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To cite this version:

Svetlana Uzbekova, S. Hanley, Chantal Cauty, Trenton G. Smith, Bernard Breton. Functional study of salmon histone H3 promoter in transgenic rainbow trout : constitutive expression of GnRH antisense mRNA could lead to a reversible sterility.. 3. IUBS Symposium on molecular aspect of fish genomes and development., Feb 2001, Singapore, Singapore. hal-02759948

HAL Id: hal-02759948

https://hal.inrae.fr/hal-02759948v1

Submitted on 4 Jun 2020

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3rd IUBS Symposium on Molecular Aspect of Fish Genomes and Development

February 18 – 21, 2001

Singapore

Organized by Department of Biological Sciences, National University of Singapore, Singapore

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FUNCTIONAL STUDY OF SALMON HISTONE H3 PROMOTER IN TRANSGENIC RAINBOW TROUT: CONSTITUTIVE EXPRESSION OF GnRH ANTISENSE mRNA COULD LEAD TO A REVERSIBLE STERILITY

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The problem of uncontrolled breeding of genetically modified farmed fish could be partly resolved if they were sterile fish. In Salmonids, salmon Gonadotropin-Releasing Hormone (sGnRH) plays a key role in the control of gonadotropins LH and FSH synthesis and secretion, necessary for the development of gametogenesis. This peptide is mainly expressed in the brain but also in gonads and other tissues. Thus, blockage of sGnRH synthesis by constitutive expression of antisense RNA could theoretically induce sterility by inhibiting gonadotropin synthesis and secretion and may be by acting directly at the gonadal level.

We characterised the expression pattern of histone H3 intergenic promoter region from salmon histone H2A-H3 gene by complex analysis of transgenic rainbow trout lines that carried marker gene CAT driven by this regulatory sequence (PhisH3-CAT). By means of specific CAT-assay, Northern blot, RT-PCR and in situ hybridization we demonstrated that histone H3 promoter drives the expression of CAT in different tissues and it was compared to the endogenous histone H3 and sGnRH mRNA distribution. The highest expression of CAT messengers was found in immature ovary and testis in which sGnRH mRNA is also strongly expressed at that stages.

Transgenic rainbow trout lines carrying both PhisH3-CAT and recombinant vector containing antisense DNA complementary to sGnRH cDNA under the control of the same promoter (PhisH3-sGnRH-antisense) were produced.

F1 and F2 progenies expressed sGnRH-antisense RNA in different tissues. The strongest expression was detected in immature gonads. The maturation rate and FSH plasma level was significantly lower in transgenic offspring. In F1 family at 2.5 years of age there were sterile males which never produced sperm naturally. In these fish spermiation was restored by hormonal treatment.

Taken together our results showed that partial inhibition of sGnRH mRNA at the gonadal level could disturb reproductive functions in transgenic trout.

Supported by EC Program BIO4-CT97-0054