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Isolation of a novel fish thymidylate kinase gene, upregulated in Atlantic salmon (*Salmo salar* L.) following infection with the monogenean parasite *Gyrodactylus salaris*

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Abstract

Analysis of differential gene expression in salmon (*Salmo salar*) blood following infection with the monogenean parasite *Gyrodactylus salaris*, resulted in the isolation of a thymidylate kinase gene not previously described from fish and which showed similarity to an LPS-inducible thymidylate kinase gene isolated from mouse macrophages. This salmon TYKi-like gene may play a role in an innate generalised response to pathogen infection as it was upregulated in salmon following infection with the parasite, and also in response to injection with the immunostimulants LPS and Poly I:C, used to emulate bacterial and viral infections, respectively. The possible role of this gene in the biosynthesis of mitochondrial DNA in activated macrophages, in response to *G. salaris* infection is discussed.

Keywords: Salmon; *Gyrodactylus*; Infection; Differential display; Gene expression; Immune response; Thymidylate kinase; RT-PCR

1. Introduction

Gyrodactylus salaris is a monogenean ectoparasite, generally associated with salmonid fish species. Experimental infection trials have shown that *G. salaris* can survive and reproduce on many salmonids, such as *Oncorhynchus mykiss*, *Thymallus thymallus*, *Salvelinus namaycush*, *Salvelinus alpinus* and *Salvelinus fontinalis* [1–6], but infection intensities on these species are low. It can cause high mortalities in wild Atlantic salmon (*Salmo salar*) through exponential growth of parasite populations on infected fish. The parasite causes damage to the epithelium of the fish through feeding and movement on the fish surface [7], which gives rise to secondary infections by bacteria and fungi.

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More significantly, salmon parr infected with thousands of parasites may suffer osmoregulatory distress due to the number of perforations of the skin, leading to death of the fish. However, certain salmon stocks seem to be able to control the infection, in particular some Baltic salmon stocks in which *G. salaris* is endemic [8,9]. This has given rise to questions concerning the host response and basis for differences in susceptibility to *G. salaris* in these salmon populations.

While differences in the pathogenicity of parasite populations and in environmental conditions, in particular water quality, have been shown to influence the ability of *G. salaris* populations to become established and expand [10,11], a strong genetic component is implicated in resistance of fish to the parasite [12,13]. To further investigate the host response to *G. salaris* infection, and differences which might give rise to increased/decreased susceptibility, we investigated gene expression in *G. salaris* challenged Atlantic salmon stocks previously found to show high or low susceptibility to *G. salaris*.

Differential display reverse transcribed polymerase chain reaction (DD RT-PCR) was first described in the early 1990s [14] and compares total mRNA from the samples being analysed. DD RT-PCR has been used to detect differentially expressed genes in numerous biological systems including genes expressed in response to pathogen exposure [15–17], resistant specific infection-induced gene expression [18], genes differentially expressed at different developmental stages [19–21], sex-specific gene expression [22], and genes differentially expressed following drug treatment [23,24]. In this paper we present a salmon gene, isolated using DD RT-PCR technology, which is similar to an LPS-inducible thymidylate kinase gene isolated from mouse macrophages. We show that this gene was upregulated in the blood of both *G. salaris*-susceptible and less susceptible salmon strains following infection with the parasite, indicating that it is involved in host response to the parasite, but not in those aspects of the response which form the basis for differences in susceptibility to *G. salaris*.

2. Materials and methods

2.1. Experimental fish

Salmon used in the *Gyrodactylus* challenge experiment were from a Baltic strain with low susceptibility and an Atlantic strain with high susceptibility to *G. salaris*. The Baltic strain was generated from eggs and milt obtained from the Norwegian Institute for Nature Research (NINA), Ims, Norway, from stock originating from the river Neva, Russia. The Atlantic strain was generated from eggs and milt obtained from the Conon District Fishery Board hatchery, Ross-shire, Scotland, from salmon originating from the Conon river, Scotland. Challenges were carried out at the National Veterinary Institute, Fish Health Section, Oslo.

2.2. Experimental setup

Neva and Conon fish, ranging in weight from 4 g to 29 g (average 15.8 g) were placed together in equal numbers, 30–35 fish per salmon strain, into four 40 × 40 cm tanks containing 10 l of dechlorinated tap water. Conon fish were distinguished by clipped adipose fins. Fins infected with approximately 2500 *G. salaris* parasites (parasite population originated from the river Lærdalselva, Norway) were placed in two of the tanks and left for 24 h. Fish in the remaining two tanks were left uninfected, serving as controls. After 24 h exposure to the parasite, the infection level on the exposed fish was recorded by counting parasite numbers on individual anaesthetised fish, under a dissecting microscope at 12–16× magnification. Fish were removed, in their original groups, to four 0.5 × 0.5 m tanks containing 200 l of water. The infection was monitored at weekly intervals over a period of 11 weeks, by counting all parasites on the fins and body surface of four salmon from each strain from each tank of infected fish. Control fish were treated similarly.

2.3. Collection of blood, extraction of RNA and cDNA synthesis

Blood was collected from eight fish per experimental group (infected Neva and Conon and their controls) at 4, 8, 24 h, 7, 14 and 71 days post infection (p.i.). Total RNA was extracted from whole blood using the TRIzol (Invitrogen) method [25] according to manufacturers' instructions. 0.25 μg of RNA from individual fish from each experimental