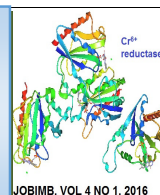


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Molecular Cloning of a Functional *Fads2* Promoter from Zebrafish

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ABSTRACT

The FADS2 catalyzes the first rate-limiting step in the long chain-polyunsaturated fatty acids (LC-PUFAs) biosynthesis pathway by converting α -linolenic acid and linoleic acid into stearidonic acid and γ -linolenic acid via the ω -3 and ω -6 pathways respectively. In mammals, PPAR α and SREBP-1c have been implicated in the polyunsaturated fatty acids (PUFAs) mediated transcriptional activation of FADS2 promoter. However, in zebrafish, not much is known regarding the regulation of *fads2* transcriptional regulation. Here, in this study, five vectors containing different promoter regions were constructed in order to analyse putative promoter activities. Through truncation analysis, it was found that the 1.2 kb promoter was able to drive luciferase activity to an approximate 40-fold in HepG2 cells. Upon mutagenesis analysis, three sites which are the putative NF-Y, SREBP and PPAR binding sites were found to be essential in driving the promoter activity. Lastly, the 1.2 kb *fads2* promoter was able to direct EGFP expression specifically to the yolk syncytial layer (YSL) when transiently expressed in microinjected zebrafish embryos.

INTRODUCTION

Mammalian desaturases can be regulated by various transcription factors; however, two most prominent *trans*-factors affecting the expression of fatty acid desaturase 2 (*FADS2*) would be peroxisome proliferator-activated receptor α (PPAR α) and sterol regulatory element-binding proteins 1c (SREBP-1c) [1,2]. Although, polyunsaturated fatty acids (PUFAs) are catalytic products of desaturases, its involvement in a feedback regulatory function via the PPAR α and SREBP-1c on desaturases expression has been noted previously. Other than PPAR α and SREBP-1c, key *cis*-acting elements and their corresponding *trans*-acting factors like nuclear transcription factor Y (NF-Y), specificity protein 1 (Sp1), GATA, CCAAT-enhancer-binding proteins (C/EBP α), retinoid X receptor (RXR) and carbohydrate-responsive element-binding protein (ChREBP) have been implicated in desaturases transcriptional activation. In fact, NF-Y and SREBP sites were remarkably conserved as previously identified in Salmon and Cod *FADS2* promoter [3].

In teleost like zebrafish, localization of high level of transcript in the liver and intestine in adult suggested that these were the primary PUFAs biosynthesis sites [4]. In early embryonic stages, localization of *fads2* transcripts in the primitive brain further proves the significance of PUFAs during early vertebrate neural development. As embryos progressed into the 72 hpf stage, the primary biosynthesis role was taken over by the now extensively differentiating hepatocytes in the primitive endodermal layer as shown through *in situ* hybridization [5]. This demands an extensive coordination among the brain, primitive liver and intestinal tissues. In this aspect, it is possible that different subsets of transcription factors are required to fully integrate the responses needed for tissue specific desaturation of PUFAs to occur in the continuously growing vertebrate.

In view of the apparent conservation of the biosynthesis pathway for PUFAs between mammals and teleost, it is reasonable to postulate that similar transcriptional regulation mechanism governs the expression of *fads2* in zebrafish. Due to the many desirable features of using zebrafish as a model such as the optical transparency and non-invasive procedures in gene