THE GENETIC BASIS OF *GYRODACTYLUS SALARIS* RESISTANCE IN ATLANTIC SALMON (*SALMO SALAR*)

FISHERIES RESEARCH SERVICES

JOHN GILBEY[1, 2], IVETA MATEJUSOVA[1, 2], TANJA SORSA-LESLIE[2], CATHERINE COLLINS[1], CAREY O. CUNNINGHAM[1], ERIC VERSPOOR[1], LESLIE R. NOBLE[2], CATHERINE S. JONES[2]

[1]*FRS Marine Laboratory, Scotland,* [2]*University of Aberdeen, Scotland*

UNIVERSITY OF ABERDEEN

INTRODUCTION

- ***Gyrodactylus salaris*** **Malmberg, 1957 (Monogenea, Platyhelminthes) is a freshwater ectoparasite of salmonid fish, and has only been found in Europe to date.**
- **Atlantic salmon stocks are very susceptible to *G. salaris* infection, often resulting in death of fish, in particular parr.**
- **First record of *G. salaris* in Norway was in 1970. Since then it has caused major losses in freshwater salmon fisheries with stocks of several rivers wiped out.**
- **Treatment measures are often environmentally harsh.**
- **Salmon x trout hybrids demonstrated a strong genetic component in susceptibility of the host to *G. salaris* (Bakke *et al*., 1999). Baltic salmon stocks also show a reduced susceptibility to *G. salaris* infection. Comparison of *G. salaris* infection and host response at the molecular level, in distinct Atlantic and Baltic salmon stocks, may show differences which form the basis for resistance to *G. salaris*.**
- **Through understanding the basis for resistance, natural variation in susceptibility to *G. salaris* infection in Atlantic salmon stocks could allow enhancement, through selective breeding, of *G. salaris* resistance, giving another option for *G. salaris* control.**

Integration of findings and development of genetic management options for *G. salaris* infections

AIM

To elucidate the molecular basis for variation in resistance to *Gyrodactylus salaris* in Atlantic salmon and the mechanisms involved in host-parasite interactions, and to provide the scientific basis for new options for controlling the impact of the parasite on susceptible salmon stocks.

An overview of the approach taken to the project, and some preliminary results for one work package are presented.

PROJECT STRUCTURE

Establishment of experimental salmon and *Gyrodactylus* stocks

Neva salmon stock from river Neva, Russia; resistance shown to *G. salaris* infection (Bakke *et al*. 1990).

Conon salmon stock from river Conon, Scotland; highly susceptible to *G. salaris* infection (Bakke & MacKenzie, 1993).

***G. salaris* population from Lærdalselva, western Norway.**

e.verspoor@marlab.ac.uk tor-atle.mo@vetinst.no

F1 and F2 salmon crosses

F1 fish generated from Conon and Neva crosses. F2 fish generated by backcrossing F1 males, with females from Conon and Neva pure stocks. Analysis of F2 fish with high and low susceptibility to *G. salaris* will reduce "non relevant" strain differences while at the same time highlighting those which confer differences in susceptibility.

e.verspoor@marlab.ac.uk

Genetic markers for host resistance

Comparison of pooled DNA from F2 susceptible and resistant fish, using QTL Mapping and Bulk Segregant Analysis, to identify genetic markers linked to genes influencing resistance. Areas of interest on the chromosomes can be analysed in greater detail.

gilbeyj@marlab.ac.uk e.verspoor@marlab.ac.uk l.r.noble@abdn.ac.uk

Genetic markers for parasites

To access the utility of different markers for *G. salaris* population discrimination.

- **Non-transcribed region of the ribosomal DNA.**
- **COI mitochondrial regions.**
- **Microsatellite markers.**

cunninghamc@marlab.ac.uk

Host immune response

Characterisation of immune response of salmon resistant and susceptible to *Gyrodactylus* infection.

Determine relative contribution of cellular and humoral immune response to resistance to *G. salaris*.

Analysis of antigenic components of *Gyrodactylus* parasites.

