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vig-1, a New Fish Gene Induced by the Rhabdovirus Glycoprotein, Has a Virus-Induced Homologue in Humans and Shares Conserved Motifs with the MoaA Family

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We used mRNA differential display methodology to analyze the shift of transcription profile induced by the fish rhabdovirus, viral hemorrhagic septicemia virus (VHSV), in rainbow trout leukocytes. We identified and characterized a new gene which is directly induced by VHSV. This VHSV-induced gene (*vig-1*) encodes a 348-amino-acid protein. *vig-1* is highly expressed during the experimental disease in lymphoid organs of the infected fish. Intramuscular injection of a plasmid vector expressing the viral glycoprotein results in *vig-1* expression, showing that the external virus protein is sufficient for the induction. *vig-1* expression is also obtained by a rainbow trout interferon-like factor, indicating that *vig-1* can be induced through different pathways. Moreover, *vig-1* is homologous to a recently described human cytomegalovirus-induced gene. Accordingly, *vig-1* activation may represent a new virus-induced activation pathway highly conserved in vertebrates. The deduced amino acid sequence of *vig-1* is significantly related to sequences required for the biosynthesis of metal cofactors. This suggests that the function of *vig-1* may be involved in the nonspecific virus-induced synthesis of enzymatic cofactors of the nitric oxide pathway.

The viral hemorrhagic septicemia virus (VHSV) is a rhabdovirus responsible for an important viral disease causing significant losses in European trout farms (9). Viral hemorrhagic septicemia, first described in 1938, is a systemic disease characterized by marked hemorrhagic lesions and exophtalmia. The rate of mortality in a juvenile stock can reach 90%. The nonsegmented RNA genome of the virus encodes six proteins (2, 4, 5, 40). The transmembrane glycoprotein (G) is the only external protein and is sufficient to induce a protective specific immune response (6, 26). The protection can be passively transferred with the serum and is ensured by neutralizing antibodies. However, nonspecific mediators are involved during the immunization (6).

The earliest antiviral response of the host is nonspecific. Upon viral infection, host cells are stimulated to change their transcription profile (16) and begin to secrete mediators as interferon and tumor necrosis factor alpha. The best-studied pathway of such cell activity modulation is the interferon system. Viruses induce interferon gene expression and then the up-regulation of various downstream interferon-responsive genes (reviewed in references 32 and 44). Some of these genes, such as 2-5 A synthetase, RNA-dependent protein kinase, RNase I, and MxA, have antiviral activity. Nitric oxide (NO) is another important compound of the nonspecific immune response to viruses. The NO synthase 2 gene is induced by gamma interferon in macrophages (11), and the antiviral effects of NO have been described in several models (19, 20, 35, 45). Although these mechanisms have been well studied in mammalian models, the cellular response to viruses is far from understood.

In the rainbow trout, *Oncorhynchus mykiss* (Walbaum), the induction of an interferon-like activity by viruses was first described in the early 1970s (10, 31). However, neither fish interferon nor cytokines involved in the regulation of the fish immune response have been unequivocally characterized so far. Among primitive vertebrates, the immune system of the rainbow trout is one of the best studied. B- and T-cell receptors have been described, and their loci have been partially characterized (3, 8, 27, 33, 37). Class I and class II major histocompatibility complex genes (12, 15, 38) and recombinating activation genes and terminal deoxytransferase have also been isolated (14). Few genes involved in the nonspecific response have been cloned in fish. Trout Mx genes (41, 42) and genes of the acute-phase proteins of the same species (18) constitute the main examples. The rainbow trout therefore constitutes a good model for the identification and characterization of new genes of immunological interest. Fundamental signaling pathways involved in innate immune mechanisms are conserved in organisms as distant from each other as *Drosophila melanogaster* and mammals (28), and several molecules of the immune system have been discovered in nonmammalian models. The Toll/cactus pathway was first described in *Drosophila* (29), several new molecules of the immunoglobulin superfamily were found in insects and mollusks (17, 25, 39), and the marker for cortical thymocyte of *Xenopus* was discovered in *Xenopus* and then retrieved in the mouse (7).

Despite increasing knowledge about the trout immune system and detailed studies about VHSV, the nonspecific host response to this pathogen is poorly described. The characterization of new key factors and pathways induced early in rainbow trout in response to viruses or by viral components is important for a better understanding of virus-host cell interactions. We used the mRNA differential display methodology (mRNA DD-PCR) (23) to isolate transcripts induced by VHSV in cells of the head kidney or pronephros, which is the most important lymphoid and hematopoietic organ in the rainbow trout. This approach led to the identification and charac-

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terization of a new rainbow trout virus-induced gene, which seems to belong to a new virus-induced pathway conserved in vertebrates.

MATERIALS AND METHODS

Viruses and reagents. The fish experiments were conducted in the Jouy-en-Josas fish experimental facilities. Pathogenic isolate 07-71 of VHSV (22) was used. When necessary, VHSV was inactivated with beta-propiolactone (BPL) at 1/4,000 for 1 h at room temperature and then overnight at 4°C. Cycloheximide (CHX) (Sigma) was used at 100 μg/ml. For fish DNA immunizations, we used the VHSV glycoprotein gene cloned in pcDNA1 (Invitrogen) or the plasmid alone as a control as described in reference 6. The fish genetic immunizations were performed as described in reference 6.

