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COMPOSITION, EXPRESSION, REGULATION AND EVOLUTION OF FISH HOX GENES FAMILY: A REVIEW

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Abstract

The Hox gene family stands as a foundation in the intricate dance of embryonic development, particularly in vertebrates, where it assumes the crucial task of coordinating the formation and patterning along the anterior-posterior axis. Fish is a diverse group within the vertebrate lineage; it offers a fascinating indication of the evolutionary processes that have shaped this family of genes. Through extensive diversification and expansion, the Hox genes in fishes have played a pivotal role in generating the surprising array of morphological forms seen among these aquatic creatures. Their evolutionary journey not only sheds light on the mechanisms underlying the emergence of vertebrate diversity but also underscores the intricate interplay between genetic regulation and phenotypic variation. Investigating the evolutionary history, functional significance, and regulatory mechanisms of fish Hox genes is therefore crucial for unraveling the genetic blueprint that underpins vertebrate development. By interpreting the shades of these genes, the evaluation of DNA sequence homology and phylogenetic relationships between species will show great significance in the conservation of biological diversity, particularly genetic diversity and explanation of the course and mechanism of biological evolution.

Key words: fish, Hox genes, composition, function, evolution, regulation

Introduction

The evolutionary progression of life on Earth has advanced from simple to increasingly complex organisms, beginning with single-celled bacteria, evolving into simple multicellular animals, transitioning from diploblasts to triploblasts, and ultimately leading to the emergence of vertebrates. The structural and functional innovations in these evolutions are partly accomplished through the expansion of the genetic toolbox, such as gene duplication. Comparisons between vertebrates and chordates have found that the expansion of gene number is closely related to the complexity and variability of morphology and anatomy (Amores et al., 1998). The Hox gene family comprises numerous evolutionary homologous genes that exhibit parallel development, with their structure and number continuously evolving across different organisms through genome duplication. This makes the Hox gene family a key model for studying biological evolution and the dynamics of genome duplication (Ferrier, 2016).

1. Overview of Hox genes

Hox genes are a highly conserved family of homeobox-containing transcription factors that play crucial roles in embryonic development and adult processes (Hubert and Wellik, 2023). These genes are essential for proper body plan formation, determining cell identity, and patterning along the anterior-posterior axis in both invertebrates and vertebrates (Kappen, 1996). The Hox gene family is characterized by its unique genomic organization, with genes arranged in clusters that dictate their spatial and temporal regulation during development (Hubert and Wellik, 2023; Peraldi and Kmita, 2024). Interestingly, Hox genes exhibit spatial collinearity, where their order within the cluster corresponds to their expression pattern along the body axis. In vertebrates, this spatial collinearity is closely linked to temporal collinearity, with genes activated in a sequential manner that aligns with the progressive emergence of axial tissues (Peraldi and Kmita, 2024). This intricate regulation of Hox genes is crucial for their diverse functions in embryogenesis and beyond. The importance of Hox genes extends beyond development, as their dysregulation has been associated with various human malignancies, including leukemia and solid tumors (Alharbi et al., 2012; Feng et al., 2021; Kelly et al., 2011). Understanding the complex regulatory mechanisms and functions of Hox genes is essential for elucidating their roles in normal development, disease progression, and potential therapeutic applications (Feng et al., 2021)

Hox genes (homeobox genes) are a type of gene containing homeoboxes that are unique to metazoans. They are genes that specifically regulate the body shape of organisms and participate in the early embryonic development of animals. The expression level in embryonic development plays an important regulatory role in forming tissues and organs. They mainly regulate other genes related to cell division, spindle direction, and the development of bristles, appendages, etc. Mutations in Hox genes can lead to ectopic growth of an organ during embryonic development, that is, other organs replace the normal structure. The Hox gene contains a conserved sequence of about 180 bp of homeobox, which can transcribe a protein containing about 60 amino acids. The conserved region, also known as the homeodomain, contains a helix-loop-helix domain (HLH) and regulates gene expression by binding to specific DNA. The expression of Hox genes can control the regional specificity of the vertebrate body and play an important regulatory role in different stages of the reproductive process of mammals. Humans have 4 gene clusters, named *HoxA*, *HoxB*, *HoxC*, and *HoxD*, containing 38 genes, each about 100 kb in length and containing 9 to 11 loci (Yu et al., 2018). Mice have 4 gene clusters, named *Hoxa*, *Hoxb*, *Hoxc*, and *Hoxd*. Homeobox genes can be divided into 13 homologous families based on sequence similarity and location on chromosomes. Members of each homologous family are expressed and regulated in similar patterns in mammalian embryos.

