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# Gene regulation during development: The roles of the transcription factors *xbp1*, *creb3l1* and *creb3l2* in axial mesoderm differentiation

## Introduction

By dividing and differentiating, a single totipotent cell – a zygote – can develop into a complex multicellular organism such as a human being. Transcription factors play an important role in this development. They are proteins that control the rate of transcription of genetic information from DNA to mRNA by binding to specific DNA sequences.

In this project, the focus lies on the development of the axial mesoderm, a type of mesoderm that lies along the central axis under the neural tube and gives rise to two tissues called the hatching gland and the notochord. To determine the genetic elements and pathways that lead to the structural changes and distinct characteristics of the axial mesoderm in early development, three transcription factors (*xbp1*, *creb3l1* and *creb3l2*) and their regulatory pathways were investigated. The zebrafish (*Danio rerio*) served as the model system.

## Methods

To investigate the role and function of the transcription factors, I edited the genome of zebrafish zygotes using CRISPR/Cas9 so that the fish no longer had a functional version of the transcription factor gene. To detect the influence of the missing transcription factor, I compared the axial mesoderm of normal zebrafish to that of the resulting knockout mutants.

To reveal the biological pathways in which the transcription factors were involved, the expression of specific genes was measured. Thus, changes in gene activity due to the missing transcription factor could be detected.

In addition to the molecular methods, I developed and used a new computational approach to predict biological pathways in which the transcription factors could be involved, and thus, I created a list of candidate genes, whose activity should be measured.

## Results

The investigation of zebrafish mutants with a knockout of the gene *xbp1* showed that the hatching gland cells, which arise from the axial mesoderm, had smaller secretory vesicles. The mutants also showed a delay or complete malfunction of hatching ability compared to controls.

Changes in gene expression due to the knockout indicated that the genes *calr3b*, *rab1ab*, *rnaseka, lrrc59*, *ssr4*, *pdia6*, *p4hb* and *hdlbpa* were regulated by *xbp1*.

Analysis of the expression of specific genes in *creb3l1*; *creb3l2* double mutants revealed that the genes *col2a1a*, *loxl1*, *p3h3*, *ggcx* and *p4hb* were downstream of at least one of the two transcription factors.

## Discussion

Overall, the experimental approach worked well for identifying transcription factor function and downstream genes. The successful prediction of regulatory pathways using a unique computational approach shows new possibilities. However, not all experiments provided conclusive results because the generated mutants were mosaic, which means that the knockout was only partially successful. Therefore, the experiments need to be repeated with stable mutants to draw clear conclusions.

## Conclusion

This project revealed new pathways and genes that are regulated by the transcription factors, which provides new knowledge about the development of the axial mesoderm.

The *xbp1*-mediated upregulation of eight genes was demonstrated. The requirement of *xbp1* for proper hatching gland development became apparent through the malformation of secretory vesicles in the hatching gland cells of *xbp1* knockout mutants.

The regulatory influence of *creb3l1* and/or *creb3l2* on four collagen biosynthesis-related genes was discovered, suggesting an important role for the *creb3l* genes in collagen production.