Analysis of factors influencing host preference in *Gyrodactylus*.

kurt.buchmann@vetmi.kvl.dk

Host genetic response

cunninghamc@marlab.ac.uk l.r.noble@abdn.ac.uk

Methodology

- **Differential Display RT-PCR was carried out on samples from *G. salaris* infected and control fish.**
- **Differentially expressed products were sequenced, and RACE-PCR carried out to obtain the full gene sequence.**
- **Semi-quantitative RT-PCR analysis was carried out on the experimental groups to verify differential expression of the isolated products.**

Results

- **One gene, upregulated in blood samples from infected susceptible Conon fish (figure 1), is presented here. The gene is not upregulated in infected resistant Neva salmon, and its constitutive expression in resistant Neva control fish is also lower compared with that in susceptible Conon controls**
- **The gene sequence has similarity with FIP2 proteins from human and chicken (figure 2), including a conserved leucine zipper motif. However, it is truncated compared to human and chicken FIP2 genes, and does not possess a second characteristic leucine zipper and zinc finger at the 3' end.**
- **FIP2 proteins are involved in the NFκB signalling pathway (Li *et al,* 1998). NFκB is an activator of genes involved in innate immune and inflammatory responses (Karin & Delhase, 2000).**

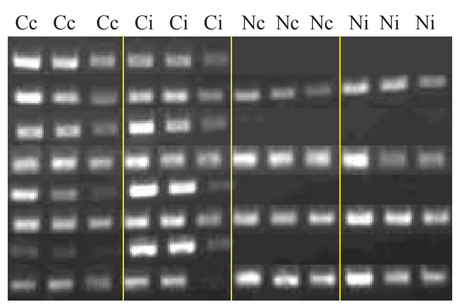
Discussion

Due to the truncated 3' end and absence of the second leucine zipper domain, the FIP-like gene presented here may not be the fish homologue of FIP2. There are however numerous proteins within this family that result from alternate splicing. These proteins, including the FIP-like protein isolated from salmon, also show regions of similarity with IKK (IκB kinase) and NEMO (NFκB essential modulator), both of which are involved in the NFκB signalling pathway. The FIP-like salmon protein may represent a novel protein within this group of regulatory proteins.

Differences in expression of the FIP-like gene, between susceptible Conon and resistant Neva salmon may be stock differences, or may be due to a different allelic form in the Neva salmon which is poorly amplified under the PCR conditions

Expression analysis will be carried out on new material and at the individual level to confirm differential expression of both genes in response to *G. salaris* infection, and gene function will be characterised further.

Figure 1



Semi-quantitative RT-PCR of FIP-like product, isolated during differential display analysis of cDNA from blood, from control and *G. salaris* infected fish.