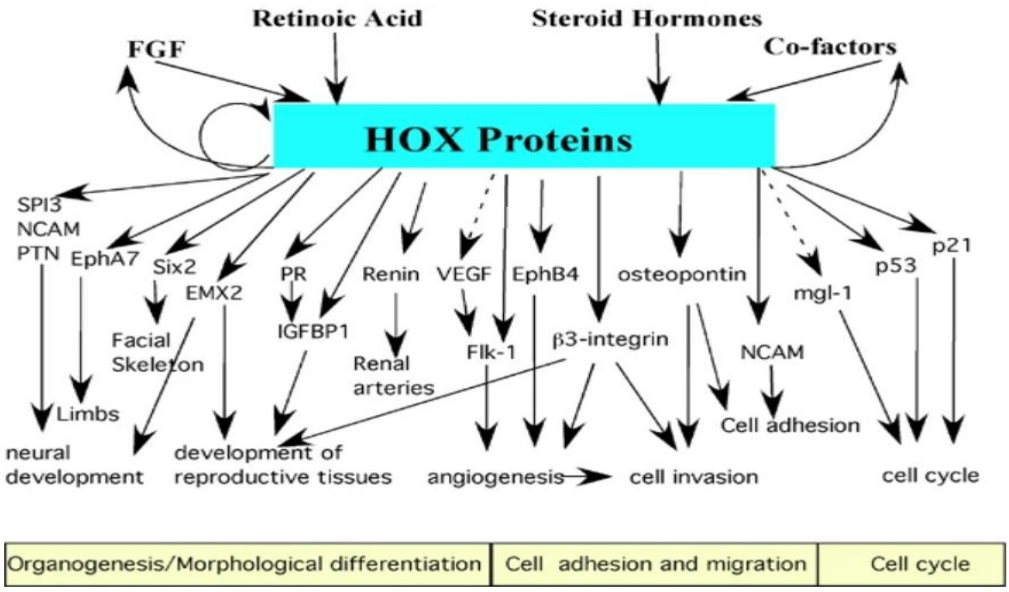

Figure 1.1. Hox transcription factors and their elusive mammalian gene targets

Hox downstream targets are involved in numerous cellular processes including organogenesis, cellular differentiation, cell adhesion and migration, cell cycle, and apoptosis. Several downstream targets play multiple roles in several pathways, with many acting as transcription factors and regulating their subset of genes. Hox proteins also participate in autoregulatory circuits in addition to regulating the expression of some binding cofactors and other factors that influence Hox expression. Targets not shown in this figure include the differentiation markers alpha-2(V) collagen, the murine hair keratin family, and the TTF-1. A dotted line indicates direct regulation has not yet been confirmed.

2. Characteristics of Hox genes

Hox genes are a specific subgroup of homeobox-containing genes characterized by their structural and functional homology to the homeotic genes of *Drosophila*. These genes play crucial roles in pattern formation along the anterior-posterior axis during embryonic development in both invertebrates and vertebrates (Hubert and Wellik, 2023; Kappen, 1996). Hox genes encode proteins containing a DNA-binding homeodomain, which act as transcription factors regulating the expression of other genes during morphogenesis (Kappen, 1996). In mammals, there are 39 Hox genes organized into four conserved clusters: A, B, C, and D (Lopez et al., 2006). These genes are essential for body segment identity, organ development, and tissue differentiation (Kappen, 1996). Interestingly, Hox genes are not only crucial during embryonic development but also play significant roles in adult processes, including hematopoiesis and reproductive tract function (Alharbi et al., 2013; Du and Taylor, 2016; Van Oostveen et al., 1999). Their expression is tightly regulated, and disruptions in these regulatory mechanisms can lead to developmental problems and diseases such as cancer. For instance, altered expression or disruption of Hox genes can result in changes in blood cell characteristics or disturbances in blood cell development (Van Oostveen et al., 1999). In conclusion, Hox genes are master regulatory transcription factors with diverse functions throughout an organism's lifespan. Their highly conserved nature and involvement in various developmental and adult processes make them a subject of great interest in fields ranging from embryology to

oncology. Understanding the complex mechanisms of Hox gene regulation and function remains an important area of research, with potential implications for developmental biology, cancer research, and therapeutic interventions (Alharbi et al., 2013; Li et al., 2019).

The clustered existence of Hox genes determines the fate of cells in the anterior-posterior axis of animal embryos. Evidence suggests that Hox genes underwent two duplications during the evolution from invertebrates to vertebrates. Invertebrate chordates possess a single Hox gene cluster, whereas mammals have four such clusters, each situated on distinct chromosomes (Koh et al., 2003). Each linkage group contains up to 13 Hox genes, and bony fish have more linkage groups. Amores et al. (1998) found that there are seven Hox gene linkage groups in the zebrafish (*Danio rerio*) genome. Studies have shown that a chromosome-doubling event occurred during the evolution of fish, forming a Hox gene linkage group that is twice that of humans. At the same time, the duplication of Hox gene clusters may have promoted the evolution and variation of the body structure of vertebrates. The evolution of Hox genes is also accompanied by the loss of linkage groups, so zebrafish have seven Hox gene linkage groups (Prince et al., 1998). Phylogenetic analysis and genetic mapping show that after the divergence of ray-finned fish and cross-finned fish and before the emergence of bony fish, a chromosome-doubling event may have occurred, and many of these events were formed through whole genome doubling. Therefore, bony fish, as the most diverse group of vertebrates, have more copies of developmental regulatory genes than mammals, although their anterior-posterior axis is less complex than that of mammals. Hox gene homology domains are 70–80% conserved and are usually found at the C-terminus of proteins, participating in gene expression regulation.

