

**INTERFERON INDUCED GENES  
IN THE RAINBOW TROUT**

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**EXTENDED ABSTRACT ONLY - DO NOT CITE**

**Introduction**

The vertebrate interferon system provides an early line of cellular defence against viral infection by inducing the production of proteins that inhibit virus replication. Mx proteins have been shown to be part of these intracellular mediators of viral resistance (see for review: Samuel, 1991).

In the rainbow trout, three Mx cDNA have been cloned and sequenced (Leong *et al.*, 1998). At the amino acid level, RBTMx1 and RBTMx3 are greater than 96 % identical and RBTMx2 differs slightly from the other two with an 88.2 %

identity to RBTMx3 and a 90.6 % identity to RBTMx1. It is not known whether these isoforms are encoded by one or more loci.

Fish had been shown to have interferon activity (De Kinkelin *et al.*, 1982) but to date interferon has neither been purified nor its coding nucleic acid sequence determined. In this study we used Mx gene as a marker of interferon activity in the rainbow trout *Oncorhynchus mykiss*.

### Expression of mx *in vitro*

An RT-PCR system for Mx genes has been developed. Primer pairs were designed from the published cDNA sequences in order to specifically amplify the three Mx forms. Genomic DNA was used as template for PCR and the products were cloned and sequenced. The three Mx sequences obtained appeared to include one or two introns, making the primers suitable for expression studies by RT-PCR. The specificity and inducibility of Mx genes were confirmed using these primer pairs for *in vitro* studies. The rainbow trout cell line RTG2 was induced by poly I:C or Interferon Containing Supernatants (ICS) for 12, 24 or 48 hours. ICS induced transiently Mx1 and Mx3 after 12 hours of incubation. PolyI:C induced Mx1 and Mx3 later, after 24 hours of incubation (Fig. 1).

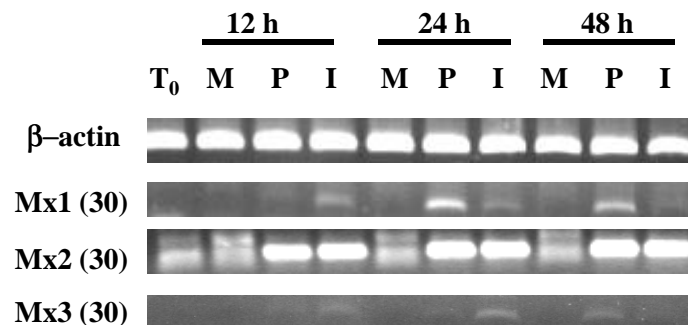


Figure1: Time course of induction of Mx expression in RTG2 cells by ICS or polyI:C. The number of PCR cycles is indicated in brackets. T<sub>0</sub>, control before incubation, M, Medium, P, PolyI:C 20 μg.ml<sup>-1</sup>, I, ICS diluted 2 fold

Induction of Mx1/3 and Mx2 have different time course patterns, and adds to argument they may have different biological functions as suggested by the cellular location of the proteins (Leong, 1998).

### **Mx expression in DNA-vaccinated fish**

Expression of Mx1 transcripts was investigated in fish which were previously injected with a DNA vaccine directed against the surface glycoprotein of the VHS virus (Lecocq-Xhonneux *et al.*, 1994). Eight weeks after vaccination, Mx was expressed in Liver and head kidney of vaccinated fish, but not in the controls (injected with vector alone).

### **Regulation sequence of mx gene**

As a way to simplify later analysis of interferon production, the promoter of the interferon-induced gene Mx1 has been investigated. Five genomic libraries were constructed using the Genewalker Kit (Clontech). Briefly, genomic DNA was purified and digested with five different blunt-end restriction enzymes (PvuII, StuI, DraI, EcoRV and ScaI) giving, after ligation of an adaptor on 3' and 5' ends, five genomic libraries. Reverse primers were designed in the 5' end of the Mx1 cDNA and used in nested PCR with the adaptor primers. Hot start and touchdown technologies were used to increase the specificity of amplification. Long distance PCR was also carried out with a mix of *Taq* and *Pfu* polymerases.

Two fragments of 620 bp and 356 bp were obtained, cloned and sequenced. The two fragments appeared to belong to the same contig. As a verification of the sequence, a forward primer were designed within this new sequence and used with a reverse primer located on the transcribed region of the gene. PCR was carried out on genomic DNA extracted from different individuals and gave a product with the expected size.

The sequence obtained has the typical structure of a promoter of an interferon induced gene: a TATA box located 30 based upstream of the putative site of initiation of transcription, a highly conserved 13 bp-long Interferon Stimulating Response Element (ISRE, see Fig 2) and a Sp1 site, responsible for induction by viruses.

Consensus	RGAAANNGAAASY
Rainbow trout	TGAAAGTGAAACA *****
Mouse	AGAAAC-GAAACT
Mouse 202	GGAAATTGAAAGC
Human 2',5'AS	GGAAAC-GAAACC
Human 6-16	GGAAAATGAAACT
Human 56 kDa	GGAAAGTGAAACT
Human ISG15	GGAAACCGAAACT
Human ISG54	GGAAAGTGAAACC
Human factor B	GGAAACAGAAACT
Human IFN- $\alpha$ 1	AGAAATGGAAAGT

Figure 2: Alignment of the Interferon Stimulating Response Element (ISRE) in rainbow trout with mammalian ISRE present in the regulating sequence of various interferon induced genes. Codes for the consensus sequence are as followed: R = A or G, Y = C or T, S = G or C, N = A, C, G or T.

This structure is similar to the mouse promoter for Mx1 (Hug *et al.*, 1988) with the difference that in rainbow trout there is a single ISRE longer than the five ISRE motifs present in the murine promoter. The ISRE shows a complete identity with the human promoter for the 56 kDa-interferon induced-2',5' oligoadenylate synthetase, another interferon-induced gene. Only the first and last base of the trout ISRE does not match the consensus (Friedman and Stark, 1985) and seem to be inverted.

## References

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