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Gene regulatory networks in morphological evolution: A developmental biology perspective

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Abstract. Morphological evolution is key to understanding biodiversity and adaptability, revealing how organisms' morphology changes during evolution. Gene regulatory networks (GRNs) play a central role in regulating gene expression and driving morphological traits. This review explores the intersection of morphological evolution and developmental biology, discussing GRNs' composition, developmental functions, and their role in shaping morphological diversity across species. Research shows GRNs are conserved yet flexible, allowing evolutionary adaptation in changing environments. The paper also highlights technological advancements, challenges, and future research directions, emphasizing systems biology and synthetic biology for deeper insights into morphological evolution.

Keywords: Morphological Evolution, Gene Regulatory Networks, Developmental Biology, Genomics, Systems Biology.

1. Introduction

Morphological evolution is key to understanding biodiversity and adaptive evolution, involving changes in organism form and structure over time. Developmental biology provides insights into these changes, particularly through the regulation of morphogenesis by gene regulatory networks (GRNs). GRNs, composed of genes, transcription factors, and signaling pathways, coordinate gene expression to control cell differentiation and organ formation. Recent studies have shown GRNs' essential role in morphological evolution, contributing to biodiversity. For example, changes in GRNs often accompany limb and organ evolution, studied through comparative and functional genomics [1]. This review summarizes the latest research on GRNs in morphological evolution, focusing on their structure, functions, and applications, along with case studies demonstrating their role in driving morphological diversity. It also explores technological advances, challenges, and future research directions, emphasizing potential applications of systems and synthetic biology [2]. This review aims to provide researchers with a comprehensive understanding of gene regulatory mechanisms in morphological evolution.

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2. Overview of morphological evolution and developmental biology

2.1. Definition and Research Significance of Morphological Evolution

Morphological evolution impacts organisms' adaptability and survival. Since Darwin, researchers have explored the causes and processes behind morphological changes through natural selection. Advances in genetics and molecular biology have deepened our understanding of these mechanisms, crucial for biodiversity and ecological adaptation [3]. Applications of morphological evolution span agriculture, medicine, and conservation, such as using genomic editing to develop more adaptable crops [4]. Developmental biology examines how organisms develop from a fertilized egg, with GRNs ensuring coordination of cell differentiation and organ formation [5]. Key signaling pathways like Wnt and Hedgehog regulate cell behavior and tissue structure [6], while epigenetics introduces gene expression regulation without altering DNA sequences [7].

2.2. Intersection of Morphological Evolution and Developmental Biology

Evolutionary developmental biology (Evo-Devo) explores how genetic changes influence morphological evolution through developmental programs. Evo-Devo research has shown that morphological changes are not merely the result of gene mutations but rather the outcome of developmental processes being re-regulated [8]. The role of GRNs in morphological evolution is a central issue in Evo-Devo. By comparing GRNs across different species, researchers can identify conserved mechanisms and variations driving morphological changes. The study of Hox gene clusters is a classic example, where Hox genes regulate developmental processes along the body axis, with slight differences in their expression patterns leading to significant morphological changes [9]. Evo-Devo also uncovers the mechanisms of morphological innovation, where new structures and traits emerge during evolution. Morphological innovation often occurs through the recombination and functional expansion of existing gene networks. For instance, the evolution of insect wings demonstrates how the multifunctionality of developmental genes promotes the formation of new traits [10]. Epigenetics also plays a crucial role in morphological evolution by regulating gene expression without changing the DNA sequence, providing mechanisms for rapid morphological diversity. Research shows that epigenetic regulation can affect adaptive changes over short timescales, especially in rapidly changing environments [11].

3. Gene Regulatory Networks

3.1. Composition of Gene Regulatory Networks

GRNs are composed of regulatory genes, transcription factors, and signaling pathways that collaborate to orchestrate gene expression, thus facilitating morphological evolution. Regulatory genes are the core elements of GRNs, influencing the expression of other genes by encoding proteins or RNA molecules. These genes are key drivers of morphological changes as they control critical steps in cell differentiation and tissue patterning. For example, Hox genes regulate body segmentation during development, maintaining evolutionary conservatism while allowing the emergence of novel morphological features in specific evolutionary lineages. Changes in the expression patterns of Hox gene clusters often result in significant morphological changes, such as the evolution of insect wings and the development of tetrapod limbs [12]. Transcription factors are key regulators in GRNs, modulating gene transcription by binding to specific DNA sequences. Changes in transcription factors can drive the emergence of new morphological traits. For instance, Pax6 is a well-studied transcription factor that plays a crucial role in eye development across different species, with changes in its expression and regulation being a major source of eye structure diversity [13]. Signaling pathways are responsible for transmitting information between cells to regulate gene expression during development. These pathways can amplify external and internal signals, ultimately influencing or even determining cell fate. Wnt, Hedgehog, and Notch are typical signaling pathways that play important roles in regulating growth, differentiation, and

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morphogenesis. For example, the Wnt signaling pathway influences cell fate determination by regulating β-catenin activity, playing a key role in limb development and central nervous system formation [14].