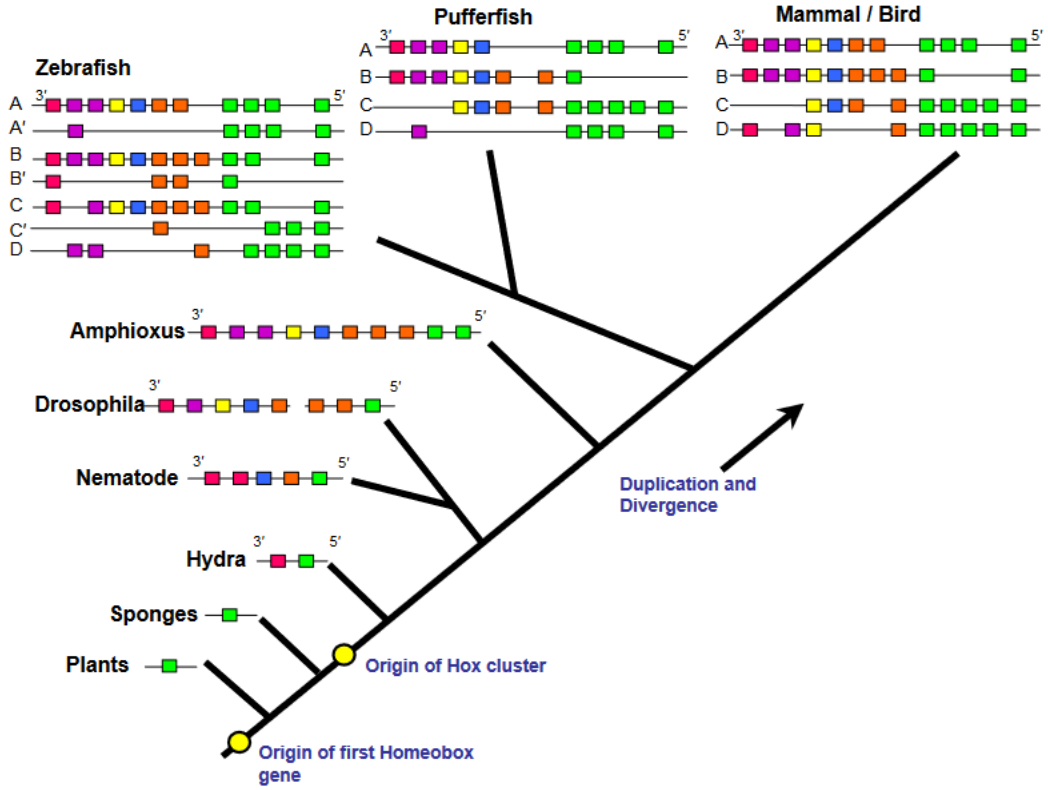

Figure 1.2. A representative dendrogram illustrating the evolution of Hox clusters. Hox gene clusters are thought to have developed by a process of duplication and divergence from a primordial homeobox gene estimated to have arisen about 1,000 million years ago

2.1. Highly conserved in evolution

Hox family genes are present in lower organisms, mammals, and humans, and they exhibit a high degree of conservation across these groups (Goodman, 2002). The evolutionary conservation of Hox gene clusters is essential for the shared use of nearby regulatory elements. Data from zebrafish show that the number of Hox gene clusters varies greatly and some common Hox genes have even been lost. These genes and pseudogenes have existed in the genome for a long evolutionary time, suggesting that the duplication and maintenance of genes in the genome are important. Or perhaps the looping is not costly and the genetic program is executed for a different purpose than one might expect. This tendency for genes to be looped around in the genome promotes the evolutionary potential of relatives, such as fish, to respond more quickly and flexibly to changing ecological environments (Aparicio et al., 1995).