In vitro transcription-translation assay. VIG-1 polypeptide was produced by in vitro transcription-translation by using the TNT T7 reticulocyte system from Promega; microsomes and endoglycosidase H were purchased from Boehringer. In vitro transcription-translation assays in the presence of ^{35}S-labelled methionine and endoglycosidase digestion (2 μU per reaction) were performed according to the manufacturer's protocols.

mRNA DD-PCR. For mRNA DD-PCR analysis, 5.10^7 to 10^8 head kidney cells from a single trout were incubated with VHSV (1 PFU per cell) or BPL-inactivated VHSV or without virus and cultured for 40 h at 14°C in RPMI medium containing 2% fetal calf serum. Total RNA was extracted with Trizol reagent (Life Technologies) and treated with RNase-free DNase I (Boehringer). First-strand cDNAs were synthesized by using an anchored deoxyribosylthymine, (dT) primer, and then PCR amplified with the same oligo(dT) primer combined with 10-mer random oligonucleotides (23). After separation on sequencing gel, differentially displayed bands were excised and reamplified under the same conditions, and the products were cloned into the pCR-Script plasmid (Stratagene).

Semiquantitative reverse transcriptase (RT)-PCR. Semiquantitative RT-PCR assays were performed as described in reference 13. Briefly, PCR conditions were 94°C for 8 min and then 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, for 25 to 30 cycles. PCRs for quantitation purposes were stopped in the exponential phase of amplification, and tests were performed on serial dilutions of templates. The total amount of cDNA was calibrated on the basis of the amplification of actin cDNA. Actin and Mx primers are defined in reference 6. *vig-1* primers were CAGTTCAGTGGCTTTGACGA and ACAAACGCCTCAAGG TATGG (amplified product, 232 bp).

Northern blot analysis. Total RNAs (20 μg) were fractionated in 1.2% agarose gel, 10% morpholinepropanesulfonic acid (MOPS) and 10% formaldehyde and blotted onto Hybond N-plus membranes (Amersham). The blots were probed with a random hexanucleotide-primed ^{32}P-labelled cDNA made from a *vig-1* clone (1.5 kb). The blots were rehybridized with a beta-actin probe to control the amounts of mRNAs loaded on the gels.

Nucleotide sequence accession number. The sequence of *vig-1* has been deposited in the GenBank database under accession no. AF076620.

RESULTS

Identification of *vig-1*, a cDNA differentially expressed following VHSV induction. In order to characterize new fish genes expressed in response to viral infection or induction, we used mRNA DD-PCR methodology because it allows comparative analysis of the whole set of transcribed genes in cells subjected to different treatments (24). We used cells from the head kidney, as the pronephros is the main lymphoid organ in fish. To avoid the effects of different genetic backgrounds, we performed differential display analysis on leukocytes from a single trout. Head kidney cells were separated on a Ficoll gradient, divided into three aliquots, and then incubated for 40 h at 14°C with live or BPL-inactivated VHSV (BPL-VHSV) or with medium alone as a control. A cDNA was synthesized with an anchored dT primer and then PCR amplified with a set of 12 arbitrary primers in the presence of [^{32}P]dCTP. Among the 12 primer combinations used for the differential display, a pair of primers (5′-CTTGATTGCC-3′ and 5′-TTTTTTTTTT CG-3′) led to the amplification of one discrete band of 650 bp from samples treated with live or inactivated VHSV but not from the control. The DNA product within this band was reamplified, cloned, and sequenced. To confirm the viral induction of the transcript, we performed a semiquantitative RT-PCR assay (13) with RNA extracted from virus-treated and untreated rainbow trout head kidney cells, using a set of primers derived from the sequence of the insert. Figure 1A shows that a specific amplification was obtained only with samples derived from VHSV-treated cells. Northern blot analysis was finally performed to authenticate the viral induction. A signal was observed only with RNA samples from VHSV-treated cells (Fig. 1B). In both tests, rainbow trout actin was used as a control of the amount of RNA. The size of the VHSV-induced transcript was estimated at 1.5 kb by comparison with RNA size markers. These results clearly establish that this transcript was induced by live or inactivated VHSV. The corresponding gene was therefore named *vig-1* for VHSV-induced gene no. 1.

