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Antiviral Research 66 (2005) 39–45
Antiviral Research
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Differential profile of genes expressed in hemocytes of White Spot Syndrome Virus-resistant shrimp (*Penaeus japonicus*) by combining suppression subtractive hybridization and differential hybridization☆

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Received 15 September 2004; accepted 15 December 2004

Abstract

White Spot Syndrome Virus (WSSV) is the major viral pathogen of culture shrimp. Although remarkable progress has been made in characterizing the WSSV genome, information concerning the antiviral process of host is still limited. To identify the genes differentially expressed along with their expression profile in the hemocytes of the virus-resistant shrimp, suppression subtractive hybridization (SSH) and differential hybridization (DH) were employed. Relying on the sequences identified in the subtractive cDNA library, 30 genes were characterized to be involved in the antiviral process as defense-relevant, among them, 22 are found for the first time in penaeid shrimp. The most interesting finding is that the interferon-like protein (IntlP) and (2′–5′) oligo(A) synthetase-like protein (data not shown) known as the antiviral factors showed increased expression in virus-resistant shrimp and the non-specific antiviral activity of IntlP protein was verified by cytotoxicity experiment. A number of proteins with certain similarities to the components of the complement and cytokines system in vertebrates were also found in the subtracted library. The high expression of redox-related factors (NADH dehydrogenase, glutathione peroxidase and transcription factor AP-1 precursor), plasma defensive protein (C-type lectin and laminin-like protein) and translationally controlled tumor protein (TCTP) in the virus-resistant shrimp suggested that they are essential components participating in the antiviral process. Our work provides a wide array of genes differentially expressed in the virus-resistant shrimp, and a framework for further studies aimed at antiviral mechanism in shrimp.

Keywords: Antiviral factor; Virus resistance; Differential profile; *Penaeus japonicus*

1. Introduction

White spot syndrome (WSS) is the most serious viral disease of culture shrimp in the world (Lightner, 1996). Since 1993, WSS has been found in most shrimp farming regions in Southeast Asia, America, Europe and Australia and causing catastrophic economic losses. Although significant progress has been made in detection and characterization of White Spot Syndrome Virus (WSSV) in recent years, information pertaining to the antiviral activities of shrimp remains elusive.

Shrimp, like other invertebrates, lacks a true adaptive immune system, instead, it relies on various innate immune responses to fight against invading pathogens. Knowledge about shrimp defense at the molecular level has extended rapidly in recent years. Several genes involved in antimicrobial activity have been cloned and characterized, such as prophenoloxidase (proPO) activating system (Söderhäll et al., 1994; Sritunyalucksana et al., 1999), and antimicrobial peptides (Destoumieux et al., 1997, 2000). In spite of these

☆ The sequences reported in this paper have been submitted to GenBank with accession respective numbers.

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doi:10.1016/j.antiviral.2004.12.010

progresses, information about genes involved in the antiviral ability of shrimp is still scanty, to date only two papers dealing with this topic were published (Luo et al., 2003; Zhang et al., 2004), thus limiting the understanding of innate immunity of shrimp, especially its antiviral mechanism.

In order to isolate genes up-regulated in virus-resistant shrimp and identify their expression profile, suppression subtractive hybridization (SSH) (Diatchenko et al., 1996) and differential hybridization (DH) were adopted to compare the genes expressed in the hemocytes of virus-sensitive (normal) and virus-resistant shrimp *Penaeus japonicus*. Hemocyte is known as an immune cell in shrimp. It produces many immune factors, such as antibacterial peptides, proPO, signal molecules and also participates in most of the immune activities. In this paper, a number of differentially expressed genes (fragments) were identified and most of them are found for the first time in shrimp. These results may facilitate the overall understanding of the virus-resistance mechanism in shrimp.

2. Materials and methods

2.1. Shrimp

Virus-sensitive (normal) and virus-resistant shrimp *P. japonicus* were collected from shrimp farms in Zhangpu, Fujian, China. In one of the ponds, most shrimp died during an outbreak of WSSV, but still a few of them survived. The activities of immune factors, such as phenoloxidase (PO), hemolysin and hemaggulutinin, were obviously higher in surviving shrimp than those in normal shrimp (data not shown). Shrimp collected from the neighbor pond were used as control (normal shrimp). These shrimp were originated from the same group of larvae and grew up under the same culture conditions, but were not infected by virus. No WSSV was detected in both normal and virus-resistant shrimp by polymerase chain reaction (PCR) (Wang et al., 1999).