2.2. Regulatory role of Hox genes in the reproductive process

Hox genes play a crucial regulatory role in the reproductive process, from embryonic development to adult function. These highly conserved transcription factors are essential for the proper development of the müllerian tract during embryogenesis and continue to be expressed in the adult uterus (Du and Taylor, 2016; Taylor, 2000). In the adult reproductive tract, Hox genes are particularly important for endometrial development and receptivity, with Hoxa10 and Hoxa11 being necessary for uterine receptivity and implantation in mice (Daftary and Taylor, 2000). The expression of Hox genes in the reproductive tract is regulated by sex steroids, including estradiol and progesterone, which mediate development in both the embryo and the adult organism (Daftary and Taylor, 2006; Du and Taylor, 2016). Interestingly, while Hox genes are primarily known for their role in embryonic patterning, their continued expression in adult tissues, particularly in the reproductive tract, sets them apart from other developmental genes (Taylor, 2000). This unique characteristic allows Hox genes to contribute to both the initial formation of reproductive structures and their ongoing functional differentiation in adulthood (Daftary and Taylor, 2006). The regulation of Hox gene expression involves complex mechanisms, including nuclear dynamics, RNA processing, microRNA regulation, and translational control, which may contribute to the robustness of their expression patterns during development and in adult tissues (Mallo and Alonso, 2013). In conclusion, Hox genes serve as master regulators in the reproductive process, orchestrating both developmental and functional aspects of the reproductive tract. Their involvement spans from early embryonic patterning to adult endometrial receptivity, highlighting their critical importance in reproductive biology. Understanding the regulatory mechanisms of Hox genes in reproduction could provide valuable insights into fertility issues and potential therapeutic approaches for reproductive disorders.

Hox genes play an important regulatory role in different stages of mammalian reproduction. Hox genes affect embryonic development and are related to the differentiation of the adult reproductive system. They play an important regulatory role in reproductive processes such as the establishment of uterine receptivity during implantation and the occurrence of uterine decidualization reaction. The abdominal region B gene family is a subfamily of Hox genes. Their expression regulates the structural domain of the posterior part of the body, including the mid-region of the reproductive domain in *Drosophila* adults and the development of the urogenital system in vertebrates (Casares et al., 1996). The female reproductive system of mammals is differentiated from the paramesonephric duct (Müllerian duct). The timing and pattern of the opening of the Hoxa axis determine the development process of the paramesonephric duct. The continuous expression of

Hox genes in female adults plays an important regulatory role in maintaining developmental plasticity and mammary gland development (Gehrke and Shubin, 2016).

2.3. Participating in the regulation of embryonic development

Hox genes play a crucial role in regulating embryonic development, particularly in determining tissue identity and patterning along the anteroposterior body axis (Du and Taylor, 2016; Taylor, 2000). These genes encode transcription factors that are essential for proper morphogenesis and cell differentiation during embryonic development (Du and Taylor, 2016; Hubert and Wellik, 2023). The expression of Hox genes is tightly controlled and follows a temporal and spatial sequential activation pattern, which is critical for proper embryonic development and patterning (Gentile and Kmita, 2020). Hox genes are involved in specifying embryonic structures along the body axis and are associated with normal cell growth (Luo et al., 2004). Interestingly, while Hox genes are primarily known for their role in axial patterning, they are not expressed in neural crest cells contributing to tooth formation. Instead, other non-Hox homeobox genes may be involved in patterning the dentition. Additionally, the regulation of Hox gene expression is influenced by various hormones and their cognate receptors, including estradiol, progesterone, testosterone, retinoic acid, and vitamin D, which mediate development in the embryo (Daftary and Taylor, 2006). In conclusion, Hox genes are fundamental regulators of embryonic development, orchestrating the formation of body structures and tissue identities. Their expression is precisely controlled through various mechanisms, including epigenetic modifications by polycomb repressive complexes (Gentile and Kmita, 2020). The importance of Hox genes in development is further emphasized by their evolutionary conservation across species and their expanded gene complement associated with the emergence of chordate and vertebrate characters (Hubert and Wellik, 2023; Pendleton et al., 1993).

All vertebrates, from salamanders to humans, have very similar early embryonic development. Among them, Hox genes function as a master regulator, determining cell fate, growth, and development. They control the transcription of all cells, regulate the position, shape, number, pattern, and arrangement of the trunk and attached limbs, and ensure the proper development of the organism's trunk, limbs, head, and other organs (Bradaschia-Correa et al., 2019). Mutations in homeotic genes can cause ectopic expression during embryonic development, resulting in the formation of new limb structures and the loss of others. This leads to the transformation of organs or body parts from one segment into those typically found in another segment. Mutations in homeotic genes initially result in minor alterations during early embryonic development. However, as tissues and organs continue to differentiate, the effects of these mutations become more pronounced, ultimately leading to substantial changes in the organism's morphology and structure. For instance, in fruit flies, mutations in homeotic genes can cause thoracic legs to develop in place of antennae on the head, and wings to form where eyes should be, or even result in the duplication of thoracic segments and legs.

