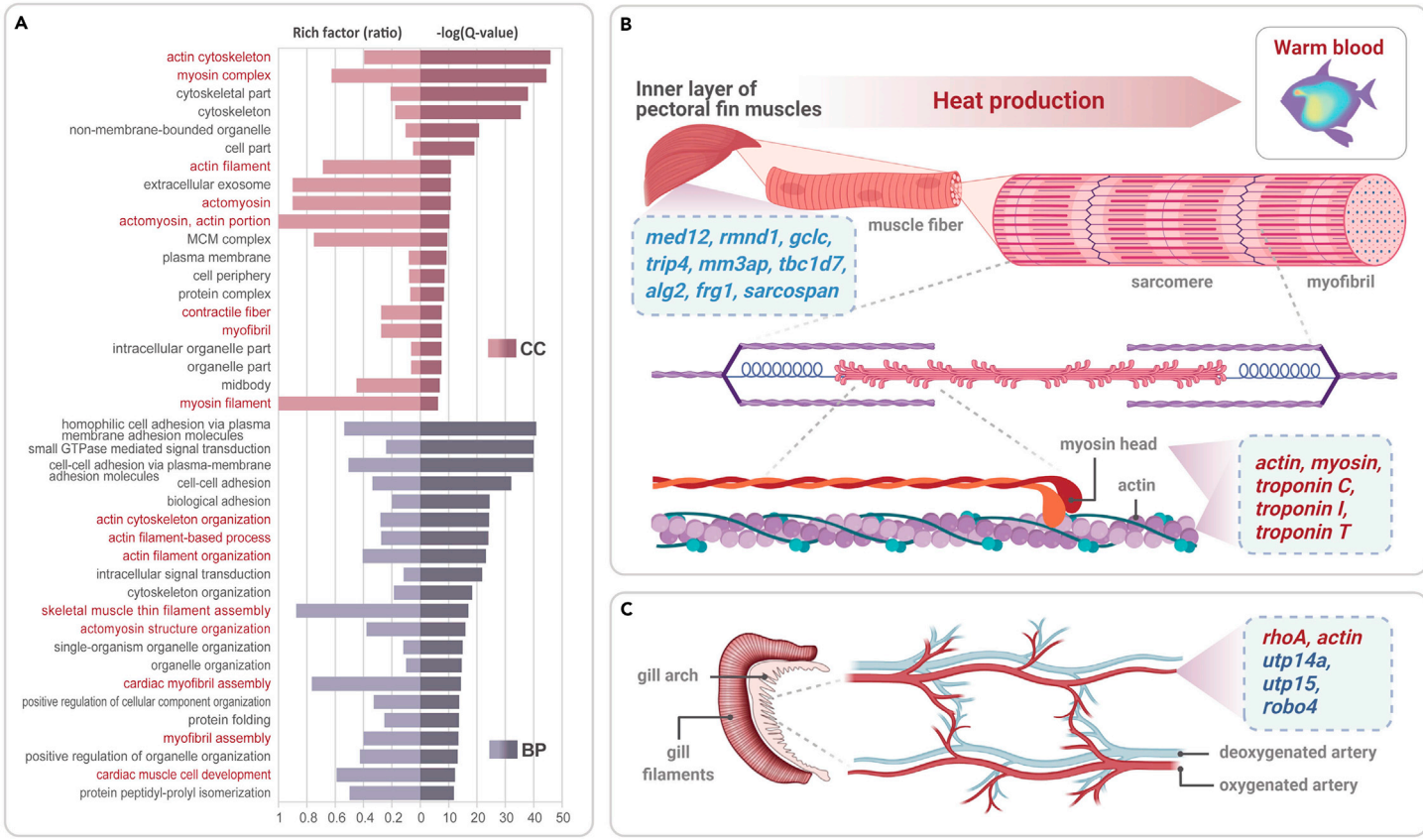

Figure 3. Genomic features related to the production and retention of metabolic heat in opah (A) The top 20 significantly enriched GO terms for expanded gene families in the opah genome are shown for cellular components (CCs) and biological processes (BPs). (B) PSGs (blue) and expanded genes (red) related to muscle development and skeletal muscle contraction. (C) PSGs (blue) and expanded genes (red) are involved in vasculature genesis, regulation, and patterning.