2.2. WSSV challenge experiment

This experiment was designed to confirm the character of virus-resistance of the surviving shrimp. Animals were maintained in 500 l tanks (at 25 °C) filled with air-pumped circulating sea water. WSSV inoculum was prepared from virus-infected shrimp tail muscle tested positively by PCR (Wang et al., 1999). Frozen infected tissue was homogenized in 1× PBS (1:5, w/v) and centrifuged at 5000 rpm for 10 min. The supernatant filtered through a 0.45-μm-pore-size filter was used for injection. Each surviving shrimp was injected with 100 μl WSSV inoculum at a point between the second and third tail tergal plates on the lateral sides by a 1-ml sterile syringe. Shrimp were challenged with WSSV four times. The interlude between two challenges is one week. (Normally, the majority of the virus-sensitive shrimp died after the one-week challenge with WSSV.)

2.3. Hemocytes collection and preparation of poly(A)$^+$ RNA

Hemolymph was taken from the ventral sinus using a 1 ml sterile syringe, with 100 μl anticoagulant (500 U/ml heparin sodium salt) preloaded. The amount of hemolymph collected ranged from 200 to 500 μl, depending on the size of the shrimp. The hemolymph was centrifuged immediately at 600 × *g* for 5 min (4 °C) to separate the hemocytes from plasma. Poly(A)$^+$ RNA was extracted from hemocytes of virus-resistant or virus-sensitive (normal) shrimp, using Oligotex mRNA kit (Qiagen, Valencia, CA) according to the manufacture's instruction.

2.4. SMART cDNA synthesis and suppression subtractive hybridization

cDNAs synthesis and subtraction were performed with SMART^{TM} PCR cDNA Synthesis Kit and PCR-Select cDNA Subtraction kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. A and B are assigned to denote the cDNAs from virus-resistant and virus-sensitive shrimp, respectively. The forward-subtracted cDNA pool ["F cDNA pool", cDNA A as tester and cDNA B as driver: A–B] and reverse-subtracted cDNA pool ["R cDNA pool", cDNA B as tester and cDNA A as driver: B–A] were generated. Subtraction of cDNA A from cDNA B will enrich for virus-resistance-relevant cDNAs. To evaluate the efficiency of cDNA subtraction, the content of the cDNA for β-actin (forward, 5′-GCCGGATCCCTCCGTCTGG ATCTGGCT-3′; reverse, 5′-TATCGAATTCTTAGAAGC ACTTCCTGTG-3′) by PCR in subtracted and unsubtracted cDNA pools were compared.

2.5. Differential hybridization

Subtracted cDNAs (F cDNA pool and R cDNA pool) were cut free of the primer sequences at both ends and labeled with Digoxigenin-11-dUTP by DIG High Prime DNA Labeling and Detection Starter Kit II (Roche) according to manufacturer's instruction. PCR products of the clones from the forward-subtracted cDNA pool were spotted identically on two 8 cm × 12 cm positive-charge nylon membranes and then analyzed by hybridization to the labeled forward- and reverse-subtracted cDNAs overnight at 65 °C, respectively, according to manufacturer's instruction.

2.6. Sequencing and FASTA homology search

The differential clones confirmed by differential hybridization were sequenced by Shanghai Sangon Co., Ltd. The sequences obtained were searched in GenBank for homology with FASTA program and then analyzed.

2.7. Virtual Northern hybridization

Virtual Northern hybridization was adopted to compare the differential expression of responsive genes in virus-resistant and virus-sensitive (normal) shrimp.