Cc = Conon control Ci = Conon infected
Nc = Neva control Ni = Neva infected

Figure 2 : CLUSTAL W multiple sequence alignment of salmon FIP-like protein with human & chicken FIP proteins

```
G.gallus.FIP-2        ---------MSSKPQIRPAENGEHCRSKMENGMDSMAPPTLSTYTPEEMVQQMKELITEN 51
huFIP-2               ---------MSHQPLSCLTEKEDSPSESTGNGPPHLAHPNLDTFTPEELLQQMKELLTEN 51
S.salar.FIP-like      KNKKYPSSFKVYLNHLVMASSSPMVNGDISRGPSSHPGHSPTTGTLEETLQQMNRLIQEN 60
                             :..     . .*    .  .  * * ** :***:.*: **

G.gallus.FIP-2        NELKEAMKLHNQAMKDRYEELSIWREKQKEEREFYETKFKEAKQCLLAKCVENEQLQQQL 111
huFIP-2               HQLKEAMKLNNQAMKGRFEELSAWTEKQKEERQFFEIQSKEAKERLMALSHENEKLKEEL 111
S.salar.FIP-like      RDLKEALKQTNMSMKERFEGLSVWREKQREEKEFLEGRLDEARGRMEALSSHNQELSMKV 120
                      .:****:*  * :** *:* ** * ***:**::* * : .**:  : *  . .*::*. ::

G.gallus.FIP-2        QSLKEREEGAEMEG-------CATPEKEARQLKSKVQRLQAEKADLLAIISELQVKLN-I 163
huFIP-2               GKLKGKSERSSEDPTDDSRLPRAEAEQEKDQLRTQVVRLQAEKADLLGIVSELQLKLNSS 171
S.salar.FIP-like      EELEGGEGEGKGQAET------ANQNAELEALHAQLLRLQAEKNDLVAMNSELQLKTG-Q 173
                        .*:  .  .. :        *  : *   *:::: ****** **.: ****:* .

G.gallus.FIP-2        ASAEDSFVEIGMNE-EVNRTARENQDN--SSEMASN--IAVYIRSKSADESKN-LESEEL 217
huFIP-2               GSSEDSFVEIRMAEGEAEGSVKEIKHSPGSTRTVSTGTALSHYRRRSADGAKNYFEHEEL 231
S.salar.FIP-like      GSEDDSFIEIRFAKEDAXKELYHNERD-----------------PRYDMSVSRHESEEL 215
                      .* :***:** : : :.     . : .                  * : .  * ***

G.gallus.FIP-2        TVSQLLCCLRNETQRREKLEKELQDHKERLSKMENETSNCLESGTQTN-------QEEES 270
huFIP-2               TVSQLLLCLREGNQKVERLEVALKEAKERVSDFEKKTSNRSEIETQTEGSTEKENDEEKG 291
S.salar.FIP-like      TVSQLLQSLRKETQRVERLQMELQATRSRITELEEKVTN-TESSTQTS------------ 262
                      ****** .**: .*: *.*:  *:  :.*::.:*::.:*  *  ***.

G.gallus.FIP-2        SEAIGSEVESLKKQICALFKELQEAHEKLKEAELIQKKLQEKCQTLEKVNSAAATELEEK 330
huFIP-2               PETVGSEVEALNLQVTSLFKELQEAHTKLSEAELMKKRLQEKCQALERKNSAIPSELNEK 351
S.salar.FIP-like      ----------------LLPEVLSPALATTACGLSQNQRRSPNRTLEGEG---------- 295
                                     *: *: ..    . . * ::: :.  ::**  .

G.gallus.FIP-2        QQLIYTIKKLELQVESVQAEVKLEQAKTQDEKTRYSSLQDAYNKLLAELTEAMKTISEMK 390
huFIP-2               QELVYPNKKLELQVESMLSEIKMEQAKTEDEKSKLTVLQMTHNKLLQEHNNALKTIEELT 411
S.salar.FIP-like      ------------------------------------------RYTDKLLPSLSLTL------- 309
                                                               :***  . . ::

G.gallus.FIP-2        VKEHDRVDKVVVEELNAKVLLAEQALAAKQLQMDEMKQLIAKQEEDLETMAVLRAQMEVY 450
huFIP-2               RKESEKVDRAVLKELSEKLELAEKALASKQLQMDEMKQTIAKQEEDLETMTILRAQMEVY 471
S.salar.FIP-like      ------------------------------------------------------------

G.gallus.FIP-2        CSDFHAERAAREKIHEEKEQLAVQLAYLLKEQQNLEDLGRSSLAEMQNRHGARAPDREHS 510
huFIP-2               CSDFHAERAAREKIHEEKEQLALQLAVLLKENDAFEDGGRQSLMEMQSRHGARTSDSDQQ 531
S.salar.FIP-like      ------------------------------------------------------------

G.gallus.FIP-2        PRLVQRGTGSQEWPEQRNISIYSCPKC-EELPDLDTLQIHVMDCIN 555
huFIP-2               AYLVQRGAEDRDWRQQRNIPIHSCPKCGEVLPDIDTLQIHVMDCII 577
S.salar.FIP-like      ------------------------------------------------
```

Leucine zipper Zinc finger

Methodology

- **Subtractive hybridisation was carried out using material from two different stocks of salmon (susceptible Conon and resistant Neva) that had been infected with *G. salaris*.**
- **Differentially expressed cDNA was cloned and sequenced. RACE-PCR was carried out to obtain the full gene sequence.**