3.2. Role of Gene Regulatory Networks in Development

GRNs coordinate gene expression during development, ensuring proper cell differentiation and complex morphological structure formation, leading to adaptive and innovative evolutionary outcomes. In embryonic development, GRNs establish cell fate through morphogen gradients like BMP and Shh, guiding differentiation according to predetermined patterns, conserved across species [15]. During organogenesis, GRNs regulate organ formation, such as heart development, ensuring proper differentiation of cardiomyocytes. In regeneration, similar GRNs promote tissue repair [16]. GRNs also enable organisms to adapt to environmental changes by modulating gene expression, facilitating rapid responses and promoting evolutionary adaptation [17]. Dysregulation of GRNs can lead to congenital defects and diseases, such as cancers linked to Hedgehog pathway mutations, offering insights into disease mechanisms and therapeutic strategies [18].

3.3. Key Roles of Gene Regulatory Networks in Morphological Evolution

3.3.1. Gene Regulatory Networks in the Development and Evolution of Tetrapod Limbs

The evolution of tetrapod limbs is a crucial topic for understanding morphological diversity and complexity. The emergence and evolution of limbs were key adaptations that allowed vertebrates to transition from aquatic to terrestrial environments and serve as a paradigm of morphological innovation. During limb development, GRNs regulate the formation and differentiation of limbs, influencing their morphology. Hox genes are the main regulatory factors in limb development, controlling the characteristics of different body segments along the body axis. hey exert control over limb formation and morphology through their specific patterns of expression. For example, the HoxA and HoxD gene clusters play essential roles in the proximal-distal axis and anterior-posterior axis differentiation of limbs. Their regulatory networks not only determine the position and shape of the limbs but also influence the number and proportion of digits [8]. The Sonic Hedgehog (Shh) signaling pathway regulates cell differentiation during limb development by establishing morphogen gradients. The expression of Shh leads to the establishment of the anterior-posterior axis of the limb, determining the formation of the thumb and little finger. Variations in Shh signaling not only affect limb morphology but are also associated with developmental anomalies such as polydactyly. Research indicates that changes in the regulation of the Shh signaling pathway during tetrapod evolution have been key drivers of limb morphological diversity [9]. The diversity of tetrapod limbs exemplifies the adaptability of GRNs in evolution. Through comparative genomics, researchers have found that the conservation and flexibility of GRNs collectively drive limb morphological evolution. In amphibians, reptiles, birds, and mammals, even minor changes in these networks can result in significant morphological differences, reflecting the diverse ecological niches and functional demands on limbs across species [10].

3.3.2. Genetic Regulation of Beak Morphology Evolution in Birds

The diversity of avian beak morphology is a classic example of adaptive radiation and niche differentiation. Changes in beak morphology reflect different species' adaptations to feeding, nesting, and social behaviors. Genetic regulatory mechanisms play a critical role in this process. The Bone Morphogenetic Protein (BMP) signaling pathway: The expression level of BMP4 is directly correlated with beak width and thickness. Experiments have shown that by manipulating BMP4 expression in chicken embryos, the beak's morphology can be remodeled to display features characteristic of different finch species. This indicates that the BMP signaling pathway is a fundamental genetic basis for the diversity of beak morphology in birds [11]. Calmodulin: This regulatory protein is involved in the regulation of beak length. Research has demonstrated that Calmodulin influences beak length in Darwin's finches by regulating bone growth. Through gene expression regulation, Calmodulin has played a significant role in the adaptive evolution of beak morphology, enabling different species to

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better adapt to their respective ecological environments [12]. The evolution of avian beaks showcases how GRNs can produce significant morphological changes in a short period through natural selection. The adaptive changes in these regulatory networks allow birds to respond rapidly to environmental changes, especially in ecosystems where resource distribution is uneven, or competition is intense. By studying the genetic basis of beak morphology in birds, people can gain deeper insights into the mechanisms of morphological evolution and the role of GRNs in adaptive radiation [13].