2.4. Regulation of Hox gene expression

Hox genes play a crucial role in embryonic development and patterning, particularly in establishing the body plan along the anterior-posterior axis. The regulation of Hox gene expression is a complex process involving multiple mechanisms that operate at various levels, including transcriptional, post-transcriptional, and translational control (Mallo and Alonso, 2013). The initial transcription of Hox genes during gastrulation is governed by molecular genetic interactions that are not yet fully understood. However, the discovery of a "cluster repressive regulation" has provided insights into the colinear and sequential initiation of Hox gene transcription. As Hox expression

domains are established, they remain labile and can be reprogrammed under the influence of more posterior locations. This process may involve retinoic acid (RA) and FGF signaling. One of the key mechanisms in Hox gene regulation is the use of alternative promoters. For instance, the human Hox-5.1 gene employs two alternative promoters to generate two classes of mRNAs with distinct tissue and subcellular distribution, RA induction patterns, and mRNA stability. This differential regulation allows for precise control of Hox gene expression in various tissues and developmental stages. The regulation of Hox genes also involves epigenetic mechanisms, particularly through the action of polycomb repressive complexes (PRCs). PRCs contribute to Hox silencing by modifying chromatin structure, which is crucial for maintaining the transcriptional state of Hox genes both within and outside their expression domains (Gentile and Kmita, 2020). The dynamic binding of PRCs and their impact on the 3D organization of the genome play significant roles in Hox regulation during development (Gentile and Kmita, 2020). MicroRNAs have emerged as important regulators of Hox gene expression. These small non-coding RNAs contribute to the fine-tuning of Hox expression patterns and are involved in the robustness of Hox regulation during development (Braekeleer et al., 2014; Mallo and Alonso, 2013). The interplay between microRNAs and Hox genes is particularly relevant in the context of leukemogenesis, where dysregulation of both can lead to malignant transformation (Braekeleer et al., 2014). Hormonal regulation of Hox genes has been identified as a crucial mechanism in both embryonic development and adult tissue function. Hormones such as estradiol, progesterone, testosterone, retinoic acid, and vitamin D have been shown to regulate Hox gene expression (Daftary and Taylor, 2006). This endocrine regulation allows for the generation of structural and functional diversity in developing and adult tissues. Importantly, disruption of this hormonal control, such as exposure to endocrine disruptors, can lead to aberrant Hox gene expression and subsequent developmental abnormalities (Daftary and Taylor, 2006). In the context of cancer, dysregulation of Hox gene expression has been observed in various malignancies, including melanoma and breast cancer (Svingen and Tonissen, 2003). The altered expression of Hox genes in cancer cells can contribute to oncogenic transformation and metastasis. Interestingly, the patterns of Hox gene dysregulation may differ depending on the specific Hox gene and cancer type (Brotto et al., 2020).

Clustered genes exhibit distinct characteristics compared to individual genes. Most genes within the same cluster share similar structures, functions, and expression patterns. The spatiotemporal expression patterns and expression levels between genes are highly coordinated, suggesting that the same cluster of genes are regulated as a unified whole and have a common regulatory mechanism. The clustered arrangement of genes is the basis for achieving spatiotemporal coordinated gene expression. It is a high-level organizational form of genetic information with strong evolutionary advantages. To reveal the basic laws of clustered gene expression regulation, overall and multi-gene interaction studies should be conducted from the levels of cis-acting elements, trans-acting factors, chromatin structure, etc. Elucidating these mechanisms is of great theoretical significance for studying cell differentiation and individual developmental programs.

In terms of value, it is an extremely important basic research field in life sciences. The Hox genes in these organisms function similarly, with those located near the 3’ end of the cluster being expressed in the anterior regions of the animal’s body, while those near the 5’ end are expressed in the posterior regions. In addition to this rule, Hox genes are also some of the reasons that they behave differently in different states. In the same gene cluster the expression of each gene is highly orderly and coordinated, and even in some gene clusters gene expression has obvious polarity that is, according to the location of genes on chromosomes, the order of arrangement shows the order of time and space. This high degree of coordination tonality is also a distinctive feature of clustered

gene expression regulation (Abbasi and Grzeschik, 2007). In chromatin domains, Hox genes share some regulatory elements, the parts restrict each other and are expressed in a coordinated manner. Complex gene clusters may form in different tissue cells or stages of development and differentiation. The structure of the mammalian Hox gene cluster is organized into four clusters, each situated on four different pairs of chromosomes (Oulion et al., 2010). Hox genes are divided into 13 groups. Except for a few genes, most of the Hox genes are transcribed in the same direction. Genes are organized into clusters, which facilitate the coordinated regulation of genes with related structural and functional roles. This organization represents one of the sophisticated mechanisms for the expression of genetic information (Larhammar et al., 2002).