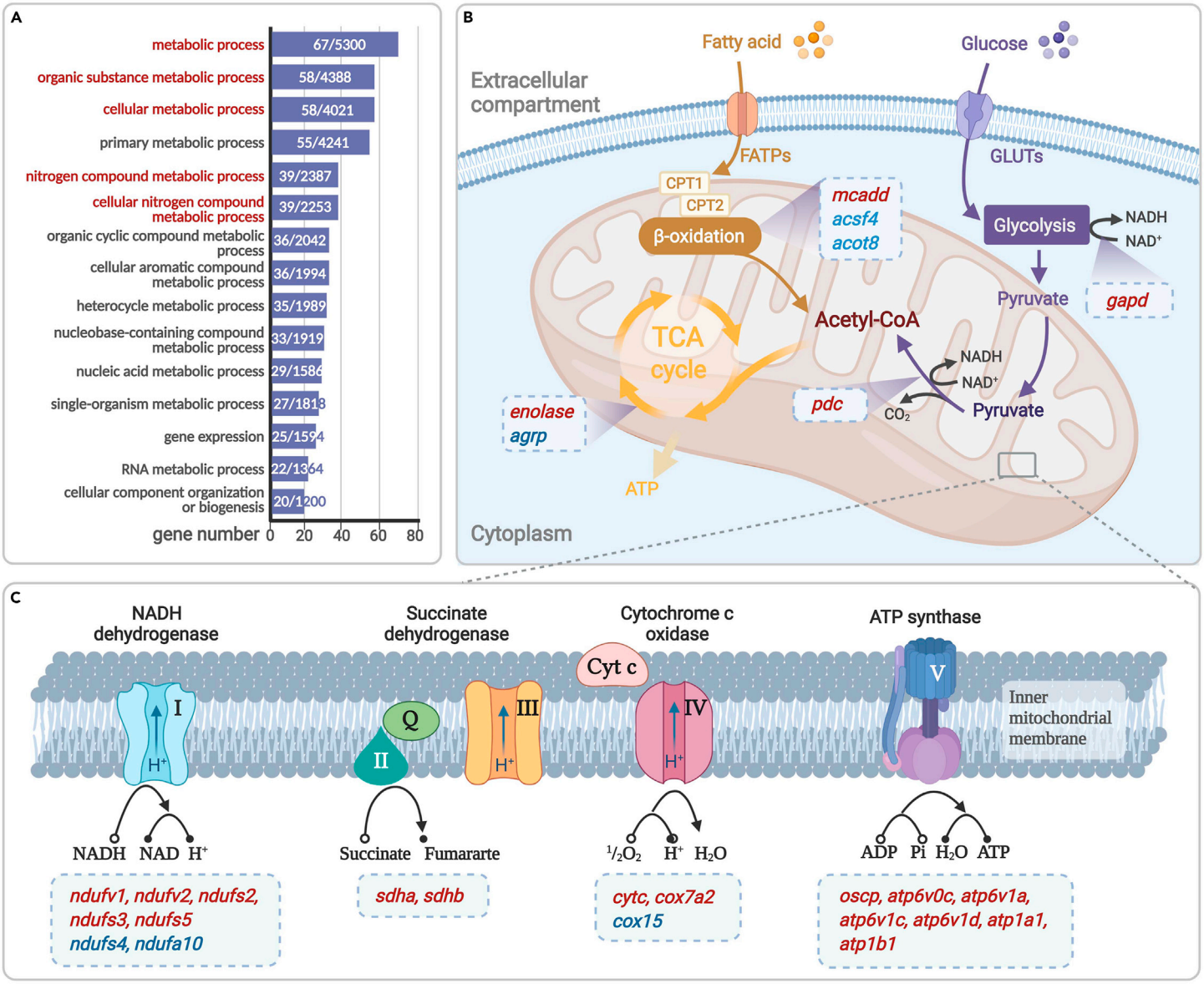

Figure 4. Genomic features related to the whole-body endothermy of opah (A) Top 15 enriched GO terms (category: biological process) among PSGs in the opah genome. (B and C) Expanded genes (red) and PSGs (blue) in opah are involved in lipid metabolism, carbohydrate metabolism, and (C) oxidative phosphorylation.