3.3.3. Analysis of Gene Networks in Flower Organ Morphological Diversity

The diversity of flower organs is a key factor in the successful adaptation of angiosperms to diverse environments. Changes in flower morphology not only promote reproductive success in plants but also drive coevolution with pollinators. In this process, GRNs play a crucial role in the formation and diversification of flower organs. The ABC model: The ABC model of flower organ development forms the basis for studying the morphological diversity of flowers. This model involves A, B, and C class genes controlling the development of sepals, petals, stamens, and carpels. These genes interact to form specific expression patterns that determine the type and arrangement of flower organs. Studies have shown that changes in the expression patterns of ABC genes are significant mechanisms driving the morphological diversity of flowers [14]. Gene duplication and functional diversification: Gene duplication events increase genomic complexity and provide opportunities for the evolution of new functions. Many plant lineages have undergone multiple whole-genome duplication events during evolution, promoting the diversification of flower organs. For example, the expansion and functional diversification of the MADS-box gene family has played a crucial role in the diversification of flower morphology. These genes achieve morphological innovation in different flower organs through various expression patterns and functional combinations [15]. The role of non-coding RNAs: Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play essential roles in regulating gene expression and flower development. miRNAs play a role in regulating the expression of transcription factors, which in turn influence the development and morphological changes of flower organs. Research has found that miRNA regulation of MADS-box genes is a significant mechanism for flower morphology variation, highlighting the regulatory role of non-coding RNAs in GRNs [16].

4. Technological advances and research methods

4.1. Genomics and Transcriptomics

Genomics and transcriptomics are crucial tools for understanding the role of GRNs in morphological evolution, enabling comprehensive analysis of genomic structure and function. In recent years, rapid advancements in genomic sequencing technologies have significantly reduced sequencing costs and increased sequencing speeds. This has allowed researchers to sequence genomes across various species, revealing the complexity of genomes and their role in morphological evolution. For instance, by sequencing the genomes of salamanders and other amphibians, scientists have identified specific gene groups associated with limb regeneration, uncovering the genetic basis of regenerative capabilities [17]. Comparative genomics, which involves comparing the genomic structures and functions of different species, is instrumental in uncovering the genetic basis of morphological differences. This technology plays an important role in exploring the conservation and diversity of GRNs. By comparing the genomes of different species, researchers can identify evolutionary changes in specific gene regulatory elements. For example, studies have shown that differences in certain GRNs between fruit flies and mosquitoes directly affect their adaptability to different ecological environments [18]. Transcriptomics, through RNA sequencing (RNA-Seq), provides a gene expression profile of cells at specific developmental stages or under specific conditions. This technology allows scientists to observe the dynamic changes in gene expression and identify key regulatory genes at different developmental stages or environmental conditions. Transcriptomic analysis helps researchers better understand the spatiotemporal dynamics of GRNs during morphological development, which is crucial for unraveling complex biological phenomena such as limb regeneration and organ development [19].

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4.2. CRISPR-Cas9 and Other Gene Editing Technologies

CRISPR-Cas9 gene editing technology has revolutionized research in morphological evolution by providing the ability to precisely intervene in and validate the functions of GRNs. CRISPR-Cas9, derived from the bacterial immune system, utilizes the Cas9 nuclease, guided by RNA, to precisely cut target DNA sequences in the genome. This technology can be used to knock out, insert, or replace genes, enabling the study of specific genes' roles in developmental processes [20]. CRISPR-Cas9 has been widely applied to explore GRNs in morphological evolution. Through gene editing, researchers can generate specific gene mutants to directly observe these genes' roles in morphological development. For example, by knocking out specific regulatory genes in zebrafish using CRISPR technology, scientists have clarified their crucial roles in fin morphology and function development [21]. Although CRISPR-Cas9 technology offers unprecedented research possibilities, its application also presents ethical and technical challenges. Gene editing may lead to off-target effects on non-target genes, resulting in unintended genetic variations. When applying these technologies to mammals and humans, particular caution is required to avoid potential ethical issues and adverse consequences. These challenges prompt researchers to pay more attention to safety and ethical considerations during the development and application of this technology [22].