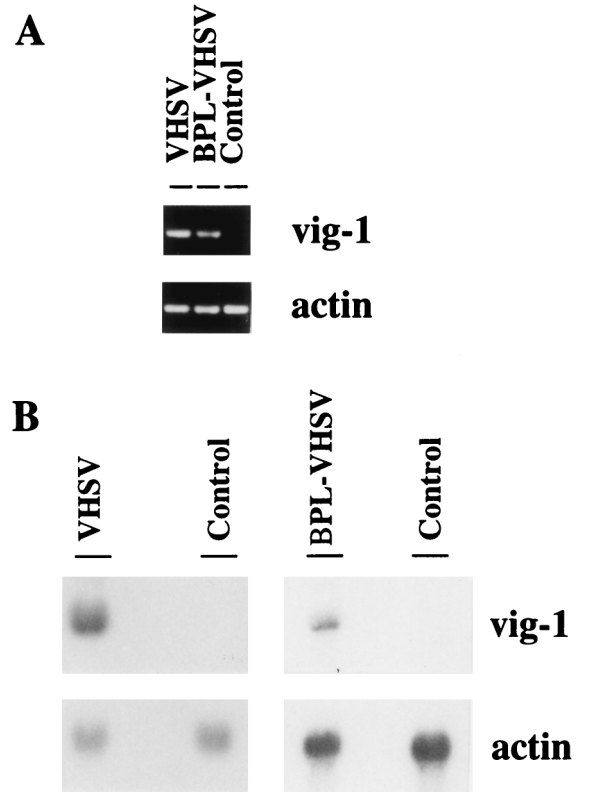

FIG. 1. VHSV-induced expression of *vig-1*. (A) Semiquantitative PCR assay on cDNA from rainbow trout head kidney cells cultured as described with VHSV (VHSV), with BPL-inactivated virus (BPL-VHSV) or without virus (Control). The samples were normalized on the basis of actin expression. (B) Northern blot analysis of *vig-1* expression in rainbow trout head kidney cells treated as described in panel A. The same blots were hybridized with an actin probe as a control for the total amounts of RNA loaded in the gels.

Characterization of *vig-1*. In order to clone the full-length cDNA of *vig-1*, we first looked for the presence of the transcript in different tissues. We analyzed *vig-1* expression by a sensitive RT-PCR assay, using internal specific primers in the fibroblast-like cell line rainbow trout gonad-2 (RTG-2) and in different trout tissues. *vig-1* was not expressed in RTG-2 cells and was not amplified from a cDNA library made from this cell line. It was weakly expressed in unstimulated spleen and head kidney but not in the muscle, suggesting lymphoid or myeloid expression (data not shown). Subsequently, *vig-1* was detected in a cDNA library made from the spleen of a naive rainbow trout. This library was therefore screened to retrieve the *vig-1* cDNA. All analyzed clones had an insert of 1.5 kb in accordance with the size of the mRNA assessed by Northern blot analysis and suggesting that they correspond to full-length cDNAs. Three clones were fully sequenced, leading to a

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                ACTCGACAGGCCAGGTGAAGACTAGTAATATTGTTCTCTACCCAAATCGTGTTTGTGAAG
1   ATGTTCCTACAGCGCTGCATGAGTTTCCTCCAGTGCATCTTTGCAGCTGTTCTGGCCTGG  60
    M  F  L  Q  R  C  M  S  F  L  Q  C  I  F  A  A  V  L  A  W
    ATCGGTGTGCGAGGACAGCAGGTGCACGGCGCATCACAGTTCTCTTCTACAGGAAAAGTC 120
    I  G  V  R  G  Q  Q  V  H  G  A  S  Q  F  S  S  T  G  K  V
    AATCCAGCTACAATCAAGGTGGTGAACAATGTTATTTCTCAAGCTTCGGCAACTCCAAGC 180
    N  P  A  T  I  K  V  V  N  N  V  I  S  Q  A  S  A  T  P  S
    AGTGTCAATTATCATTTTACCCGACAGTGCAATTATAAGTGCGGTTTTTGTTTCCACACT 240
    S  V  N  Y  H  F  T  R  Q  C  N  Y  K  C  G  F  C  F  H  T
    GCAAAAACGTCTTTTGTCCTACCTATTGAGGAAGCAAAGAGAGGTTTACAGCTTCTGAAG 300
    A  K  T  S  F  V  L  P  I  E  E  A  K  R  G  L  Q  L  L  K
    GAATCAGGACTGGAAAAAATAAACTTTTCTGGCGGAGAGCCTTTCATACACGACAGAGGA 360
    E  S  G  L  E  K  I  N* F  S  G  G  E  P  F  I  H  D  R  G
    GATTTCTTGGGGAAATTAGTCCAATACTGCAAACACGACCTCCAGCTCCCAAGTGTCAGT 420
    D  F  L  G  K  L  V  Q  Y  C  K  H  D  L  Q  L  P  S  V  S
    ATTGTCAGCAATGGCAGCATGATCAGAGAAAAGTGGTTCCAAACATATGGCGAATACCTG 480
    I  V  S  N* G  S  M  I  R  E  K  W  F  Q  T  Y  G  E  Y  L
    GACATTCTGGCCATTTCTTGTGACAGCTTTGACGAAGACACCAACCAGACTATTGGCAGA 540
    D  I  L  A  I  S  C  D  S  F  D  E  D  T  N* Q  T  I  G  R
    GCCCAGGGCAGGAAGAGCCACCTGGACAACCTCTTCAAGGTCCGTGACTGGTGCCGGAAG 600
    A  Q  G  R  K  S  H  L  D  N  L  F  K  V  R  D  W  C  R  K
    TACAAAGTGGCGTTCAAAATCAACTCTGTGATCAACACCTTCAATGTGGATGAAGACATG 660
    Y  K  V  A  F  K  I  N  S  V  I  N  T  F  N  V  D  E  D  M
    AGAGAAAACATCACAGAACTCAACCCTGTACGCTGGAAGGTGTTCCAGTGTCTGCTGATC 720
    R  E  N* I  T  E  L  N  P  V  R  W  K  V  F  Q  C  L  L  I
     GATGGCGAGAACGCTGGGGAGAACAGTCTGAGGGAGGCAGAGAGGTTTCTCATCAGTGAT 780
    D  G  E  N  A  G  E  N  S  L  R  E  A  E  R  F  L  I  S  D
    CAGCAGTTTCAGGACTTCCTGGAAAGGCACAGCAGCATCAGCTGCCTGGTACCAGAGTCC 840
    Q  Q  F  Q  D  F  L  E  R  H  S  S  I  S  C  L  V  P  E  S
    AATCAGAAGATGAGAGATTCCTACCTCATTCTGGATGAATATATGCGTTTCCTGGATTGC 900
    N  Q  K  M  R  D  S  Y  L  I  L  D  E  Y  M  R  F  L  D  C
    CGAGAGGGGAGGAAAGATCCATCCAAGTCCATCCTTGATGTTGGCGTGGAAGAGGCCATT 960
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    K  A  D  M  R  L  E  W
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    TGTATGAATTGCAGTGTGTTACATCTCTCTGTGTTTGTAATGTATCTCAAATAAATACAA 1500
    AACGATGACTTTGCATGATACAAAAAAAAAAAAAAA                          1536
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FIG. 2. Sequence of *vig-1* cDNA. Rainbow trout *vig-1* cDNA nucleotidic and deduced amino acid sequences. The initiation codon, the termination codon, and the polyadenylation signal are in bold. Potential glycosylation sites are indicated by a bold-faced N*.

