Molecular cloning, Tissue distribution and Functional studies of Asprosin in Fish Model

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Fasting-induced hormones are essential for growth, especially in commercial fish species. As a novel feeding hormone, it is imperative to find the regulatory targets of Asprosin in goldfish. In this study, we compared Asprosin structure between goldfish and humans, distribution of Asprosin in peripheral and brain tissues, and possible regulatory targets of Asprosin. Molecular cloning demonstrates that Asprosin is highly conserved in fish species and the 3D protein structure of Asprosin is highly comparable between goldfish and humans. Our experiment presents evidence that Asprosin is ubiquitously expressed in the liver and highly expressed in the different brain regions. Time course study in goldfish hepatocytes demonstrates that Asprosin stimulates Spexin, Adiponectin and Phoenixin mRNA expression. There is a constant stimulation of Spexin by human Asprosin, however, the effect of Asprosin on Adiponectin and Phoenixin decrease with time. We also found evidence of Asprosin autoregulation, and the effect is clearly observed at the 6-hour time point. In the future, more studies needs to be carried out to find the regulators of Asprosin and the signaling mechanism that controls Asprosin regulation.

Introduction

- Fasting-induced glucogenic hormone
- Discovered by Romere et.al in 2016
- Adipokine produced by white adipose tissues
- Has a precursor – Fibrillin 1 (FBN1)
- Cleaved from FBN1 by furin mediated cleavage at the C-terminus
- Encodes 2 exons – exons 65 and exon 66 in humans
- Has receptor - olfactory receptor OLFR734 (G-protein coupled receptor)
- Known to directly act on liver and appetite– regulating neurons in the brain
- Induces hepatic glucose production by using cAMP as a second messenger
- Promotes insulin resistance in skeletal muscles
- Play a role in a number of cardiometabolic diseases, type-2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS), non-alcoholic fatty liver disese (NAFLD), heart disease

Objectives
- In silico protein modeling of goldfish Asprosin
- Distribution of Asprosin in goldfish
- Regulatory targets of Asprosin

Molecular Cloning

Step 1

Designed primers with restriction sites → High Fidelity PCR → Gel purification → Ligation and transformation into DH5α cells → Select strains - Blue white screening → Sequencing

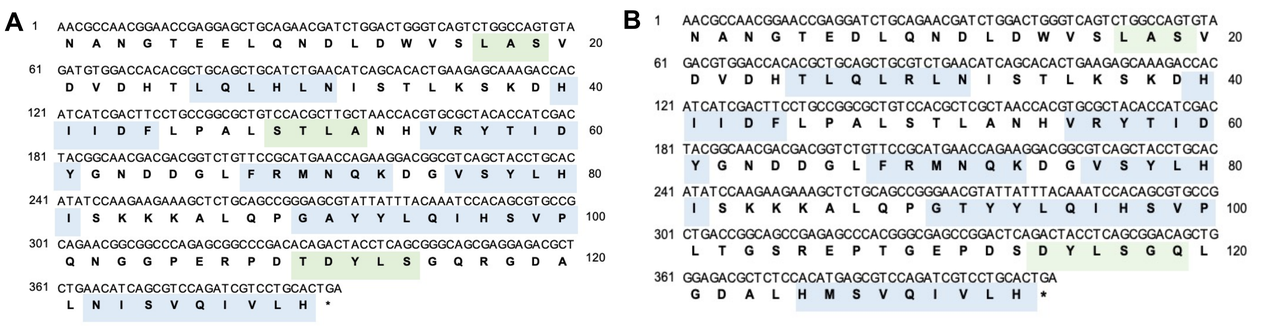

Fig. 1 **Goldfish FBN1a cDNA (A) and FBN1b cDNA (B) sequence corresponding to the Asprosin CDS.** Sequences shaded green represent helices, blue represent beta-pleated sheets, and the uncolored regions are coils.