In virtual Northern hybridization (Hämmerle et al., 2003), a fraction of the generated first-strand Driver and Tester cDNA were used for PCR. The number of PCR cycles was 18. Five microlitres of PCR product was electrophoresed on 1.0% agarose gel. The cDNA was transferred onto a positive-charge nylon membrane using the MINIVE BLOTTER (Amersham Biosciences, San Francisco, USA) and a UV crosslinker (BIO-LINK type BLX). The blots were prehybridized in DIG Easy Hyb solution at 68 °C for 30 min. The probes, using the amplified SMART cDNA as template, were random primer labeled with Digoxigenin-11-dUTP according to manufacturer's instruction. After hybridization at 68 °C overnight, the membrane was washed two times in 2× SSC–0.1% SDS at room temperature and two times in 0.5× SSC–0.1% SDS at 68 °C. Blots were autoradiographed for empirically optimized exposure times. Probes for a house-keeping gene, β-actin, were used as control in standard and virtual Northern blots.

2.8. Antiviral assay of IntlP protein

An open reading frame (ORF) of interferon-like protein (IntlP) cDNA was amplified from the subtractive library with specific primers (forward, 5′-CGC*GGATCC*ATGAGAAGAAAGCCCAGT-3′; reverse, 5′-GCGC-*GAATTC*TCACCATGTGCTTCTGCTCAA-3′) containing *Bam*HI and *Eco*RI sites, respectively (as shown in italic letters). The PCR fragment was inserted into the GST-fused expression vector pGEX-20T. Recombinant plasmid was transformed into *Escherichia coli* XL1-Blue, and confirmed by sequencing. The fused protein was purified by Glutathione Sepharose 4B (Amersham Biosciences) following the manufacturer's instruction. The antiviral activity was assayed by cytotoxicity experiment (see ref. Luo et al., 2003). The concentration of the IntlP protein required to inhibit virus-induced cytopathic effect (CPE) to 50% of the virus-infected control (without the IntlP protein) was estimated as the 50% effective concentration (EC_{50}). Cytotoxicity of the IntlP protein was evaluated as CC_{50}, which corresponds to the concentration required to reduce the viable cells to 50% of the control (without the IntlP protein).

3. Results

3.1. Suppression subtractive hybridization and differential hybridization

To identify virus-resistance-relevant genes differentially expressed in virus-resistant shrimp, *P. japonicus*, subtractive hybridization by SSH and DH between virus-resistant and virus-sensitive shrimp were performed. This subtraction generated a forward-subtracted cDNA pool (named "F cDNA pool" hereafter) and a reverse-subtracted cDNA pool ("R cDNA pool") (Fig. 1). First of all, the efficiency of the SSH was evaluated by PCR with two primers set specifically for a house-keeping gene, β-actin. The β-actin product could be observed for the unsubtracted cDNA after 25 cycles, while no PCR product was observed for the subtracted sample even after 35 cycles. It suggested that a drastic reduction of highly abundant cDNAs was achieved.

Among the 1500 spots on the membrane, which correspond essentially to the separate genes, only 148 spots were detected by the F cDNA pool probe. Those clones were sequenced and then compared with the sequences in the GenBank using the FASTA program, among them, 30 genes are supposed to be immune-relevant (Table 1). Most sequences