GO: 0007015, skeletal muscle thin filament assembly GO: 0030240) (Figure 3A, Table S23). In addition, many PSGs, such as *med12*, *rmnd1*, and *alg2*, are considered to be involved in the regulation of muscle formation and development. The expanded gene families of the structural proteins myosin, actin, and troponin encode key components of the sarcomere structure (Figure 3B). The expanded gene families were also enriched in the term cardiac muscle cell development (GO: 0055013), and three PSGs, *utp15* (U3 small nucleolar RNA-associated protein 15), *utp14a* (U3 small nucleolar RNA-associated protein 14 homolog A), and *robo4* (roundabout homolog 4) (Figures 3C and S7), were related to the genesis, regulation, and patterning of vasculature. A mutation in *utp15* disrupts vascular patterning in zebrafish embryos,[21] *utp14a* promotes angiogenesis,[22] and *robo4* is expressed in emerging blood vessels and can neutralize signaling to maintain vessel integrity through an angiogenic factor.[23] These expanded gene families and PSGs found in opah might thus be related to the evolution of the counter-current retia mirabilia.

The identified 233 PSGs were significantly enriched in organic substance metabolic processes and nitrogen compound metabolic processes (Figure 4A, Table S24). Among them, *agrp* regulates energy homeostasis;[24] *acsf4* and *acot8* are involved in lipid metabolism (Figure 4B); and *ndufa10*, *ndufs4*, and *cox15* encode core proteins in the mitochondrial electron transport chain (Figure 4C), which is crucial for oxidative phosphorylation. Remarkably, *ndufa10* also exhibited convergent amino acid changes among the fast-running ruminant species, which require a sufficient quantity of red muscle and efficient contractions to guarantee a supply of metabolic heat.[25] Moreover, gene families involved in lipid metabolism (*mcadd*), carbohydrate metabolism (*gapd*, *enolase*), and oxidative phosphorylation (*ndufv*, *cytc*, *cox7a2*, and *atp1a1*) were found to be expanded in the opah genome (Figure 4C). The genetic changes detected in opah indicate enhanced physiological functions, including increased aerobic performance, food digestion, and assimilation.

Transcriptomic and proteomic profiles of opah pectoral musculature

Being the major "heat motor" for the whole-body endothermy of opah, further studies were conducted to evaluate the evolutionary origin of the dark red, aerobic pectoral musculature using its transcriptomic and proteomic profiles. Seven tissues were used to identify the tissue-specific expressed genes as follows: the inner (PMI) and superficial layer (PMS) of the pectoral musculature, dorsal musculature (DM), ventral musculature (VM), liver, kidney, and gill (Table S25). Additionally, five tissues were used to identify the tissue-specific expressed proteins, namely PMI, PMS, VM, liver, and gill. Transcriptomic profiles revealed that the 100 genes with the highest expression in PMI, which included many expanded gene families such as cytochrome c oxidase (*cytc*), ATP synthase F0 subunit 6 (*atp6*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), and troponin (*troponin*) (Figure 5, Table S26). Consistent with the transcriptomic profiles, the 100 most abundant proteins included Cytc, Atp6, Gapdh, troponin, and myosin

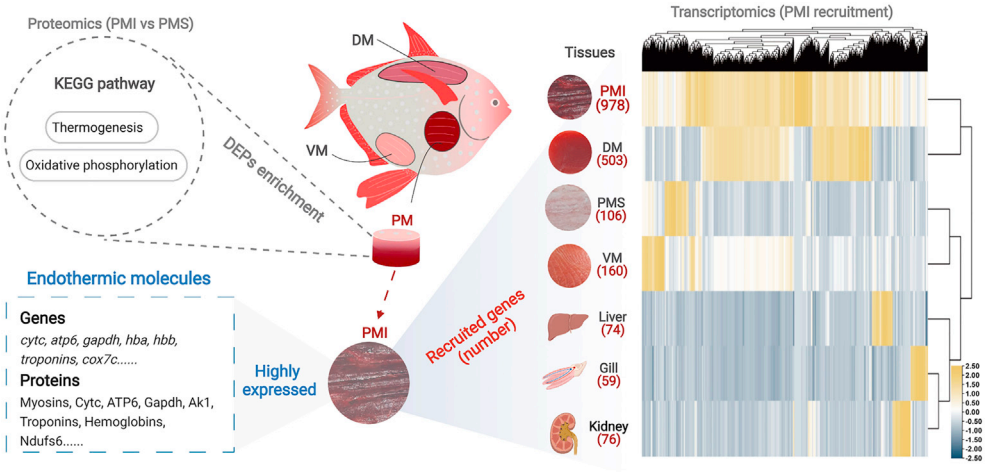

Figure 5. Transcriptomic and proteomic profiles of the novel pectoral musculature that was co-opted for heat production Proteomics: KEGG pathway enrichment analysis of the upregulated DEPs of the PMI and the PMS, including thermogenesis and oxidative phosphorylation. Endothermic molecules: the 100 most highly expressed genes and proteins in the PMI contributing to whole-body endothermy. Transcriptomics: the number and expression profiles of genes recruited from other tissues for the development of the dark red, aerobic PMI.

