The Cloning and Regulatory Analysis of Tilapia (Oreochromis mossambicus) Hepatocyte Nuclear Factor-3b Promoter

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ABSTRACT

The hepatocyte nuclear factors-3 (HNF-3) family members HNF-3?? HNF-3? and HNF-3? are hepatocyte-enriched transcription factors, they play important roles in controlling development, cell differentiation, organogenesis and in the regulation of metabolism. In our previous study, the expression of insulin-like growth factor-I (IGF-I), IGF-II, HNF-1a, -1b, and -3b?烅ere detected in the liver and gonads of tilapia, and the expression level of HNF-3b? was higher than others and it could be regulated by 17b-estradiol. Based on the results, the aim of this study was to clone and analyze the tilapia HNF-3b promoter, and to provide a foundation for further studies on the relation between HNFs and gonad/gamete development in tilapia, and on biomedical and developmental studies in the future. The 5 ’ -flanking region of tilapia HNF-3b promoter containing 2364 bp was cloned and sequenced, and on which 373 putative transcription factor binding sites for HNFs (HNF-1, -3, -4 and -6), CCAAT/enhancer binding protein(C/EBP), cAMP responsive element binding (CREB), sterol regulatory element, steroid hormones receptor binding site, signal transducer and activator of transcription (STAT), GATA-binding factor and other factors were found. These factors are important for embryo development and gonadal function. Four deletion fragments (2, 1,5, 1 and 0.5 kb) of tilapia HNF-3b promoter were constructed with green fluorescent protein (GFP) gene for biological activity assay by cell line (TO-2 and Hep3B) transfection or microinjection into zebrafish eggs. The GFP was mainly expressed in yolk and somite 10 h after injection, and in somite, notochord and floor plate 72 h after injection, but that of 0.5 kb fragment was mainly expressed in eye and head. And the expression rates of 2 kb and 1.5 kb groups seemed better than those of 1 kb and 0.5 kb (p

Keywords : tilapia, gonad, hepatocyte nuclear factors-3 (HNF-3), insulin-like growth factors(IGFs), steroid hormones

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