Step 2

Multiple sequence Alignment → Phylogenetic Analysis → Protein modeling

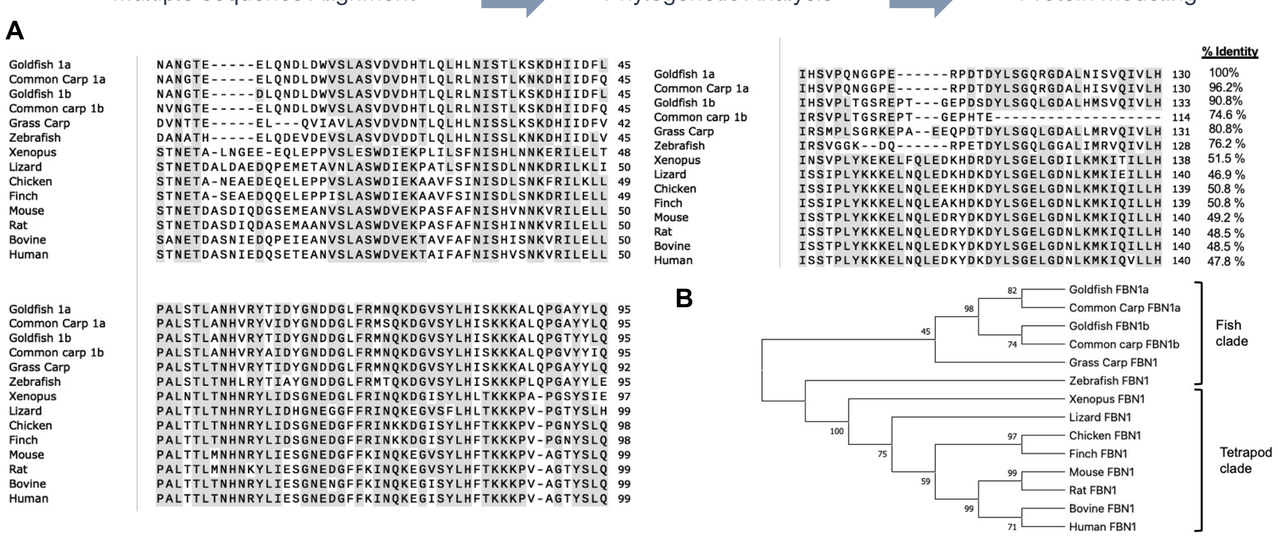

Fig. 2 **Alignment of FBN1 protein sequences (A).** Goldfish asprosin protein sequences were aligned with homologous sequences from other vertebrate species in SnapGene using the T-Coffee algorithm. Amino acid sequences shaded in grey represent conserved residues. Percentage identity is listed on the right. **Phylogenetic analysis of FBN1 sequences (B).** Vertebrate FBN1 coding sequences was used to construct the guide tree for evolution. Neighbor-joining method in MEGA X was carried out with bootstrap values derived from 1000 replications as indicated by the number on each individual node.