Results

- **Six potential candidate transcripts were retrieved.**
- **Semiquantitative RT-PCR confirmed differential expression.**
- **Clone 54T is down-regulated in *G. salaris* infected samples. This downregulation is greater in resistant Neva fish than in susceptible Conon (figure 3).**
- **The sequence shows similarity to a gene that is upregulated in viral haemorrhagic septicaemia infections of rainbow trout (figure 4) (O'Farrell *et al*., 2002).**

Figure 3

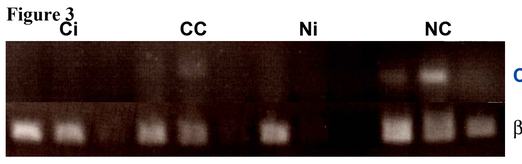


Figure 4

```
Salmon        SDLSNSCVLFSPHLSLFSP---EHKSKLSMGMGEDLDLKLHTPQAMRVVFQGRNSTEWKV 57
Oncorhynchus  MKMYGIDRLFIHLHCLWIVGYVDHTSEESLRVGETLDLKLHTPQAKKVVFQGRNSTEMTV 60

Salmon        LWMRGDEKVNRGHLEEAEGVVVLRGLRMDNSGTYKVLDSHGLSVSTVKLEVE--EVAKLQ 115
Oncorhynchus  LWMRGEEEVNSGRLKEVEGFVVLKGLKIDDCGTYTVLDSHGLLVSTVHLTVEAYQVDETQ 120

Salmon        KMIHIQ--DKPVGKRLKIKIIEMTYSRQVYQVHDTVEDWTIIVHFEHVSHLLRLS 168
Oncorhynchus  KFHEIQGKQKPIGK--------STYNR-----SSSLCMLFVLLFSPLIQHLL--- 159
```

Discussion

The 54T clone may represent a novel gene. Its expression has now been found to be modified by both VHS (viral) and *G. salaris* (parasitic) infection, but in opposite ways, upregulated in viral infection, and downregulated in *G. salaris* infection. Different compartments of the immune system can react to different pathogen types, and it is feasible that due to interactions between them, these compartments can be upregulated or downregulated depending on the type of response.

ACKNOWLEDGEMENT

This project has been funded by EU Contract No. QLRT-2000-01631

REFERENCES CITED

Bakke, T.A., Jansen, P.A. & Hansen, L.P. (1990). Differences in the host resistance of Atlantic salmon, *Salmo salar* L., stocks to the monogenean *Gyrodactylus* Malmberg, 1957. Journal of Fish Biology, 37: 577-587.

Bakke T.A. & MacKenzie, K. (1993). Comparative susceptibility of Native Scottish and Norwegian stocks of Atlantic salmon, *Salmo salar* L., to *Gyrodactylus* Malmberg: laboratory experiments. Fisheries Research, 17:69-85.

Bakke, T.A., Soleng, A. &Harris, P.D. (1999). The susceptibility of Atlantic salmon (*Salar salar* L.) x brown trout (*Salmo trutta* L.) hybrids to *Gyrodactylus* Malmberg and *Gyrodactylus derjavini* Mikailov. Parasitology, 119:467-481.

Karin, M. & Delhase, M.(2000). The IκB kinase (IKK) and NFκB: key elements of proinflammatory signalling. Seminars in Immunology, 12: 85-98.

Li. Y., Kang, J. & Horwitz (1998). Interaction of an adenovirus E3 14.7-kilodalton protein with a novel tumor necrosis factor alpha-inducible cellular protein containing leucine zipper domains. Mol. Cell. Biol., 18 (3), 1601-1610.

Michelmore, R.W., Paran, I. & Kesseli, R.V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proceedings of the National Academy of Sciences, 88: 9828-9832.

O' Farrell, C., Vaghefi, N., Cantonnet, M., Buteau, B, Boudinot, P. and Benmansour, A. (2002). Survey of transcript expression in Rainbow trout leukocytes reveals a major contribution of interferon-responsive genes in the response to a rhabdovirus infection. Journal of Virology, 76 (16):8040-8049.