Circular RNA Expression in Retinal Tissue of Developing Chicken (*Gallus gallus*) Embryos

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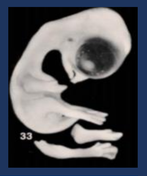

Introduction

- Circular RNA (circRNA) is a highly stable, noncoding RNA molecule.
- circRNA is formed through backsplicing—causing the linear RNA to fold back on itself, creating a circular shape.
- Functions of circRNA include sponging microRNA[1], influencing stem cell differentiation[2], and influencing development of bodily structures[3].
- circRNA has been found in large amounts within the nervous system—including the retina[4].
- A previous study found circFat3 and circRERE to be expressed in human body tissues[5].
- The developmental expression patterns of circFat3 and circRERE are largely unknown.

Objectives

- **To determine the presence of circFat3 and circRERE in developing chicken retinas as well as developing zebrafish embryos.**
- **To determine the expression patterns of circFat3 and circRERE within the developing chicken retina.**

Methods

- Chicken retinas were dissected from embryonic chickens at stages 23, 27, 30, 33, 35, and 40[6]. Stages 27 and 33 can be seen above.
- Zebrafish embryos at 24 hours post fertilization (hpf), 72 hpf, and 5 days post fertilization were obtained.
- RNA was extracted from the chicken retinas using Trizol (Invitrogen). RNA was extracted from zebrafish embryos using GITC-containing buffer.
- cDNA was synthesized through reverse transcription.
- Outward-facing primers were designed to amplify circFat3 and circRERE using the UCSC Genome Browser and Primer3.
- PCR was used to determine the presence of circFat3 and circRERE in both the embryonic chicken retinas and the zebrafish embryos.
- PCR products were sequenced to determine amplification of the correct circRNAs.
- Quantitative Real-Time PCR was performed to determine the expression patterns of circFat3 and circRERE as well as their parent linear forms—Fat3 and RERE—within the developing chicken retinas across stages 23, 27, 30, 33, 35, and 40[6]. GAPDH was used as a control gene.
- ΔΔ CT values of circFat3, circRERE, Fat3, and RERE across the six developmental stages were calculated along with GAPDH CT values. All ΔΔ CT values were compared using a one-way ANOVA with GraphPad Prism version 8.

All research activities in this study were approved by the York College of Pennsylvania Institutional Animal Care and Use Committee (IACUC).

Overall Conclusions

1. **circFat3 and circRERE are both expressed in the developing chicken retina. → Future studies should aim to determine which retinal cells are expressing the circRNA.**
2. **circFat3 and circRERE exhibit expression patterns that are different from their linear forms.**
3. **circFat3 is expressed in zebrafish embryos. → Future studies should aim to determine which zebrafish organs are expressing circFat3.**

Results

Outward-Facing Primer Diagram

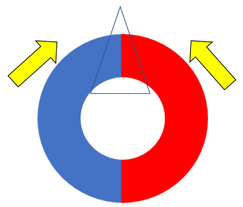

Figure 1. Diagram indicating how outward-facing primers (yellow arrows) amplify the connection points of exons comprising the circRNA. Blue and red halves both represent individual exons that create the circRNA. Triangle represents the connection of the two exons.

PCR Analysis

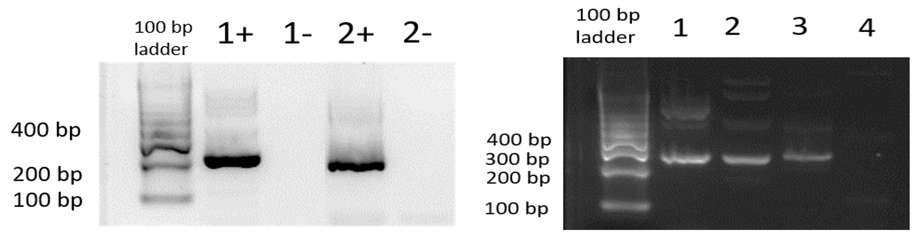

Figure 2. 1.5% agarose gels containing PCR products of amplification of circFat3 (214 bp) and circRERE (180 bp) from cDNA synthesized from embryonic chicken (*Gallus gallus*) retinas (Left Image) and circFat3 (238 bp) from zebrafish (*Danio rerio*) cDNA (Right Image). Numbers at the top of the lanes indicate circRNA primers used (1+, 1-=circFat3 primers; 2+, 2-=circRERE primers) (Left Image). Right image shows three developmental timepoints (1= 24 hours post fertilization; 2= 72 hours post fertilization; 3= 5 days post fertilization; 4= reverse transcriptase negative control). Negative symbols indicate cDNA negative control containing the absence of reverse transcriptase. A 100 base pair ladder was used to determine base pair sizes.

