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Integrative Transcriptomic Profiling Reveals Core Genetic Networks and Hub Genes Governing Tail Regeneration in *Clinotarsus curtipes*

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ABSTRACT

Regeneration is a complex biological process involving coordinated cellular and molecular mechanisms that enable organisms to restore lost tissues. Amphibian larval stages provide a powerful model for studying regenerative biology due to their remarkable capacity for complete tissue restoration. In the present study, we investigated the molecular basis of tail regeneration in *Clinotarsus curtipes*, an endemic amphibian species of the Western Ghats. Whole-transcriptome RNA sequencing (RNA-seq) was performed at four distinct time points: 0, 5, 15, and 30 days post-amputation (dpa), representing key regenerative phases. De novo transcriptome assembly followed by differential expression analysis identified 281 significantly differentially expressed transcripts across stages. A conserved set of 48 core genes (10.8%) was found to be consistently expressed throughout regeneration. Protein–protein interaction (PPI) network analysis revealed key hub genes, including *FOS*, *EGR1*, *NR4A1*, *JUND*, *DUSP5*, and *CIRBP*, which are primarily associated with transcriptional regulation, stress response, and cellular proliferation. Functional enrichment analysis indicated significant involvement of MAPK, Wnt, and TGF-β signaling pathways. These findings provide novel insights into the coordinated gene regulatory networks underlying amphibian regeneration and establish *C. curtipes* as a valuable non-model organism for regenerative biology research.

1. Introduction

Regeneration is a fundamental biological phenomenon through which organisms replace or restore damaged or lost tissues. While this ability is highly restricted in mammals, several lower vertebrates, particularly amphibians, exhibit remarkable regenerative capacities (Tanaka and Reddien, 2011). Amphibians are capable of

regenerating complex structures such as limbs, tails, and even portions of the central nervous system through a process known as epimorphic regeneration, which involves wound healing, blastema formation, cellular proliferation, and differentiation (Brockes and Kumar, 2008).

Among amphibians, anuran larvae (tadpoles) exhibit a unique developmental window during which regeneration occurs efficiently. This regenerative capacity declines significantly after metamorphosis, making larval stages ideal for studying the molecular mechanisms governing tissue regeneration (Beck *et al.,* 2003). The regenerative process involves dynamic interactions between cellular signaling pathways, extracellular matrix remodeling, and transcriptional regulation.

Clinotarsus curtipes, an endemic frog species of the Western Ghats, demonstrates a remarkable ability to regenerate its tail within approximately 30 days following amputation . The regenerative process in this species involves well-defined stages, including wound healing, blastema formation, tissue differentiation, and structural restoration. Previous studies have highlighted the roles of reactive oxygen species (ROS), extracellular matrix components such as collagen and elastin, and cellular proliferation in facilitating regeneration (Sithijameela and Ramesh Kumar, 2024; Das and Peerzade, 2018).

Despite these advances, the transcriptional regulation underlying tail regeneration in *C. curtipes* remains poorly understood. High-throughput RNA sequencing (RNA-seq) has emerged as a powerful tool for elucidating global gene expression patterns during regeneration (Sánchez Alvarado and Tsonis, 2006). Studies in model organisms such as *Xenopus laevis* and axolotls have identified key signaling pathways, including Wnt, Notch, and MAPK, that regulate regeneration (Gemberling *et al.,* 2015). However, non-model endemic species may exhibit distinct gene regulatory networks.

Therefore, the present study aims to perform an integrative transcriptomic analysis of tail regeneration in *Clinotarsus curtipes* to identify differentially expressed genes, core genetic networks, and hub genes that govern regeneration.

2. Materials and Methods

2.1 Experimental Design and Sample Collection

Tadpoles of *Clinotarsus curtipes* were collected from their natural habitat in the

Western Ghats and maintained under controlled laboratory conditions. Tail amputation was performed under sterile conditions, and regenerating tissues were collected at four time points:

0 dpa (control), 5 dpa (blastema stage), 15 dpa (proliferative stage), 30 dpa (maturation stage). These stages represent critical phases of regeneration.

2.2 RNA Isolation and Quality Assessment

Total RNA was extracted using the TRIzol method following standard protocols. RNA concentration and purity were assessed using a NanoDrop spectrophotometer, and RNA integrity was confirmed using an Agilent 2100 Bioanalyzer. Only samples with high RNA integrity were used for sequencing.

2.3 Library Preparation and RNA Sequencing

RNA libraries were prepared using the Illumina TruSeq RNA Library Preparation Kit. mRNA was isolated using poly-T oligo-attached magnetic beads, fragmented, and converted into cDNA. Libraries were sequenced on the Illumina HiSeq platform to generate paired-end reads.

2.4 De Novo Transcriptome Assembly and Annotation

Due to the absence of a reference genome for *C. curtipes*, de novo transcriptome assembly was performed using Trinity. The assembled transcripts were annotated against public databases, including NCBI non-redundant (Nr) and Swiss-Prot, to assign functional identities.