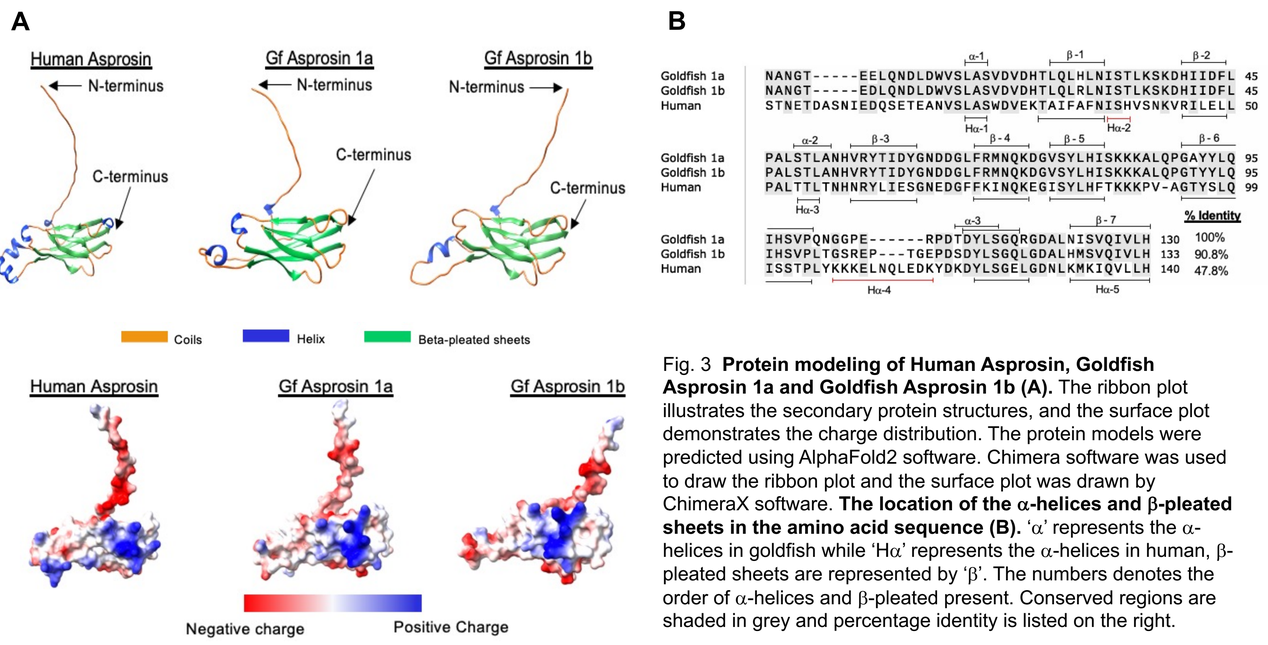

Fig. 3 **Protein modeling of Human Asprosin, Goldfish Asprosin 1a and Goldfish Asprosin 1b (A).** The ribbon plot illustrates the secondary protein structures, and the surface plot demonstrates the charge distribution. The protein models were predicted using AlphaFold2 software. Chimera software was used to draw the ribbon plot and the surface plot was drawn by ChimeraX software. **The location of the α-helices and β-pleated sheets in the amino acid sequence (B).** 'α' represents the α-helices in goldfish while 'Hα' represents the α-helices in human, β-pleated sheets are represented by 'β'. The numbers denotes the order of α-helices and β-pleated present. Conserved regions are shaded in grey and percentage identity is listed on the right.

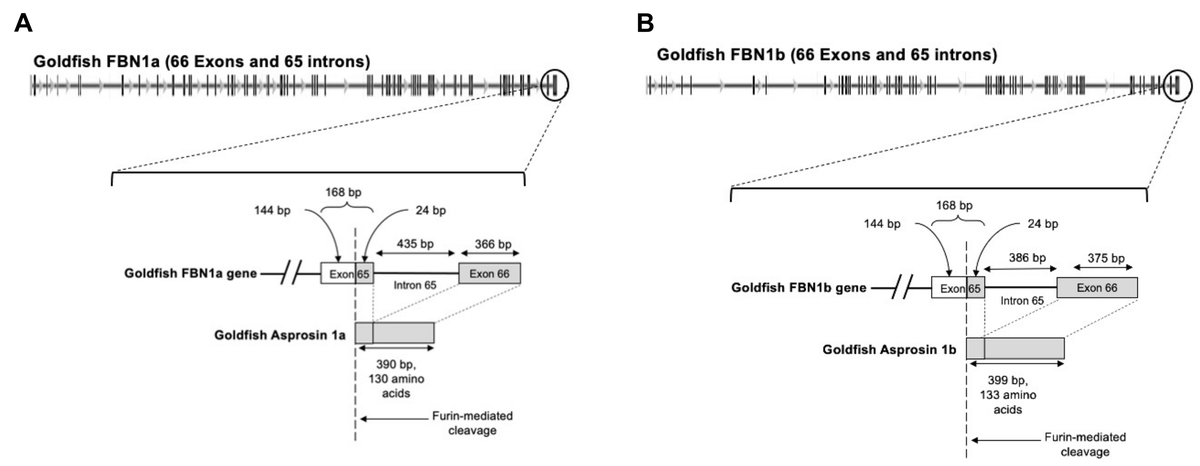

Fig. 4 **Gene structure of goldfish FBN 1a and FBN 1b.** Schematic diagram showing the Asprosin coding region of Goldfish FBN 1a gene (A) and Goldfish FBN 1b gene (B). The Goldfish FBN1a gene encoding 2869 amino acids is 55348 bp in length while Goldfish FBN1b gene is 55978 bp long and encodes 2864 amino acids. The complete gene structure was obtained from NCBI.