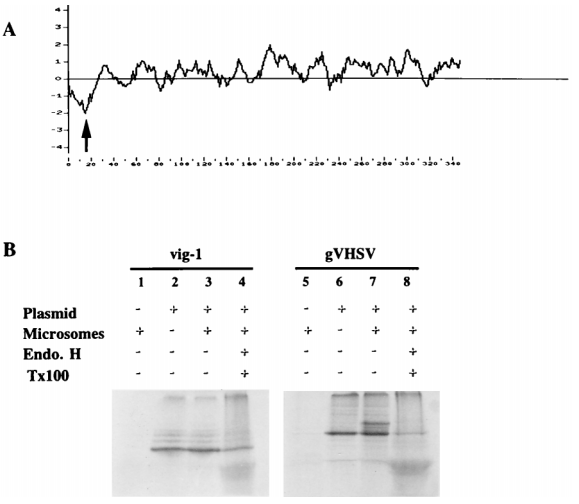

FIG. 3. VIG-1 has no signal peptide. (A) The VIG-1 Kyte-Doolittle hydropathic profile shows a hydrophobic stretch at the N terminus of the protein (amino acids 6 to 23, arrow). (B) In vitro transcription-translation and microsome assays were performed with a plasmid containing *vig-1* cDNA. The same experiments were performed with a plasmid containing gVHSV cDNA in order to control the efficiency of microsome assay. When the extract was supplemented with dog pancreatic microsomes, a mobility shift was detected for gVHSV but not for VIG-1 (lanes 3 and 7). This shift can be abolished by endoglycosidase H treatment (lane 8), which has no effect on the VIG-1 product (lane 4).

1,535-bp cDNA sequence. The sequence starts 60 nucleotides (nt) upstream from the first ATG codon, and contains a 1,044-bp open reading frame (ORF) encoding 348-amino-acid residues (Fig. 2). An AATAAA poly(A) consensus signal is present 445 nt downstream of the termination codon and 32 nt upstream of the poly(A) stretch. Sequence analysis of the polypeptide deduced from the *vig-1* sequence shows the presence of a short hydrophobic N-terminal region at positions 6 to 11 which could constitute a signal peptide (Fig. 3A). However, VIG-1 is probably not a type I transmembrane protein, since no other hydrophobic region was observed. The presence of four putative N-glycosylation sites (at positions 108, 144, 175, and 223) may suggest that VIG-1 is a secreted glycoprotein. To determine if the protein can enter the rough endoplasmic reticulum-Golgi pathway, we performed in vitro-coupled transcription-translation of *vig-1* in the presence of microsomes. The in vitro translation assay produced a 39-kDa protein product, consistent with the predicted amino acid sequence from the cDNA (Fig. 3B, lane 2). When the microsomes were added to the extracts, we could not detect any shift of the VIG-1 protein, while two bands were detected for the glycoprotein of VHSV under the same conditions (Fig. 3B, lane 7). Endoglycosidase H treatment showed that a part of VHSV glycoprotein was indeed glycosylated in the presence of microsomes. Thus, these results suggest that the short hydrophobic stretch at the N-terminal end of VIG-1 does not allow the protein to enter the rough endoplasmic reticulum-Golgi pathway. Thus, VIG-1 is probably a cytoplasmic protein.

***vig-1* is induced during VHSV infection.** To determine if *vig-1* is induced in vivo during the VHSV infection, we experimentally infected juvenile rainbow trouts with laboratory strain 07-71 of VHSV. *vig-1* induction was then searched for in infected trout tissues by a RT-PCR assay. *vig-1* mRNA was strongly expressed in the head kidney on day 6 after infection, before the onset of clinical signs of the disease but after the virus had fully replicated and accumulated in this lymphoid organ (Fig. 4). On the contrary, *vig-1* was not detected at day 1 postinoculation, indicating that *vig-1* induction was most probably dependent on direct viral induction of head kidney cells. Weak *vig-1* expression was observed in the muscle tissue at the site of virus injection (data not shown) and could have resulted from direct induction of a few infiltrating cells.