2.5 Differential Gene Expression Analysis

Gene expression levels were quantified using Transcripts Per Million (TPM). Differential expression analysis between control and regenerating stages was performed using DESeq2. Genes with $|\log_2 \text{fold change}| \geq 1$ and adjusted p-value < 0.05 were considered significantly differentially expressed.

2.6 Functional Enrichment Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted to determine the biological functions and pathways associated with differentially expressed genes.

2.7 Protein–Protein Interaction Network and Hub Gene Analysis

Common differentially expressed genes across all stages were mapped to the STRING database (v11.5) to construct a protein–protein interaction (PPI) network. The

network was visualized using Cytoscape, and hub genes were identified using the cytoHubba plugin based on degree centrality and betweenness measures.

3. Results

3.1 Global Transcriptomic Changes During Regeneration

RNA-seq analysis revealed extensive transcriptional changes during tail regeneration in *Clinotarsus curtipes*. A total of 281 genes were identified as significantly differentially expressed across the four stages.

During the early phase (5 dpa), genes associated with metabolic activation and oxidative stress response, including *ACCS* and *COX6B1*, were significantly upregulated. This suggests rapid cellular reprogramming immediately following injury.

In the proliferative phase (15 dpa), transcription factors such as *EGR1* and *FOS* showed high expression levels, indicating active cell proliferation and blastema formation.

During the maturation phase (30 dpa), genes involved in structural organization and tissue stabilization, such as *ANKR2*, were upregulated, reflecting tissue differentiation and functional restoration.

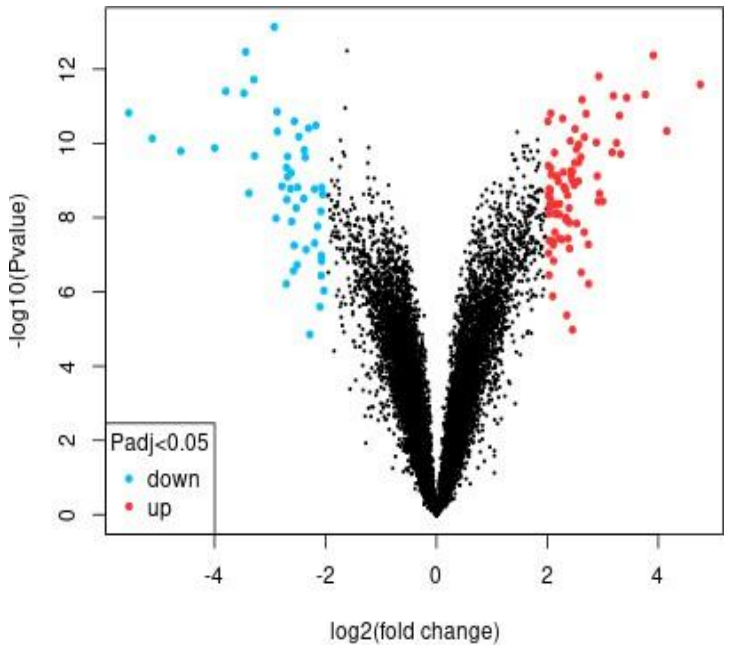

Figure 1. Volcano plot representing differential gene expression during tail regeneration in *Clinotarsus curtipes*. The x-axis shows $\log_2$ fold change, and the y-axis represents $-\log_{10}$ adjusted p-values. Red dots indicate significantly upregulated genes, while blue dots represent downregulated genes (Padj < 0.05), highlighting dynamic transcriptional changes across regenerative stages.

3.2 Identification of Core Regeneration Gene Network

To identify genes consistently involved in regeneration, a Venn analysis was performed across all time points. A total of 48 genes (10.8%) were found to be commonly expressed across all stages.

Among these, *NR4A1* and *MUC19* were consistently upregulated, while *TGM3* was downregulated throughout regeneration, suggesting its role as a negative regulator.

The persistence of these genes indicates a conserved genetic program essential for sustaining regeneration.

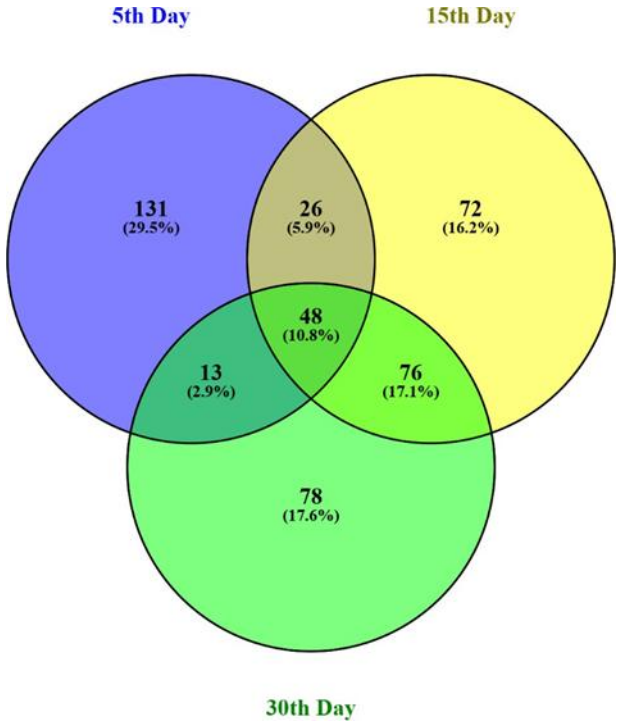

Figure 2. Venn diagram showing the overlap of differentially expressed genes (DEGs) across three regenerative stages (5, 15, and 30 days post-amputation) in *Clinotarsus curtipes*. A total of 48

genes were commonly expressed across all stages, representing a conserved core genetic network involved in tail regeneration.