Tissue Distribution

Collect samples → RNA extraction → Reverse transcription → RT-PCR → Southern blot

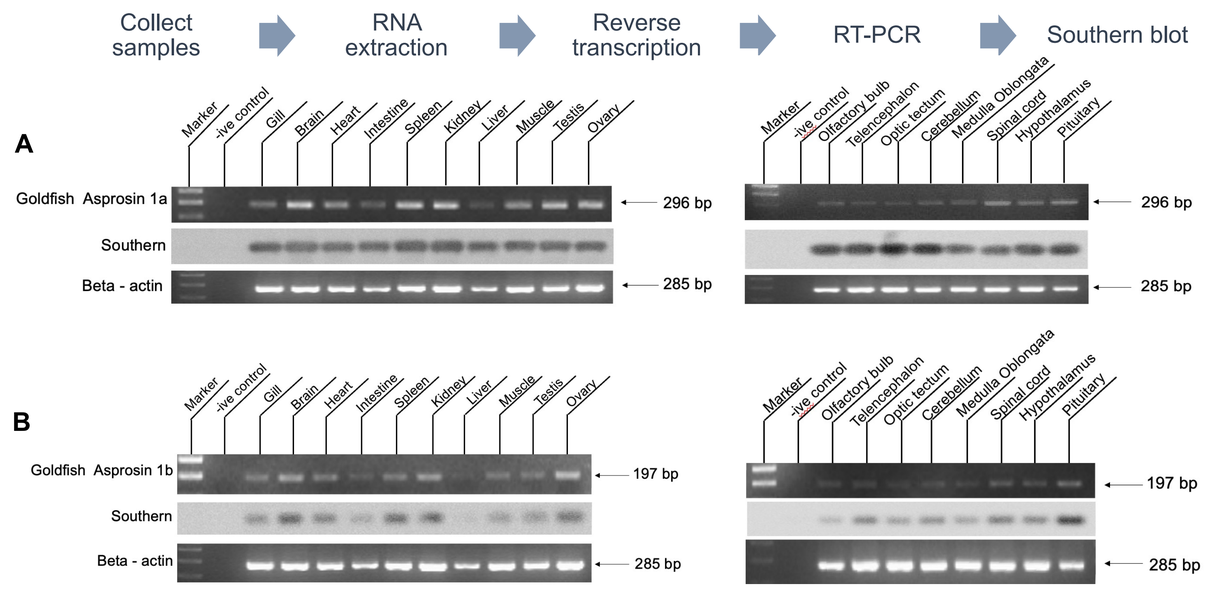

Fig. 5 **Tissue distribution of Goldfish Asprosin 1a (A) and Asprosin 1b (B).** The gel pictures demonstrate the distribution of Asprosin in peripheral tissues and brain in goldfish. Beta-actin has been used as internal control and water was used as template for negative control.

Functional Studies

Collect samples → Hepatocytes cell culture → RNA extraction → Reverse transcription → qPCR

