Phylogenetic and Expression Analysis of Fos Transcription

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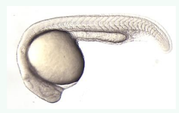
INTRODUCTION

- The mammalian FOS proteins, c-FOS, FOSB, FOSL1, and FOSL2, belong to the activator protein-1 (AP-1) family of transcription factors which share conserved bZIP functional domain.
- Members of the FOS protein family regulate gene expression responses to a multitude of extracellular signals and are dysregulated in several pathological states including melanoma (skin cancer).
- Despite providing important insights into the in vivo functions of specific FOS proteins in normal and disease states, the use of mouse models to investigate the mechanisms regulating the activities of individual FOS isoforms in vivo is challenging.



Zebrafish embryo

- In addition, mouse models have provided limited insight into the functions of FOS isoforms during early vertebrate development.
- The zebrafish has emerged as a robust in vivo model for early developmental studies, as its embryos are transparent and develop externally, facilitating easy observation of key processes while shares 70% of genes with us.

OBJECTIVES

- Identification and characterization of zebrafish Fos family orthologues.
- Expression analysis of zebrafish Fos transcription factors during early embryonic development and in zebrafish melanoma.

METHODS

As *In silico* studies, NCBI, BLAST, ZFIN, Genomicus, Phosphosite databases; Clustal X 2.1 and NJplot software were used for the identification and characterization of zebrafish Fos family orthologues.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed for quantitate expression analysis. Data was normalized to *actb* and the relative fold changes were determined by using ddct method.

Whole-Mount In Situ Hybridization (WISH) was performed for qualitative expression analysis.

Zebrafish maintenance was done following standard husbandry practices and microinjection was performed using MiniCoopR *mitfa:BRAFV600E* vector system to develop zebrafish melanoma

RESULTS

Identification and characterization of zebrafish Fos family orthologues

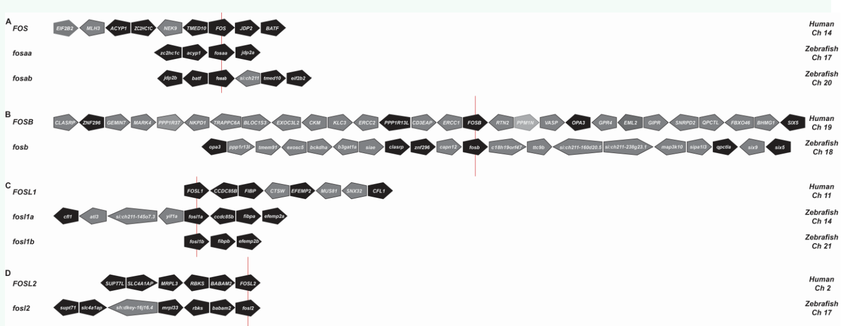


Figure 1 : Synteny analysis of *FOS* genes.

- Analysis of genomic databases identified six zebrafish *fos* genes.
- Synteny analysis indicated single *fosb* and *fosl2* orthologues, but duplicated *fosaa/fosab* and *fosl1a/fosl1b* paralogues.

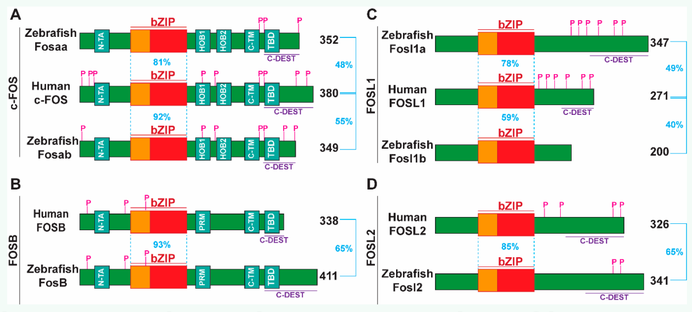


Figure 3 : Schematic representation of human and zebrafish FOS proteins.

- Sequence alignment of full-length human, mouse, and zebrafish FOS proteins revealed that, in addition to the bZIP domain, other major N- and C-terminal functional domains and phosphorylation sites of human FOS proteins are present in their corresponding zebrafish proteins.
- There are significant percentages of identity and similarity of the ZF and human amino acid sequences for each protein, specially in the functional bZIP domain as revealed using BLAST analysis.

Zebrafish Fos Genes Show Distinct Expression Patterns during Embryonic Development

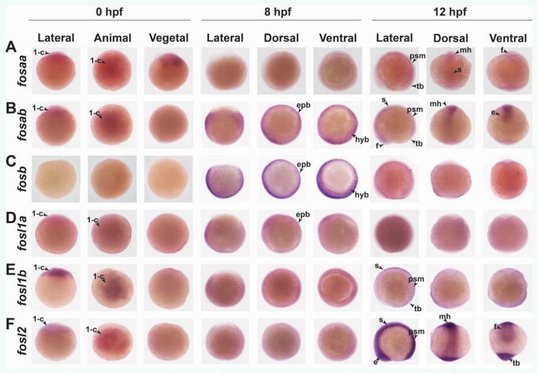


Figure 4 : Spatiotemporal expression of *fos* genes during early zebrafish embryonic development.