3. Material and Methods

3.1. Evolution and variation of Hox genes

Hox genes play a crucial role in determining regional identity and body plan development across diverse organisms. These genes exhibit remarkable evolutionary conservation, yet their variation contributes significantly to morphological diversity (Pick and Heffer, 2012). The evolution of Hox genes involves several mechanisms, including changes in gene expression patterns, alterations in downstream target gene regulation, modifications in protein-coding sequences, and posttranscriptional regulation (Pick and Heffer, 2012). Interestingly, despite the importance of Hox genes in development, studies have shown that population-level variation in Hox gene expression does not always correlate with phenotypic differences. This suggests a complex relationship between Hox gene variation and morphological evolution. The evolution of Hox genes is also characterized by gene duplication events, resulting in paralogous genes that can perform overlapping functions (Greer et al., 2000). The evolutionary history of Hox genes is further complicated by the discovery of the ParaHox gene cluster, an ancient paralogue of the Hox cluster. This finding suggests that both clusters arose from a duplication of a ProtoHox gene cluster, potentially facilitating increased body complexity during the Cambrian explosion. Understanding the evolution and variation of Hox genes remains crucial for unraveling the mechanisms underlying developmental diversity and morphological innovations in the animal kingdom.

The evolution of homeobox genes in vertebrates occurred in two main stages: First, during the Precambrian period, before the divergence of arthropods and vertebrates, the ancestral homeobox genes underwent a series of linear duplications, resulting in the formation of a complex array of gene linkage groups. Concurrently, each gene experienced variations, including duplications or losses. Second, as chordates emerged, DNA replication and chromosome doubling led to the creation of additional linkage groups, accompanied by further variations such as gene loss and the elimination of certain linkage groups (Simakov et al., 2013). Genes located at corresponding positions within each gene cluster are referred to as paralogous genes. For instance, *HoxA5*, *HoxB5*, and *HoxC5a* represent a set of paralogous genes. Branchiostoma is a typical example of the original Hox gene cluster, containing only one gene cluster. Fruit flies have generated two gene clusters during evolution, while the Hox gene clusters of mammals have undergone duplication events. Mice and humans have 4 gene clusters, and fish have more. Although various organisms in the animal kingdom have significant differences in body structure, the arrangement of body structure is controlled by similar gene systems. For example, in branchiostoma and roundworms, homeobox gene clusters have similar functions and expression patterns. This suggests that the common ancestor of vertebrates and invertebrates (i.e., early multicellular metazoans) may have possessed certain homeobox genes in the earliest stages of animal evolution (Amores et al., 2004). Homeobox

genes and their unique expression patterns are one of the evolutionary characteristics shared by metazoans. In the history of animal evolution, the variation and evolution of vertebrate morphological structure may be closely related to the continuous evolution and duplication of homeobox genes over hundreds of millions of years. Phylogenetic trees can be used to analyze the evolution of Hox genes and verify their roles. If the Hox gene cluster undergoes two genome duplications, a tree containing four homologous genes should ideally display an (*AB*)(*CD*) topology. This would reflect the first genome duplication producing ancestral pairs *A/B* and *C/D*, followed by the second duplication separating these paired relationships. However, many phylogenetic analyses do not reveal a significant (*AB*)(*CD*) topology. Instead, they often exhibit a high proportion of an asymmetric (*A*)(*BCD*) structure, where one homologous gene consistently deviates earlier than the others. Many gene families have 2 or multiple homologous genes linked to Hox genes, suggesting that these genes are related to Hox genes. Because of the parallel evolution of cluster linkage, homologous genes may be composed of a complete produced by the duplication of chromosome segments (Amores et al., 1998).

4. Results and Discussion

4.1 Research status of fish Hox genes

Research has shown that all invertebrates (including lancelets) have only one Hox gene cluster in their genomes, while all vertebrates have multiple gene clusters (4 to 13). The latest research shows that there is only one Hox gene cluster in the genome of lancelets, which consists of 15 genes. The Hox gene structure and expression of lancelets have the commonality of vertebrate embryogenesis and are a transitional type from lower animals to higher animals (Simakov et al., 2013). The Hox gene family of teleost fish such as zebrafish, medaka, and pufferfish contains 7 gene subfamilies (Figure 1.2.). Each gene subfamily is located on the same chromosome (or linkage group), and their composition varies across different fish species. The latest research shows that carp has at least 8 gene clusters and Atlantic salmon has 13 gene clusters. As the fish genome project continues to be completed, more and more Hox genes may be discovered. By comparing the Hox genes of these closely related fish, it was found that the gene composition in each gene cluster was very different, indicating that some Hox gene clusters underwent deletion mutations after duplication. The deletion of Hox genes occurred independently in the evolution of different fish and was different from each other (Pick and Heffer, 2012; Naville et al., 2017). Atlantic salmon added 6 gene clusters due to chromosome doubling events and lost a complete gene cluster *HoxDb*. Comparing the Hox gene clusters of fish with those of mice, humans, etc. it was found that the evolution of fish genomes has a clear trend of acceleration, which is consistent with the evolutionary speed of fish morphological diversity and variability (Meyer and Mélaga-Trillo, 1999). The natural deletion of the *HoxC* gene cluster was recently discovered in elasmobranchs. Through high-throughput sequencing, it was demonstrated that all *HoxC* transcripts were missing in sharks (*Scyliorhinus canicula*) and rays (*Leucoraja erinacea*), and that all *HoxC* genes and two HoxC-related microRNAs were missing in the ray genome (Pascual-Anaya et al., 2013).