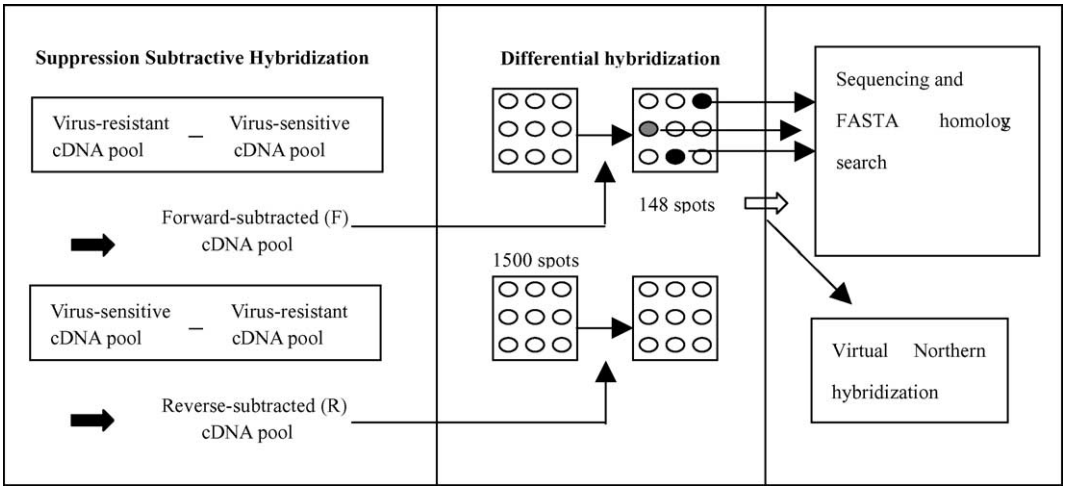

Fig. 1. Summary of our screening scheme for the virus-resistance-relevant genes in shrimp *P. japonicus*. cDNA subtraction by SSH was performed with cDNAs from mRNA sources as shown, to generate forward (F)- and reverse (R)-subtracted cDNA pools. These subtracted cDNA pools were as probes for differential hybridization, on which 1500 clones had been spotted. Several clones that gave differential signals were confirmed by virtual Northern hybridization.

Table 1
Putative virus-resistant-relevant peptides of *Penaeus japonicus*

Serial number	Homologous to genes in database (species)	Number of clones (*n*)	Percentage identity %:length
JP-31	NADH dehydrogenase NP_038288 (*Penaeus monodon*)	6	54.4:207aa
JP-32	Translationally controlled tumor protein AAO61938 (*Penaeus monodon*)	5	87.0:125aa
JP-33	Glutathione peroxidase P52033 (*Dirofilaria immitis*)	5	52.4:166aa
JP-34	C-type Lectin BAC54022 (*Anguilla japonica*)	3	37:76aa
JP-35	(2′–5′) Oligo(A) synthetase-like protein SYMSO3 (mouse)	4	32.1:50aa
JP-36	Interferon alpha-I-B IVBOIB (bovine)	1	40.0:79aa
JP-37	Ubiquitin/ribosomal 27a UQFFR7 (*Drosophila melanogaster*)	1	94.7:155aa
JP-38	CBF1 interacting corepressor NP_080130 (*Mus musculus*)	1	57.0:146aa
JP-39	Translation elongation factor eEF-1 EFBY1A (*Saccharomyces cerevisiae*)	1	67.9:82aa
JP-40	Basic proteinase inhibitor TITTOR (*Caretta caretta*)	1	33.0:111aa
JP-41	Cofilin A44397 (*Saccharomyces cerevisiae*)	2	42.2:125aa
JP-42	Transcription factor AP-1 precursor TVHUJN (human)	1	31.4:154aa
JP-43	Prothymosin alpha TNHUA (human)	2	42.9:54aa
JP-44	Heat shock protein 90 HHBY90 (*Saccharomyces cerevisiae*)	1	39.1:145aa
JP-45	Laminin gamma-1 MMHUB2 (human)	1	35.6:80aa
JP-46	Translation initiation factor IF-3 FIEC3 (*Escherichia coli*)	1	34.5: 92aa
JP-47	Transcription factor pdm2 A56564 (*Drosophila melanogaster*)	1	61.3:56aa
JP-48	Annexin II LUHU36 (human)	1	38.6:106aa
JP-49	Granulocyte–macrophage colony-stimulating factor precursor FQHUGM (human)	1	31.8:46aa
JP-50	Complement factor B precursor BBHU (human)	1	25.7:39aa
JP-51	Coagulation factor Xa EXBO (bovine)	1	45:50aa
JP-52	Collagen alpha 1 CGHU1V (human)	1	38.6:79aa
JP-53	Kinesin heavy chain A35075 (longfin squid)	1	28.2:184aa
JP-54	Transforming growth factor beta precursor WFMS2 (mouse)	1	52.0:50aa
JP-55	RAD1 protein BWBYDL (*Saccharomyces cerevisiae*)	1	28.2:168aa
JP-56	Macrophage colony-stimulating factor 1 receptor precursor WFMS2 (mouse)	1	39.1:60aa
JP-57	Cytochrome *c* CCBTS (*Miniopterus schreibersii*)	1	31.1:105aa
JP-58	Ubiquinol–cytochrome *c* reductase S26019 (mitochondrion *Ascaris suum*)	2	29.5:113aa
JP-59	Vitronectin precursor SGMSV (mouse)	1	59.3:56aa
JP-60	Protein kinase raf TVMVF6 (Murine sarcoma virus)	1	41.9:149aa

(22 out of 30) are found for the first time in penaeid shrimp, such as ubiquitin protein, interferon-like protein, transcription factor AP-1, protein kinase raf and the peptides sharing certain similarities with the components of the complement and cytokines system.