The VHSV glycoprotein is sufficient to induce *vig-1* in vivo. The accumulation of *vig-1* mRNA was observed after incubation of head kidney cells with BPL-inactivated VHSV, indicating that neither virus replication nor viral protein synthesis is necessary for *vig-1* induction. VHSV has a unique transmembrane glycoprotein (G protein), which is the protein most likely to interact with host cells. In order to determine whether the G protein alone is able to induce *vig-1*, we used DNA immunization of fish with a plasmid encoding for the VHSV glyco-

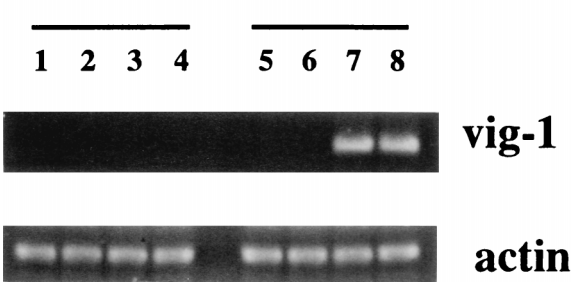

FIG. 4. *vig-1* induction during the experimental disease. *vig-1* mRNA expression was assessed by RT-PCR in the head kidney during the course of VHSV infection. *vig-1* transcripts were searched for 24 h (lanes 3 and 4) or 6 days (lanes 7 and 8) after trout were infected with VHSV. Trout injected with phosphate-buffered saline were used as controls at 24 h (lanes 1 and 2) or at 6 days (lanes 5 and 6). Total amounts of cDNA were controlled by the amplification of actin mRNA from the same samples.

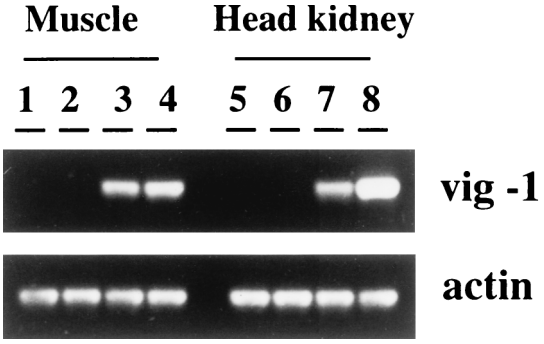

FIG. 5. *vig-1* induction after genetic immunization against the glycoprotein of the VHSV. *vig-1* mRNAs were detected in the muscle at the site of injection (lanes 3 and 4) and in the head kidney (lanes 7 and 8) by RT-PCR, 7 days after genetic immunization with pCDNA1_GVHSV. Trout injected with pCDNA1 were used as controls (lanes 1 and 2 for muscle and 5 and 6 for head kidney). Total amounts of mRNA were controlled by the amplification of actin mRNA from the same samples.

protein (pcDNA1_GVHSV). We have previously shown that intramuscular immunization of trout with pcDNA1_GVHSV led to the expression of the protein at the site of injection and to the elicitation of a strong protective immunity (6). By using a RT-PCR assay, we analyzed *vig-1* expression after pcDNA1_GVHSV or pcDNA1 injection. *vig-1* was induced both in muscle tissue at the site of plasmid injection and in the head kidney on day 7 after immunization with pcDNA1_GVHSV (Fig. 5, lanes 3 and 4 and 7 and 8). Conversely, fish injected with the control pcDNA1 plasmid did not express *vig-1* (Fig. 5, lanes 1 and 2 and 5 and 6). The expression of actin mRNA was assessed in all samples in order to check for the presence of similar amounts of RNA. The VHSV glycoprotein expressed by transfected cells in the muscle tissue is probably sufficient to induce the in vivo expression of *vig-1*.

***vig-1* is induced through different pathways.** *vig-1* induction could be directly mediated by VHSV or through a secondary cellular mediator. An indication that the expression of *vig-1* may be independent of the interferon pathway was obtained from experiments with the fibroblast-like cell line RTG-2. *vig-1* was not inducible in these cells, although they produced an interferon-like activity and expressed *Mx* mRNAs following treatment with live or inactivated VHSV (Fig. 7A). Thus, the activation of the interferon-like pathway alone is not sufficient to induce the expression of *vig-1* in RTG-2 cells.

To test the hypothesis of direct induction, we analyzed the kinetics of *vig-1* expression in virus-stimulated rainbow trout head kidney cells. As a time scale for secondary induction, we used the *Mx* gene, which is interferon responsive in mammals. Figure 6A shows that *vig-1* was expressed as early as 6 h after in vitro infection of head kidney cells with VHSV at 20°C, while Mx transcripts were not detected before 10 h. To further ascertain that *vig-1* is directly induced by VHSV, we infected head kidney cells in the presence of CHX, a potent inhibitor of protein synthesis. CHX did not prevent the accumulation of *vig-1* transcripts (Fig. 6B). Thus, no new protein synthesis is necessary for *vig-1* induction by VHSV. These observations are consistent with the direct induction of *vig-1*.