(Figure 5, Table S27). These proteins have been reported to be involved in cytochrome heat production, ATP biosynthesis, glycolysis, and skeletal muscle contraction.[26–28] In addition, hemoglobins were also highly expressed, which might indicate a type of functional compensation, considering the high oxygen consumption (Tables S26 and S27).[29] Taken together, the PMI highly expressed proteins are likely involved in endothermy, providing insights into the genetic basis for the evolutive "whole-body endothermy" of opah.

At both the structural and molecular levels, the PMI is a unique tissue that differs from muscles in other opah regions. Previous studies have indicated that the evolutionary origin of "new organs" or key innovations such as pectoral musculature often depends on the recruitment and co-option of genes that were originally expressed in other tissues.[30] In this study, the comparative transcriptome analysis indicated that the PMI depends largely on genes recruited from the segmental swimming musculature, specifically the DM (Table S28), whereas the gene expression profile of the PMS was closest to that of VM. To determine the disparity between the PMI and other musculature, we conducted further analyses and identified 1,480, 6,645, and 4,771 differentially expressed genes in PMI versus DM, PMI versus VM, and PMI versus PMS, respectively (Figures S8 and S9, Tables S29, 30 , and S31). In addition, a comparative label-free proteomic approach identified 470 (149 downregulated, 321 upregulated) and 573 (402 downregulated, 171 upregulated) differentially expressed proteins (DEPs) in PMI versus VM and PMI versus PMS, respectively (Tables S32 and S33). A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of these DEPs revealed that the highly expressed proteins in the PMI were mainly involved in thermogenesis and oxidative phosphorylation, indicating that there has been an adaptive change in the heat production of the evolved musculature (Figure S8).

Adaptive sensory and immune systems pave the way to the deep sea

In opah, approximately 5.6% (13 out of 234 genes) of the identified PSGs play important roles in the visual system (Figure 6A, Table S22): the centrosomal protein POC5 (*poc5*) is a causative gene for retinal disease, and previous studies have shown that the loss of *poc5* caused retinal degeneration in zebrafish;[31] the mutant dynamin binding protein (*dnmbp*) and mitochondrial isoleucyl-tRNA synthetase 2 (*iars2*) are associated with cataracts in mammals;[32,33] tripartite motif-containing protein 44 (*trim44*) is crucial for iris development, and the variants in *trim44* are considered the major cause of aniridia;[34] biallelic mutations in the S-phase cyclin-A-associated protein in the ER (*scaper*) can cause autosomal recessive retinitis pigmentosa with intellectual disability;[35] optic atrophy protein 1 (*opa1*) plays an important role in optic nerve development.[36] In addition, significant expansions of the β- and γ-crystallin family have been found in the opah genome, which also provide support for the enhanced visual capability of opah (Figure 6A). However, in contrast to their enhanced eyesight, opah might have a reduced olfactory performance, as they have the lowest number of olfactory receptor genes reported to date for teleosts (only 24, compared with 26 in *Hippocampus comes* and 156 in *Danio rerio*; Figure 6B).[37,38] Additionally, in the opah genome, *MHCII* genes were significantly expanded, especially in the DA group of the *MHCII* β-chain (Figure S10). Compared with the DB and DE groups, the DA group exhibited the classical characteristics of *MHCII*, and these genes were found to be specifically and highly expressed in the immune tissues (liver, kidney, and gill) of opah (Figure S11).

DISCUSSION

The evolution of endothermy in mammals and birds has been intensively studied during the last few decades, and it has been hypothesized to confer numerous physiological and ecological advantages.[15,39] Different thermogenic strategies have been adopted by those vertebrate clades that evolved endothermy independently;[1] however, the adaptive changes that accompanied the evolution of endothermy exhibit many similarities, including a higher metabolic rate, a higher capability of sustained aerobic performance, and enhanced neural conductance.[7,15,16] Adaptive phenotypic convergence between distantly related taxa has occurred repeatedly in nature and has generally been attributed to convergent genetic changes driven by similar selective pressures.[40–42] Several lineages of elasmobranchs and teleosts independently evolved specific forms of muscular endothermy allowing them to elevate their body temperature relative to that of the ambient water.[1] Previous studies on tuna and the great white shark revealed convergent genetic changes involved in oxidative phosphorylation, the Krebs cycle, muscle contraction, and lipid and fat metabolism,[15,16] and a recent study on tuna and billfish observed convergent amino acid replacements in proteins related to heat production and the visual system.[43]