3.2. Confirmation of SSH by virtual Northern hybridization

To confirm the result of SSH, four different clones were randomly selected for virtual Northern blotting. Overexpression of the four genes was observed in the virus-resistant shrimp compared to virus-sensitive shrimp (Fig. 2). These genes include cofilin, translationally controlled tumor protein (TCTP), interferon-like protein and hepatic lectin (HL). The result confirmed the efficiency of the SSH.

3.3. Antiviral assay of recombinant protein IntlP

Because of the similarity between the IntlP and mammalian interferons (IFNs) in protein sequence, we wonder whether this protein holds some functions like the IFNs. To accomplish this, IntlP gene was inserted into the vector pGEX-20T and expressed. The recombinant protein was purified by Glutathione Sepharose 4B. To date, since no shrimp cell line is available for function study, the fish cell (GP) is used instead in the antiviral test. After 48 h virus inoculation, GP cells (Fig. 3A) were completely destroyed by Singapore grouper iridovirus (SGIV) (Fig. 3B). However, while incubated in the SGIV and IntlP protein mixture for 48 h, parts of the cells remained alive (Fig. 3C).

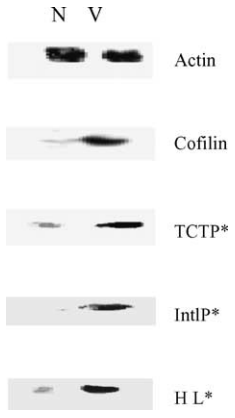

Fig. 2. Virtual Northern blot analysis of the over-expressed genes in the virus-resistant shrimp. Equal amounts of cDNAs from the normal and virus-resistant shrimp were used. N: normal group, V: virus-resistant group. *TCTP: translationally controlled tumor protein; *IntlP: interferon-like protein; *HL: hepatic lectin.

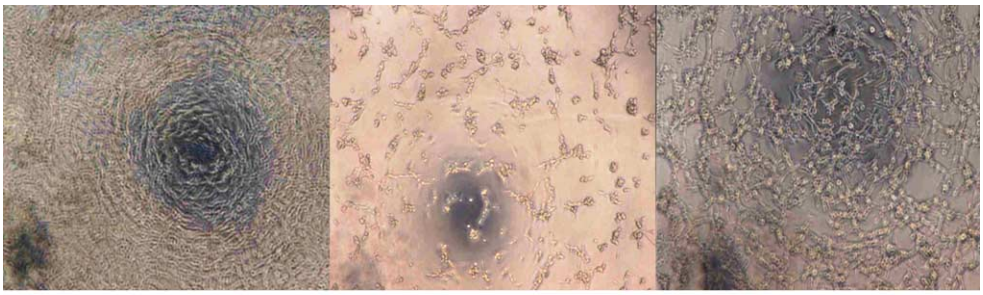

Fig. 3. Antiviral assay of recombinant IntlP protein. (A) Normal fish cells (GP) cultured for 48 h. (B) SGIV ($TCID_{50}$ ml^{-1}: 10^5) virus infection only for 48 h. (C) IntlP (5 μg/ml) mixed with SGIV virus ($TCID_{50}$ ml^{-1}: 10^5) for 48 h (SGIV: Singapore grouper iridovirus).

