Development of *in vitro* systems to study IFN signalling in gilthead seabream (*Sparus aurata*)

J. A. González-Mariscal, M. A. Fernández-Trujillo, T. Méndez, M. C. Alonso, E. García-Rosado, M. C. Álvarez and J. Béjar*

Type I interferon (IFN I) triggers specific signalling pathways leading to the activation of the innate immune defence of vertebrates against viral infections. In contrats, type II IFN (IFN II) is generally accepted to be part of the adaptive response. Among IFN I-stimulated genes, those coding the Mx proteins play a main role due to the direct antiviral activity of these proteins. The study of *Mx* genes in gilthead seabream, one of the most important species in the Mediterranean aquaculture, is especially interesting, as this species displays a high natural resistance to viral diseases, and behaves as asymptomatic carrier and/or reservoir of several viruses, such as viral nervous necrosis virus (VNNV), infectious pancreatic necrosis virus (IPNV), and viral haemorrhagic septicaemia virus (VHSV), which are pathogenic to other fish species.

Three Mx genes (*Mx1*, *Mx2*, and *Mx3*) have been identified in *S. aurata*, showing the three proteins a wide spectrum of antiviral activity. The structure of the three promoters (pMx1, pMx2 and pMx3) has been disclosed, and their response to IFN I, IPNV and VHSV indicated a clear induction of the three promoters, with some differences in the kinetics and magnitude of the response.

Several studies evidenced the important role of *Mx* transcription regulation on virus-host interaction: i) *Mx* promoters can respond to both IFN I and IFN II, thus *Mx* might be the link between innate and adaptive immunity; ii) *Mx* activation is blocked by several viruses, thus *Mx* transcription is the target of their IFN I antagonistic activity; and iii) A fish cell line modified with the promoter of a fish *Mx* gene was used to measure viraemia in serum with high sensitivity. Therefore, assessing the regulatory mechanisms controlling the transcription of fish Mx genes could significantly contribute to both, understanding virus-host interactions, and designing strategies to control viral infections. In our case, this approach can also give light to understand the successful antiviral strategies developed by gilthead seabream in nature.

Thus, the purpose of the present work was to develop three stable transgenic cell lines expressing the firefly luciferase gene under the control of the gilthead seabream *Mx* promoters. These *in vitro* systems were established and their response to poly I:C, and to two viral infections was characterized.

In the case of IPNV, a clear antagonistic activity was observed for pMx2, as the activity of the promoter was 78.53% lower, however, this effect was not observed for pMx1 and pMx3. When cells were infected with VHSV, no changes in the promoters' activity were detected, thus indicating that seabream Mx promoters are not targeted by VHSV antagonistic activity. These results confirm the specificity of the interactions between each virus/promoter combination, and support the use of the three cell lines developed as useful tools to characterize virus-host interactions in this species. Further studies aimed at the identification of the molecular mechanisms behind our observations will allow us to get more insight into this complex system.