Fish endothermy is mainly dependent on red muscle activity, which produces heat as a by-product during muscle contraction[7,15] and is located close to the center of the body, in most endothermic species (e.g., tuna and great white sharks).[44] To achieve the extraordinarily high respiratory metabolic rate, endothermic fishes have evolved a number of key adaptations. In this study, we found a convergent amino acid substitution at the V198A site located in the conserved GRX domain of *glrx3* for the three endothermic fishes among the 51 fishes investigated. As *glrx3* is indispensable for hemoglobin maturation, the replaced amino acid in the convergent site of the endothermic species, relative to that in ectothermic groups, might change the ability to provide oxygen to the musculature, thus affecting muscle strength and endurance in the opah, bluefin tuna, and great white sharks.

As tuna, billfish, and the great white shark are only able to warm up specific regions of their bodies, they are not typically considered warm blooded. Opah exhibit "whole-body endothermy," through the production of vast amounts of heat, by constantly flapping their enlarged pectoral fins when continuously swimming in a labrid fashion.[7,8] In the opah genome, a substantial portion of PSGs and expanded gene families have been identified as involved in not only skeletal muscle contraction but also lipid metabolism, carbohydrate metabolism, and oxidative phosphorylation, which coincides with expectations that endothermic species have increased metabolic rates and aerobic capacities.[15] Furthermore, the expansion of LTR retrotransposons in opah was also suspected to regulate the transposition and expression of genes related to heat-generating processes. Genes involved in muscle contraction and energy metabolism were found to have high expression levels in the PMI, which have thick fibers and show a much deeper red color compared with muscles in other regions. Interestingly, the genes in the PMI were largely recruited from the DM rather than the PMS, suggesting that the PMI is more derived and specialized in opah. Hence, we agree with previous studies suggesting that the novel structure and function of PMI is the key to the evolution of endothermy in opah.

Adaptive changes have been hypothesized to have arisen in the sensory systems of opah alongside endothermy, including enhanced temporal resolution and neural conductance for the eye and brain.[7] As a high-performance predator,