4.3. Importance of Model Organisms in Gene Regulation Research

Model organisms are key tools for studying the functions and evolution of GRNs. These organisms are ideal subjects for research due to their clear genetic backgrounds, rapid reproduction rates, and high experimental controllability. Commonly used model organisms include fruit flies (Drosophila melanogaster), zebrafish (Danio rerio), mice (Mus musculus), and Arabidopsis thaliana, which occupy significant positions in genetic and developmental biology research. Selecting an appropriate model organism involves considering the operability of its genome, its life cycle, and its similarity to the target species. Fruit flies and zebrafish have become the primary choices for GRN research, owing to their widespread use in gene function studies [23]. By using model organisms, scientists can conduct genetic manipulations to elucidate gene functions. For example, through gene knockout and overexpression experiments in fruit flies, researchers have revealed the roles of Hox genes in segment formation and regulation. In zebrafish, researchers have used CRISPR technology to edit genes to study regulatory genes' roles in embryonic development, providing new perspectives for understanding the molecular mechanisms underlying morphological changes [24]. Model organisms are not only used to elucidate gene functions but also play important roles in evolutionary biology. By comparing the genomes and developmental processes of different model organisms, researchers can identify patterns of conservation and variation in GRNs during evolution. For instance, the comparison of adaptive differences between fruit flies and mosquitoes in various environments has revealed how GRNs drive the development of morphological diversity [25].

5. Current challenges and future prospects

5.1. Major Challenges in Morphological Evolution Research

The complexity and diversity of GRNs present core challenges in morphological evolution research. GRNs consist of numerous interacting genes, transcription factors, and signaling pathways, and their complexity makes it difficult to study the function of individual genes in isolation. Additionally, the diversity of these networks varies across different species and even within different tissues of the same species, further complicating research efforts. The complexity of GRNs is reflected in their highly dynamic and non-linear regulatory relationships. Multiple regulatory elements can influence the same gene differently under various environmental conditions, making it challenging for researchers to predict the final impact of gene mutations or environmental changes on morphology. For instance, limb development involves multiple layers of regulatory networks, where alterations in any single layer can lead to significant morphological changes [26].

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The diversity of regulatory networks is key to understanding morphological differences between species. However, this diversity also implies that regulatory mechanisms identified in one species may not be directly applicable to others. The plasticity and adaptability of GRNs indicate that a single gene can fulfill multiple functions in various evolutionary contexts, thereby contributing to the complexity of research [27].

5.2. Future Research Directions

Future research should integrate the strengths of systems biology and synthetic biology to better understand the dynamic characteristics and evolutionary potential of GRNs. Systems biology aims to model and analyze the dynamic behavior of biological networks through quantitative models. By integrating genomic, transcriptomic, and proteomic data, researchers can construct precise network models to predict the responses of GRNs under different conditions. This approach can reveal underlying patterns and principles in morphological evolution [28].

Synthetic biology offers new avenues for exploring gene functions by designing and constructing new biological components, devices, and systems. Through synthetic biology, researchers can synthesize and test hypothetical GRNs in the laboratory to validate their roles in morphological evolution. This approach not only aids in understanding the origins of natural networks but also provides a new platform for biotechnological applications [29].

Technological advancements are crucial for overcoming current challenges and driving research in morphological evolution. High-throughput sequencing technologies, single-cell analysis techniques, and artificial intelligence and machine learning tools will further accelerate GRN research, uncovering more secrets of morphological evolution.

6. Conclusion

In this review, the central role of gene regulatory networks in morphological evolution and developmental biology has been examined. These networks, through precise regulation of gene expression, not only shape the morphological characteristics of organisms but also drive the evolution of biodiversity and adaptability. Through specific cases such as tetrapod limb development, avian beak morphology changes, and the diversification of plant flower organs, the conservation and flexibility of GRNs in facilitating morphological innovation in response to changing environments have been demonstrated.

Technological advancements, such as genomics, transcriptomics, and CRISPR-Cas9 gene editing, have provided powerful tools for unraveling these complex networks. However, the complexity and diversity of GRNs remain major challenges, particularly in understanding their dynamic changes across different species and developmental stages.

Future research needs to leverage the combined strengths of systems biology and synthetic biology to construct and simulate biological models, offering a more comprehensive understanding of the mechanisms by which GRNs drive morphological evolution. This interdisciplinary collaboration will advance both biotechnology and ecological conservation, providing innovative solutions. In this process, innovative experimental designs and cutting-edge technologies will be crucial in unlocking the complexities of life. This review provides researchers with a comprehensive perspective on morphological evolution and gene regulatory networks, with the hope of inspiring further exploration and discovery. It aims to deepen our understanding of life evolution and provide new approaches to address global ecological challenges.

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