The fact that *vig-1* is directly induced by the viral G protein does not exclude a role for soluble factors in the control of *vig-1* expression. To clarify the possible role of soluble factors, we treated head kidney cells with the supernatant from fish cells conditioned to produce an interferon-like activity. The supernatant from RTG-2 cells treated with vesicular stomatitis virus was extensively centrifuged to eliminate viral particles,

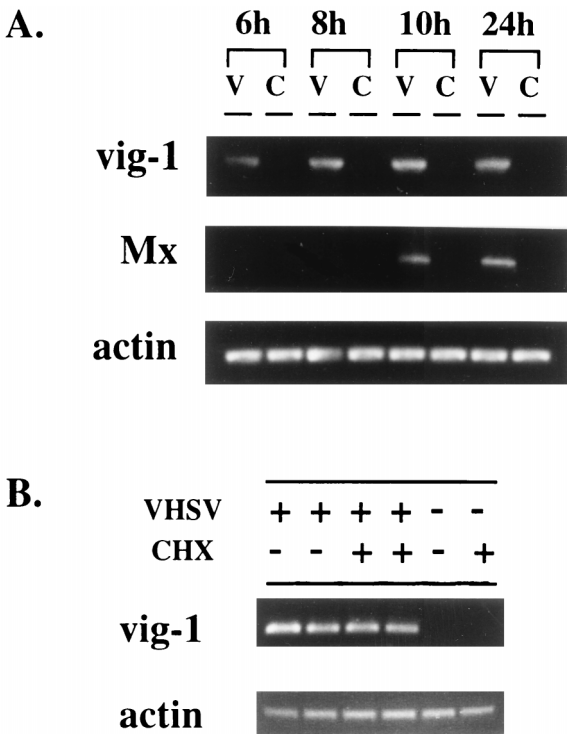

FIG. 6. The induction of *vig-1* is directly mediated by viral particles. (A) Kinetics of *vig-1* induction in rainbow trout head kidney cells cultured in the presence of VHSV (V) or without virus (C) at 20°C. *vig-1* and *Mx* mRNAs were searched for by a RT-PCR assay at 6, 8, 10, and 24 h. Samples were normalized on the basis of the actin expression. (B) Effect of CHX on *vig-1* mRNA induction. *vig-1* mRNAs were detected by RT-PCR assay from cells cultured 7 h with VHSV, with or without CHX. Cells cultured without virus in the presence or absence of CHX were used as controls, and samples were normalized on the basis of actin expression.

subjected to acid treatment at a pH of 2.2, and titrated for its antiviral activity. This conditioned supernatant was used to stimulate rainbow trout head kidney cells. Figure 7B shows that both *vig-1* and *Mx* mRNAs were strongly induced in the leukocytes that were treated with it. Under semiquantitative RT-PCR conditions, a dose effect was observed at high dilutions (lanes 3 to 5), while neither *vig-1* nor *Mx* transcripts were detected in RNAs from the cells incubated with the nonconditioned medium (lane 6).

Together, these observations suggest that two pathways are available for *vig-1* induction. The first pathway is directly mediated by the viral particles and most probably by the G protein, and the second is correlated with the presence of an interferon-like activity and probably requires virus replication. To confirm the latter possibility, we tested the effects of a virus devoid of a transmembrane glycoprotein, using a nonenveloped fish birnavirus, infectious pancreatic necrosis virus (IPNV). Figure 7C shows that *vig-1* is induced in head kidney cells by live IPNV but not by the inactivated virus, confirming that viral replication results in *vig-1* expression.

***vig-1* is a member of a group of virus-induced genes conserved in vertebrates and related to sequences involved in the biosynthesis of enzymatic cofactors.** Sequence homology searches with the entire *vig-1* sequence through dbEST database indicated that *vig-1* is homologous to four human EST sequences and one mouse EST sequence, showing that genes similar to *vig-1* are expressed in mammals. EST sequences AA054298 and AA036920 are from human pregnant uterus,

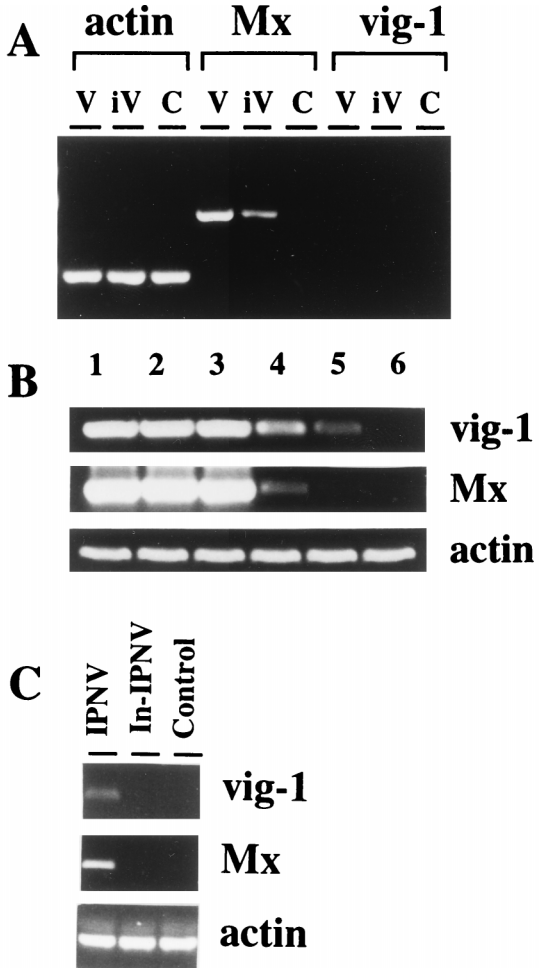

FIG. 7. Induction of *vig-1* in response to interferon. *Actin*, *Mx*, and *vig-1* mRNAs were assessed by a RT-PCR assay in different experiments. (A) RTG-2 cells incubated with live VHSV (V) or BPL-inactivated virus (iV) or without virus (C). (B) Head kidney cells were stimulated by serial dilutions of conditioned medium displaying a trout interferon-like activity (lanes: 1, 1/2; 2, 1/10; 3, 1/100; 4, 1/1,000; 5, 1/10,000). Cells cultured with unconditioned medium were processed similarly as a control (lane 6). (C) Head kidney cells cultured with live IPNV (IPNV) or inactivated IPNV (In-IPNV) or without virus (Control).