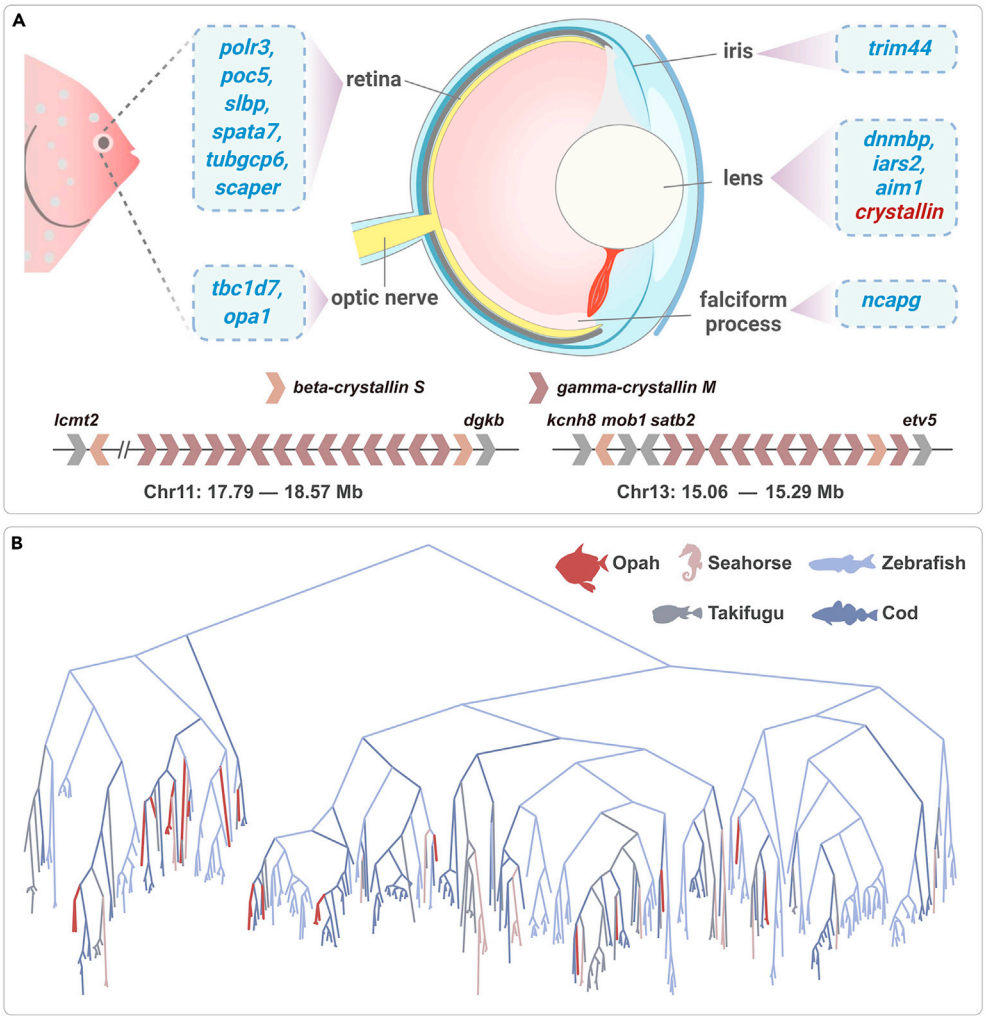

Figure 6. Genomic analysis of genes related to vision and olfaction in opah (A) Expanded genes (red) and PSGs (blue) in opah are involved in visual development. (B) Gene phylogeny of olfactory receptors across the genomes of opah, zebrafish, fugu, and cod.

opah have large eyes and are thought to rely heavily on vision to seek and capture active prey such as fast swimming squid and fish.[45] This was also reflected by its genome, as crystallin genes were duplicated, and several PSGs related to the visual system were identified; this enhanced visual capability is likely one of the benefits from endothermy, since an improvement in vision has also been reported in billfish and tuna, which are able to warm their brains and eyes.[43,46] The reduced olfactory performance might be compensated by the enhanced vision, which has been reported to be an adaptation to facilitate habitat utilization of deep and cold waters.[38] Enhanced vision, along with an expanded thermal tolerance to the nutrient-poor pelagic environment, would enable increased access to high-energy prey and provide a strong selective benefit.[39] The MHCII molecules are heterodimeric surface proteins involved in the presentation of exogenous antigens during the adaptive immune response.[47] Since endothermy represents an optimum in the trade-off between metabolism and fitness of immunity,[9,10] the adaptive genetic changes in the immune system might be coordinated with the evolution of endothermy in opah. The unique endothermy of opah has put the species in a unique situation where it can exploit cold, nutrient-rich deep waters without the shortcomings of reduced metabolic rates.

CONCLUSIONS

In this study, we generated a high-quality chromosome-level genome assembly of the Smalleye Pacific Opah (*Lampris incognitus*). By comparing the opah with mammals, birds, and other regionally endothermic fishes, strong genomic signatures of convergent evolution in the heat production process and visual system were identified. We identified a large number of positively selective genes and expanded gene families in the opah genome that are involved in the processes of skeletal muscle contraction, lipid metabolism, carbohydrate metabolism, and oxidative phosphorylation, among others, indicating that they are part of the genomic basis for whole-body endothermy. Our transcriptomic and proteomic analyses showed that genes involved in muscle contraction and energy metabolism have high expression levels in the PMI, which might be the evolutionary key innovation involved in the evolution of whole-body endothermy. These adaptations might explain how opahs can have such an active lifestyle in cold and deep-sea environments.

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AUTHOR CONTRIBUTIONS

Qiang Lin, Dazhi Wang, and Axel Meyer conceived and designed the study. Xin Wang, Shaobo Ma, and Shuaishuai Liu collected the samples. Xin Wang, Meng Qu, Yali Liu, Yue Song, Ralf Schneider, Zelin Chen, Haiyan Yu, Geng Qin, Jianping Yin, Guangyi Fan, Axel Meyer, and Jia Zhong performed genome analyses. Zelin Chen, Yanhong Zhang, and Haiyan Yu performed TE analysis. Suyu Zhang, Meng Qu, and Xin Wang performed convergent analyses. Yali Liu and Haiyan Yu performed transcriptomic analysis. Hao Zhang and Dongxu Li performed proteomic analysis. Xin Wang, Meng Qu, Yali Liu, Dazhi Wang, and Qiang Lin wrote the manuscript with input from all other authors. All authors reviewed and contributed to the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interest.

LEAD CONTACT WEBSITE

Prof. Qiang LIN http://people.ucas.ac.cn/~0018009?language=en.

SUPPLEMENTAL INFORMATION

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