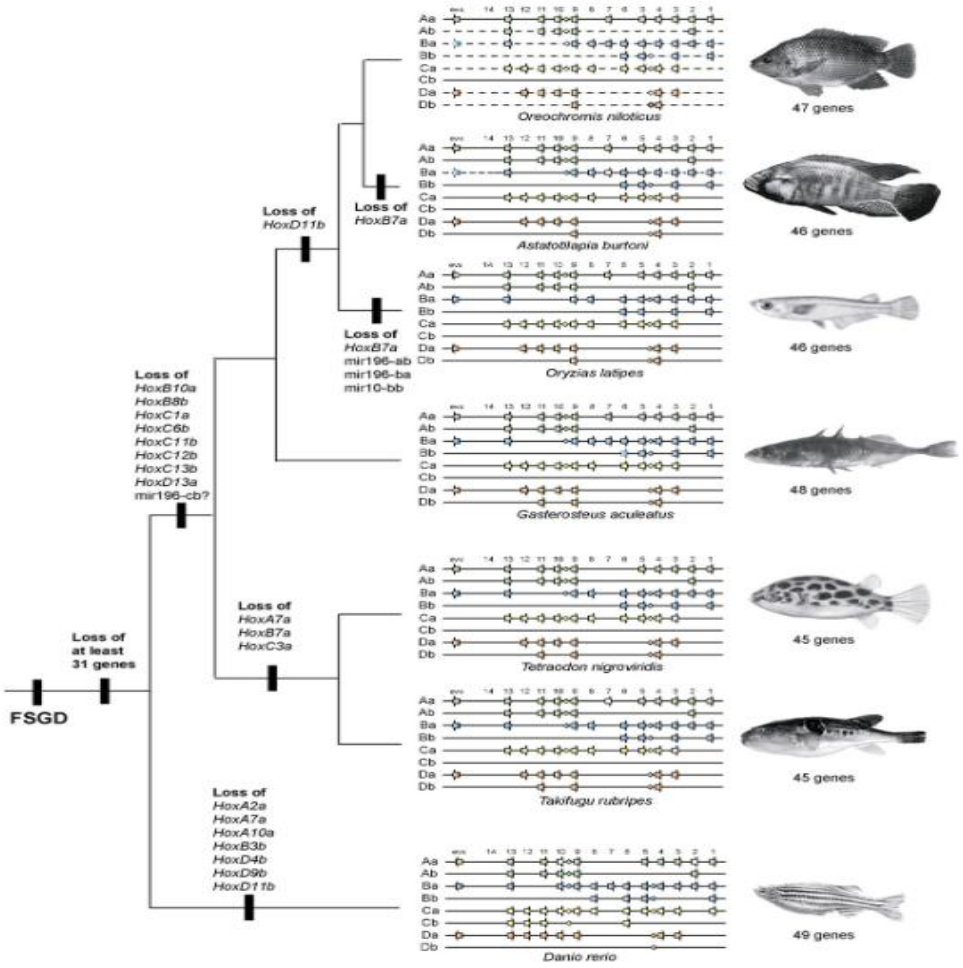

Figure 1.3. Phylogenetic tree of Hox genes family in teleost

Based on the structure of parallel evolution homologous regions in the genes of humans and other vertebrates, it is believed that multiple gene duplication events occurred in the early stages of the evolution of vertebrates from their invertebrate-like lancelet ancestors, and many hypotheses have been established. Pascual-Anaya et al. (2023) proposed the 2R hypothesis (two-round genome duplication), which believes that in vertebrates during the evolutionary process, the whole genome has been repeated at least twice. After the ancestors of teleosts separated from mammals but before they diverged significantly, they may have undergone a specific whole genome duplication, which resulted in the existence of two different copies of genes that are single copies in mammals today in teleosts. In 2011, Schneider et al. found through fossil research that the fin rays of finned fish are homologous to the wrist bones and tarsal bones of vertebrates. Based on morphological evidence, it is speculated that the limbs are the primitive form of the limbs of tetrapods, although the fin rays do not show any morphological homology with the limbs. They detected a functional copy of the Hoxd CsB enhancer, a key regulatory component that determines *HoxD* limb expression in quadrupeds, in fish such as zebrafish and rays. They also proved that both cis-acting factors and trans-acting factors are involved in the development of additional limbs in vertebrates. Some researchers sequenced the *Hoxd12* and *Hoxd13* genes of whales and other

mammals and found that whales and hippos, cattle, pigs and other artiodactyls have the same ancestor, and believed that the evolution of Hox genes is the result of natural selection.