3.3 Hub Gene Identification and Network Analysis

PPI network analysis identified six major hub genes:

- *FOS*
- *EGR1*
- *NR4A1*
- *JUND*
- *DUSP5*
- *CIRBP*

These genes exhibited high connectivity and are known to regulate transcriptional responses, stress signaling, and cellular proliferation.

The dominance of immediate-early genes (*FOS*, *EGR1*) suggests that regeneration is initiated through rapid transcriptional activation.

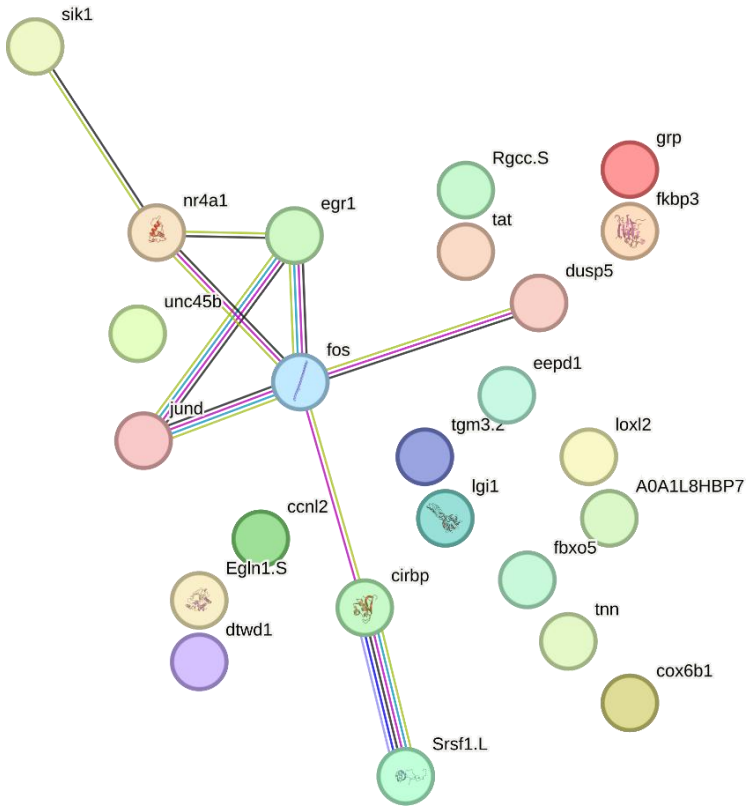

Figure 3. Protein–protein interaction (PPI) network of core differentially expressed genes during tail regeneration in Clinotarsus curtipes.

The network was constructed using the STRING database and visualized in Cytoscape. Nodes represent proteins, and edges represent interactions. Key hub genes, including FOS, EGR1, NR4A1, JUND, DUSP5, and CIRBP, exhibit high connectivity, indicating their central role in regulating transcriptional responses and cellular processes during regeneration.

3.4 Functional Enrichment Analysis

GO enrichment analysis revealed that differentially expressed genes were primarily involved in:

- Cell proliferation
- Extracellular matrix organization
- Response to oxidative stress

KEGG pathway analysis showed significant enrichment in:

- MAPK signaling pathway
- Wnt signaling pathway
- TGF-β signaling pathway

These pathways are known to regulate cellular growth, differentiation, and tissue remodeling during regeneration.

4. Discussion

The present study provides a comprehensive transcriptomic framework for tail regeneration in *Clinotarsus curtipes*. The identification of 281 differentially expressed genes demonstrates a highly dynamic gene expression profile during regeneration.

The early activation of stress-responsive genes supports the role of reactive oxygen species (ROS) in initiating regeneration, as previously reported. ROS-mediated signaling likely triggers downstream transcriptional events necessary for blastema formation.

The identification of a conserved 48-gene core network suggests the existence of a stable genetic program essential for regeneration. Hub genes such as *FOS* and *EGR1* belong to immediate-early genes, which act as key regulators in response to injury and stress.

The enrichment of MAPK and Wnt signaling pathways highlights their conserved roles in regulating regeneration across species (Gemberling *et al.,* 2015). These pathways coordinate cellular proliferation, differentiation, and tissue organization.

Overall, this study provides novel insights into the molecular mechanisms underlying amphibian regeneration and emphasizes the potential of *C. curtipes* as a model organism.

5. Conclusion

This study reveals a coordinated transcriptomic landscape governing tail regeneration in *Clinotarsus curtipes*. The identification of core genetic networks and hub genes provides new insights into regenerative biology and offers potential targets for therapeutic applications in regenerative medicine.

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