AA360817 is from a human T-cell lymphoma, AA542387 is from a mouse T-cell clone, and AA263079 is from the acute human myelogenous leukemia KG1-a. The 3′ end of the ORF in the mouse EST sequence AA542387 has 72% similarity with the *vig-1* ORF over 236 bp. Moreover, a cytomegalovirus-induced human gene (*cig-5*) described in a recent study is similar to *vig-1* (46). Although the reported partial sequence of the human transcript is longer than *vig-1* mRNA (3.1 versus 1.5 kb), the C termini of the deduced amino acid sequences of *vig-1* and *cig-5* are highly homologous (80% identity over 291 amino acids) as shown in Fig. 8A. These similarities suggest that *vig-1* is probably a member of a group of virus-induced genes.

Given the remarkable conservation of *vig-1* during vertebrate evolution, we searched for related sequences in other organisms. We found proteins showing highly significant homology with VIG-1 belonging to three families, MoaA, NIRJ, and PQQIII. MoaA proteins, described in bacteria, plants, fungi, and vertebrates, are involved in the synthesis of molybdopterin cofactors. NIRJ is necessary for the synthesis of heme d1, a cofactor of bacterial nitrite reductase, and PQQIII is required for the synthesis of the bacterial cofactor pyrroloquinoline-quinone. Four motifs are conserved in VIG-1, CIG-5, MoaA, NIRJ, and PQQIII (Fig. 8B), and the pattern CNXXCXXC (motif I, at positions 69 to 76 in VIG-1) corresponds to the so-called MoaA/PQQIII signature (prosite entry, PDO01009). The cysteines were shown to be important to the biological function of the MoaA protein, most probably for the coordination of an Fe-S cluster (30).

DISCUSSION

In this work we describe the identification and characterization of a rainbow trout gene which is induced in vitro and in vivo during infection with the fish rhabdovirus VHSV. The gene was named *vig-1* for VHSV-induced gene number 1.

vig-1 was identified in VHSV-induced rainbow trout leukocytes fractionated from the head kidney. It is expressed at a low level in the spleen and the head kidney, the main hematopoietic organs in trout. *vig-1* is not expressed in the muscle or in the fibroblast-like trout cell line RTG-2. Moreover, *vig-1* cDNA was retrieved from a spleen cDNA library, but it was absent in a cDNA library made from RTG-2 cells. *vig-1* therefore seems to be specifically expressed in lymphomyeloid cells. Further characterization of the tissular distribution awaits specific markers of fish immune cells and appropriate fish cell lines.

Since we observed the induction of *vig-1* with inactivated VHSV, the question of which viral component is involved was raised. Our conviction that the G protein can induce *vig-1* is based on several arguments. First, the G protein is the only external protein of the virus, and second, DNA immunization by a plasmid bearing the gene of G protein induces *vig-1*. We cannot exclude the possibility that specific DNA sequences in the G gene induce local inflammation and *vig-1* expression. However, this possibility is unlikely, since the plasmid DNA alone had no effect. Moreover, inactivated IPNV, a nonenveloped birnavirus devoid of external glycoprotein, does not induce *vig-1*.

Induction of *vig-1* appears to occur through two different pathways; it can be obtained directly through a viral component or indirectly through a soluble factor, probably the fish interferon. The direct pathway probably involves the G protein, since we have shown that it is the most likely viral *vig-1* inducer. The indirect *vig-1* induction pathway may also involve the G protein, since viral glycoproteins are well known to induce the expression of cellular factors, including alpha interferon (1, 21). Since we have shown that a fish interferon-like product induces *vig-1*, all known interferon inducers, nucleic acids, viral glycoproteins, and cytokines should normally induce *vig-1*, as observed following infection with live IPNV. The use of several pathways for the induction of *vig-1* supports an important role for the host response to viral infections.

vig-1 is highly homologous to murine and human EST sequences, indicating that it corresponds to a sequence conserved during the evolution of fish and mammals. Furthermore, using a similar approach with human cells, Zhu et al. (46) identified a cytomegalovirus-induced gene (*cig-5*) which has important characteristics in common with *vig-1* (46), showing the potential importance of these genes. Besides the high similarity of the deduced amino acid sequences, *cig-5* is induced directly by the inactivated virus and indirectly through interferon alpha (46). The conservation of the gene and its activation pathway suggests that *vig-1* and *cig-5* may correspond to important components of the nonspecific antiviral response.