Whole genome duplication caused by polyploidization has played a very important role in the evolution of vertebrates and is the optimal way to produce a large amount of genetic material. Compared with large module duplication events, the fluctuating small gene duplication pattern (including single genes or chromosome fragments) can be used to explain the changes in the number of parallel evolutionary homologous genes in vertebrates (Falco et al., 2009). Polyploidy is still widely present in different fish groups. For example, many species in Acipenser and Cypriniformes are polyploid. Cyprinidae are mostly diploid, triploid and tetraploid, while sturgeons are more likely to be tetraploid, octoploid or even 16ploid. This may be an important feature of fish evolution. Therefore, polyploidization events may have occurred repeatedly in the Cyprinidae, Cypriniformes and Schizothoracinae. Polyploidization causes the duplication of a large number of functional genes in the genome (Sung et al., 2008). The important functional genes that are duplicated are completely redundant in function. Whether the selection pressure of natural selection on these duplicate genes is eliminated and whether the evolution rate of duplicate genes is the same are all issues that need further study in polyploid vertebrates. Polyploidization is not only an increase in the number of genes, but also a corresponding change in gene function and expression, which is also a problem we hope to solve in the future.

5. Research prospects of Hox genes

In order to truly and comprehensively elucidate the general rules of clustered gene expression regulation, researchers have proposed research on the functional and structural evolution of Hox gene clusters. First, the genome structure map (such as gene clusters) evolves towards a more ordered state; second, with the emergence and evolution of vertebrates, the overall evolution of Hox gene clusters has changed significantly during genome duplication. After being stimulated or strengthened, can similar hypotheses be proposed for the evolution of other conservative gene clusters? The precise spatiotemporal expression regulation of clustered genes is the result of the interaction of multiple genes and multiple factors. In addition to the interaction of cis- and trans-acting factors, it also includes regulatory mechanisms such as chromatin level and multi-gene interaction. In-depth research on the regulation of clustered gene expression will help to gain a deeper understanding of the regulation of eukaryotic gene expression in vertebrates, and will help to explore the essence of life at a higher level and lay the foundation for the study of biological evolution. With the completion of the whole genome sequencing of a large number of organisms, the composition and evolution of the Hox gene family can be compared horizontally within and between species. Through microarray expression analysis, knock-out/RNAi function loss analysis, yeast two-hybrid protein interaction and other technical means, the Hox gene family has been studied in depth to better explain the formation of new genes, species differentiation and the maintenance of genetic system stability. The sequencing and assembly of the carp genome have been completed and continuously improved. The composition and evolution of the Hox gene family in the carp genome are about to be completed. The research results can be used to explain the contents of the carp genome sequencing, verify the sequencing results, and further explore the molecular mechanism of chromosome doubling and genome doubling during the evolution of carp (Bergsson, 2005; Chen et al., 2009).

6. Conclusion and future direction

In summary, the fish Hox gene family offers a captivating window into the evolutionary processes that have sculpted the genetic landscape of vertebrates. Through a combination of gene duplication events and subsequent diversification, teleosts have amassed a rich repertoire of Hox genes, each with its own unique role in coordinating embryonic development and patterning along the anterior-posterior axis. This process of gene duplication and diversification not only underscores the remarkable adaptive potential of teleosts but also highlights the intricate interplay between genetic redundancy and sub-functionalization. The phenomenon of genetic redundancy, wherein duplicated genes retain overlapping functions, provides teleosts with evolutionary flexibility and robustness against genetic perturbations. However, it also poses challenges for understanding the functional significance of individual Hox genes and their contributions to developmental processes. Through sub-functionalization, duplicated Hox genes may acquire novel roles or become specialized for distinct functions, thereby diversifying their functional repertoire and expanding the phenotypic diversity of teleosts. Moving forward, continued research efforts aimed at unraveling the regulatory networks governing teleost Hox gene expression will be crucial for gaining deeper insights into the mechanisms underlying vertebrate development and evolution. By deciphering the molecular cues and signaling pathways that govern Hox gene expression dynamics, researchers can uncover the regulatory logic that underpins the precise spatiotemporal patterning of tissues and organs during embryogenesis.

Furthermore, understanding the regulatory mechanisms that govern teleost Hox genes holds promise for applications in various fields, including evolutionary biology, developmental genetics, and regenerative medicine. By harnessing the insights gleaned from studying teleost Hox genes, researchers can develop novel strategies for manipulating developmental pathways, guiding tissue regeneration, and engineering complex biological structures. In essence, the teleost Hox gene family serves as a paradigmatic system for investigating the evolutionary dynamics of gene duplication, diversification, and functional specialization. As our understanding of teleost Hox genes continues to deepen, so too will our appreciation of the intricate genetic machinery that governs vertebrate development and evolution.

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