Sequencing

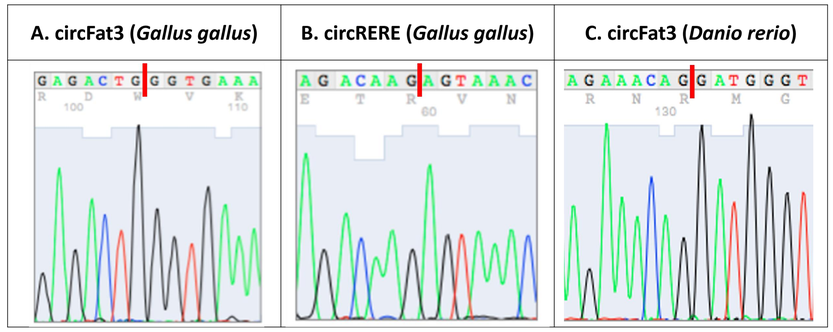

Figure 3. Sequencing results of PCR products showing connection points—shown with red rectangles—of the exons comprising circFat3 and circRERE in chicken embryos (*Gallus gallus)* and circFat3 in zebrafish embryos (*Danio rerio*).

Quantitative Real-Time PCR

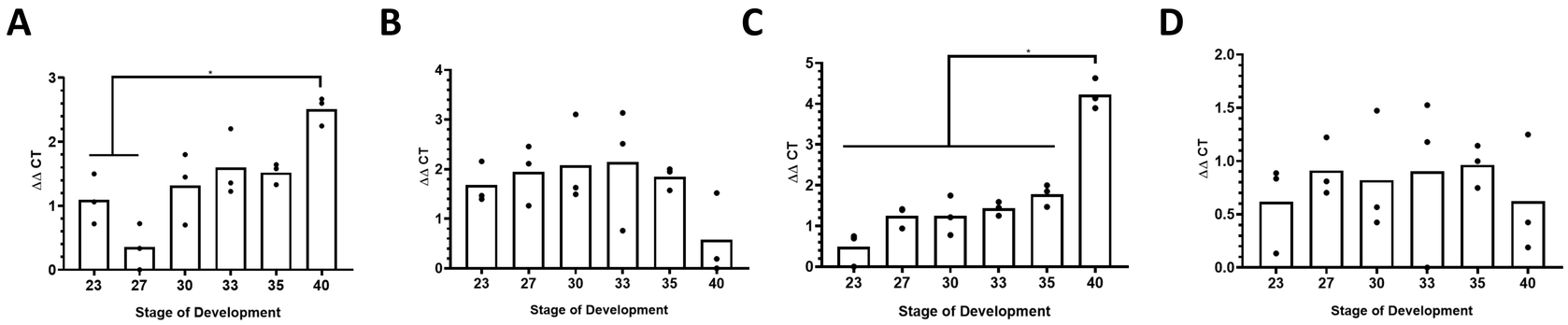

Figure 4. ΔΔCT data generated from Quantitative Real-Time PCR of circFat3 **(Panel A)**, Fat3 **(Panel B)**, circRERE **(Panel C)**, and RERE **(Panel D)** performed across six stages of retinal development of chicken (*Gallus gallus*) embryos. Graphed data indicates average value of triplicate retinal samples at each developmental stage (n=3). ΔΔCT data was calculated by subtracting CT values from GAPDH CT values. The highest ΔCT was then set to zero, and all other ΔCT values were subtracted from the highest ΔCT value. Asterisks indicate significant difference of expression levels at various stages based on a Tukey Multiple Comparisons Test ($P<0.05$). Significance among the developmental stages was determined with a one-way ANOVA: **(Panel A)=** ($F(5,12)=9.284$, $P=0.0008$); **(Panel B)=** ($F(5,12)=1.685$, $P=0.2126$); **(Panel C)=** ($F(5,12)=41.27$, $P<0.0001$); **(Panel D)=** ($F(5,12)=0.2643$, $P=0.9241$).

Literature Cited

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