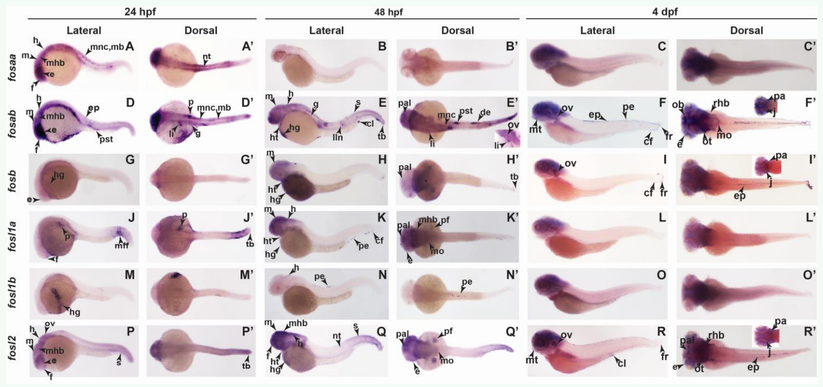


Figure 5: Spatiotemporal expression of *fos* genes in later stages of zebrafish embryonic development.

- Most of the *fos* genes have expressed at different tissue and organs of zebrafish during early and later stage of zebrafish embryonic development detected as dark purple colour by whole mount in-situ hybridization (WISH) analysis.
- These observations suggest a role of *fos* genes during the development of multiple organs like eye, brain, heart, liver, pancreas and cell lineages like melanocytes, epidermal cells.
- 1-c, one cell; cf, caudal fin; cl, cloaca; de, distal early of nephron; e, eye; ep, epidermis; epb, epiblast; f, forebrain; fr, fin ray; g, gut; h, hindbrain; hyb, hypoblast; hg, hatching gland; ht, heart; j, jaw; li, liver; lln, lateral line neuromasts; m, midbrain; mb, melanoblast; mff, median fin fold; mhb, midbrain hind brain barrier; mnc, migratory neural crest cell; mo, medulla obolongata; mt, mouth; nt, notochord; ob, olfactory bulb; ot, optic tectum; ov, otic vesicle; p, pancreas; pa, pharyngeal arches; pal, pallium; pe, peridermis; pf, pectoral fin; psm, presomitic mesoderm; pnc, posterior notochord; pst, proximal straight tubule of nephron; rhb, rostral hindbrain; s, somite; tb, tailbud.

Expression of FOS Genes in Adult Zebrafish Tissues

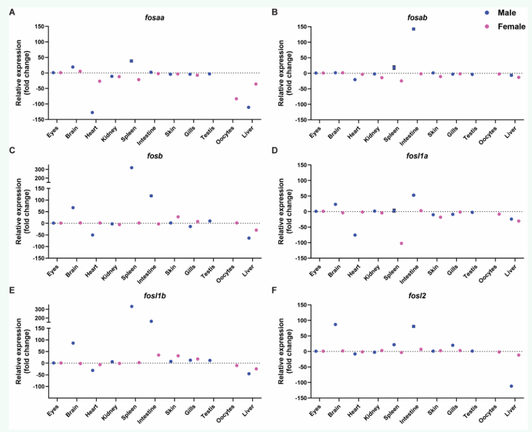


Figure 6: Relative expression of *fos* genes in male and female zebrafish adult tissues.

- The expression of *fosaa, fosab, fosb, fosl1a, fosl1b*, and *fosl2* was similar in adult zebrafish female tissues, but with modest variations compared to males, revealed by qRT-PCR analysis comparing with the expression in male eyes.

fos Genes Are Induced in BRAF-Driven Melanoma in Zebrafish

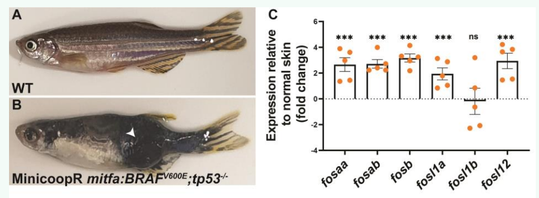


Figure 7: Relative expression of *fos* genes in human BRAFV600E oncogene driven melanoma in zebrafish.

- Gene expression analysis by qRT-PCR on zebrafish melanoma samples revealed higher transcript levels of all *fos* genes except *fosl1b*, with *fosb* displaying the highest relative expression.

DISCUSSION AND CONCLUSION

- ZF have orthologs of all human *FOS* genes. Key functional and regulatory regions are conserved in ZF Fos orthologues suggesting the functionality of each FOS isoform is likely to be regulated via similar mechanisms in both species.
- During embryogenesis, zebrafish *fos* genes exhibit both overlapping and distinct spatiotemporal patterns of expression in specific cell types and tissues suggesting their important role in embryonic development.
- .Most *fos* genes are also expressed in a variety of adult zebrafish tissues. Differences in expression pattern in male and female tissues, most notably in spleen suggest a potential role of *fos* genes in regulating sexual dimorphism in the immune system, a possibility that warrants further investigation.
- As in humans, expression of zebrafish FOS orthologs are induced by oncogenic BRAF-ERK signalling in zebrafish melanomas indicating functional conservation of key pathways regulating expression of these genes in humans and zebrafish.
- These findings suggest that zebrafish represents an alternate model to mice for investigating the regulation and functions of Fos proteins in vertebrate embryonic and adult tissues, and cancer.

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1. Kubra K, Gaddu GK, Liongue C, Heidary S, Ward AC, Dhillon AS, Basheer F. Phylogenetic and Expression Analysis of Fos Transcription Factors in Zebrafish. International journal of molecular sciences. 2022 Sep 3;23(17):10098.

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