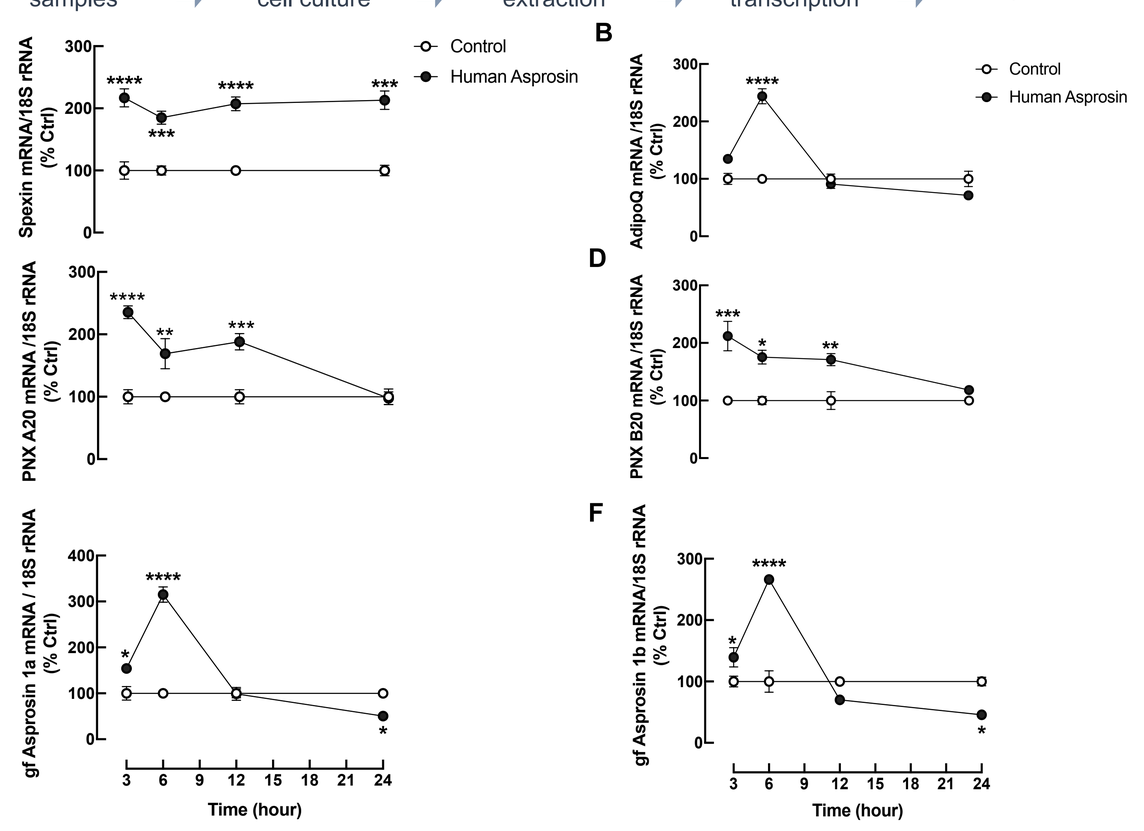

Fig. 6 **Time course effect of Human Asprosin (50 nM) on goldfish Spexin (A), Adiponectin (B), PNX A20 (C), PNX B20 (D), Asprosin 1a (E) and Asprosin 1b(F) mRNA expression from 3hr to 24 hr.** Data are normalized with 18S rRNA and are expressed as Mean ± SEM. Sidak Test followed and '****' indicates p < 0. by Two-way ANOVA was performed to determine a statistical significance of p<0.05 as denoted by the different letters on treatment groups. Significance is denoted by '*'; where '*' indicates p = 0.0332, '**' indicates p = 0.0021, '***' indicates p = 0.0002001.

Conclusion

- Asprosin is highly conserved among species
- In goldfish, Asprosin 1a has 3 α-helices and 7 β-pleated sheets, while Asprosin 1b has 2 α-helices and 7 β-pleated sheets
- Protein modeling demonstrates that human Asprosin and goldfish Asprosin are highly comparable.
- Asprosin is ubiquitously expressed in goldfish hepatocytes and highly expressed in the brain
- Asprosin can regulate Spexin, Adiponectin, Phoenixin A20 and B20 in goldfish.
- Presence of Asprosin autoregulation

Future Direction

- Dose dependency with human Asprosin
- Make recombinant goldfish Asprosin 1a/1b
- Check for comparable results with goldfish Asprosin treatment
- Carry out experiments with pituitary cells
- Neuroendocrine regulation of asprosin
- Signal transduction study

References

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