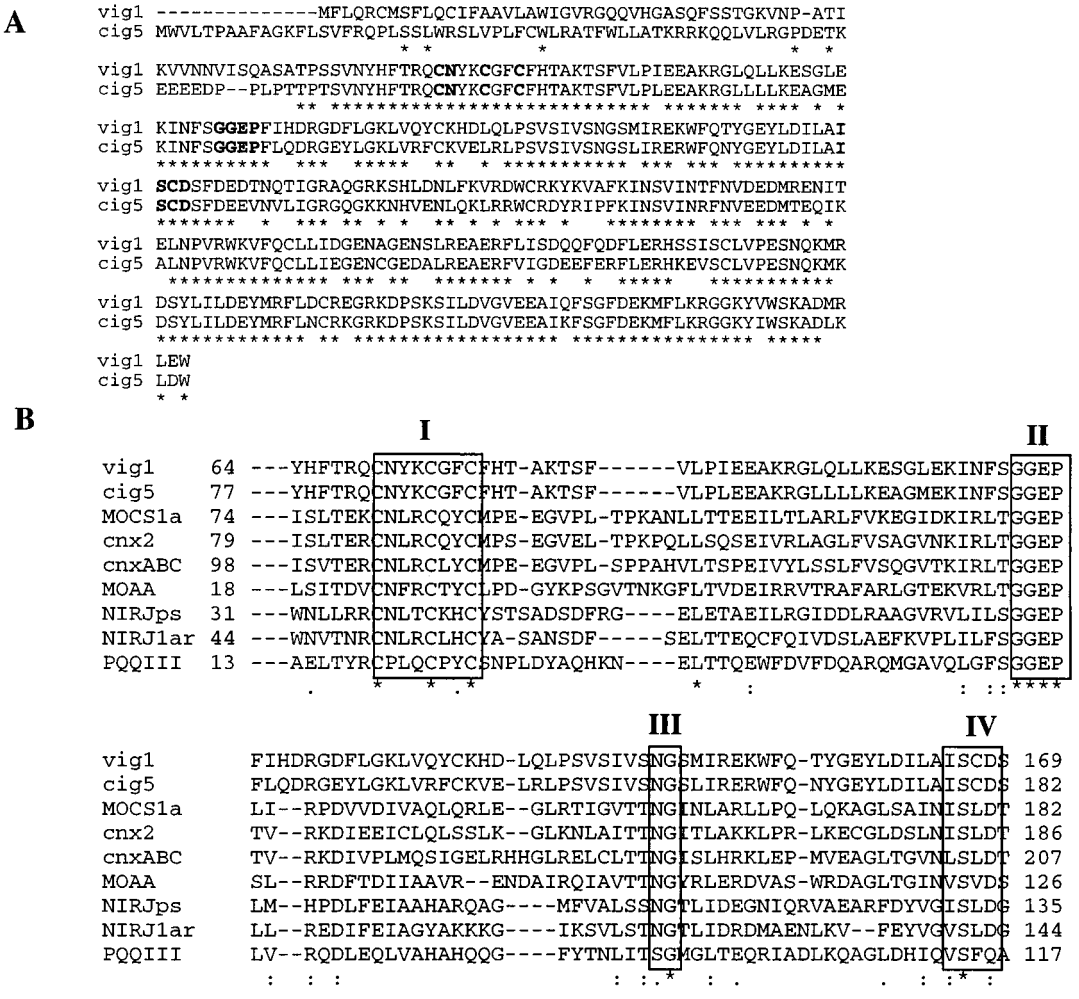

FIG. 8. Multiple alignments of the predicted amino acid sequence of VIG-1 with related proteins. (A) VIG-1 is aligned with the sequence deduced from human *cig-5* gene (accession no. AF026941). (B) The following proteins are aligned with VIG-1 and CIG-5: MoaA proteins from human (MOCS1a, accession no. AF034374), *Arabidopsis thaliana* (cnx2, accession no. Z48047), *Aspergillus nidulans* (cnxABC, accession no. AF027213), and *Escherichia coli* (MoaA, accession no. AE000181), NIRJ proteins from *Pseudomonas aeruginosa* (NIRJps, accession no. D84475) and *Archaeoglobus fulgidus* (NIRJ1ar, accession no. AE001026), and coenzyme pyrroloquinoline-quinone biosynthesis protein III from *Acinetobacter calcoaceticus* (PQQIII, accession no. X06452). Grey shaded boxes represent conserved motifs, and conserved residues are indicated by an asterisk. The three cysteines in the first box (I) constitute a potential iron-sulfur metal binding site.

In addition, we found that the VIG-1 amino acid sequence is significantly homologous to proteins required for the synthesis of molybdopterin (MoaA family), heme d1 (NIRJ), PQQ (PQQIII), and enzymatic cofactors. The iron-sulfur motif CNXXCXXC was strictly conserved in all these sequences during the evolution of prokaryotes and eukaryotes. MoaA proteins are required for the synthesis of an early precursor of the pterin compounds involved in the biosynthesis of molybdopterin cofactors (34, 36). Interestingly, the inducible NO synthase, which is involved in antiviral defense, binds a tetrahydrobiopterin cofactor. Furthermore, this cofactor regulates the balance of NO versus superoxide production by NO synthase (43). Conservation of the MoaA signature in VIG-1 could therefore indicate that this virus-induced protein may be required for the synthesis of an enzymatic cofactor modulating the efficiency of NO synthesis in vertebrates. Obviously, further studies are necessary to assess the precise significance of these homologies and to resolve the question of the VIG-1 protein function. However, the connection between nitrogen metabolism and resistance to pathogens is the most evident link between the intrinsic characteristics of VIG-1 and its virus-induced expression.

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