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1         ACTTGGACAAGGAGATTGAGGACATTGAAGGCAGCTGGGTGAAGTATCGTCAGGAGAGTG

61        TTGAAGGTCTTAAGAAGGCTGTCGAAGATGAGAAGAAAGCCCAGTGGGAGGCTGAAGGTC
21                                  M  R  R  K  P  S  G  R  L  K  V

121       AGAAGATGCTTTTTGATGCCAAGCGAGAAAATGTTGCACTCCAGCTTGAAGCTGCTTACA
41         R  R  C  F  L  M  P  S  E  K  M  L  H  S  S  L  K  L  L  T

181       GAGAGAGGCTGGCAACTGTTCATGCTGAGGTCAAGAAAAGGTTGGACTACCAGCTGGAGA
61         E  R  G  W  Q  L  F  M  L  R  S  R  K  G  W  T  T  S  W  R

241       CCGCCAATGTCAAAACTCGCATTGAGCAGAAGCACATGGTGAACTGGATTGTAAACAATG
81         P  P  M  S  K  L  A  L  S  R  S  T  W  *

301       TCAAGACTTCCATCACCCCAGCTCAAGAGGCTGCTGCCCTGAAGCAGTGCTTTGCTGACC
361       TCAAATCTCTTGCTCCCAAAACTGCCTAAATTTTAAATATTTTTCTATGTTGAGATTTTT
421       TTTCATCTTTTGTCCACAGTCGGTTCATCTTGAGCTTGATTGTATAGAAATGGATATCCC
481       GTAACCTTTTGGCAGTAGGGCTTATTGGGTTCAAGACACCAGCTTCGTGGTTGTGGTTAA
541       TAGTGGAAATGATGAGCTTCTCATTCCAATGTCATTTATGTAGTTTACTTGATGCTCCAC
601       AACTCACCCATGAAAAAAATAACTACCAGTGACAAAACCAAGTGTACCTGCCCG
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(a)

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268'                ........................................          0
alpha-Saimiri_sc    MALTFPFLVALVLLSYKSSCSMGCDLPQTHSLGNRRTLML         40
alpha_-_sheep       MAQLLPLLTALVLCSYGPVGSLGCDLPHNSAPLSRSTLVL         40
alpha-Mus_muscul    MARPFAFLMVLVVISYWSTCSLGCDLPQTHNLRNKKILTL         40
Consensus

268'                ...MRRK.PSGRLKVRRCFLMPSEKMLHSSLK......LL         30
alpha-Saimiri_sc    LAQMRRISPFSCLKDRHDFEFPQEEFDGSQLQKARAIFVL         80
alpha_-_sheep       LDQMRRVSPVLCLKDRRDFQFPREVVNGSQFQKNQTVSVL         80
alpha-Mus_muscul    LAQMRRLSPLSCLKDRKDFGFPQEKVDAQQIQEAQAIPVL         80
Consensus              mrr  p   lk r  f  p e              l

268'                TERGWQLFMLRSRKGWTTSWRP...................       52
alpha-Saimiri_sc    HEMIQQTFNLFSTKDSSAAWDETLLDKFYTELYQQLNELE        120
alpha_-_sheep       HEMLQQIFNLLHTARSSAAWNNTLLEELHTALHQQLQGLE        120
alpha-Mus_muscul    SELTQQILTLFTSKDSSAAWNATLLDSFCTGLHQLLNDLQ        120
Consensus            e   q   l         w

268'                ............PMS....KLALSR................       61
alpha-Saimiri_sc    ACVMQEVGVTDTPLMNEDSILTVRKYFQRITLYLKEKKYS        160
alpha_-_sheep       TCLVQAMGEEDSVLTADSPTLMLKRYFQRIRLYLDEKKHS        160
alpha-Mus_muscul    GCLMQLVGMKELPLTQEDSQLAMKKYFHRITVYLREKKHS        160
Consensus                                 l

268'                .STW.............................           64
alpha-Saimiri_sc    ACAWEVVRAEIMRS...................           174
alpha_-_sheep       GCAWELVRMEIRRAFSSTADLQESLRSKDGDLAS          194
alpha-Mus_muscul    PCAWEVVRAEVWRALSSSVNLLARLSEEKE....          190
Consensus              w
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(b)

Fig. 4. (a) Consensus nucleotide sequence of the cDNA encoding the interferon-like protein (IntlP) of *Penaeus japonicus* and the deduced amino acid sequence. The nucleotide sequence has been submitted to GenBank with accession number AY695938. (b) Alignment of the IntlP (268) with that of the *Saimiri sciureus*, sheep and *Mus musculus* alpha interferon.

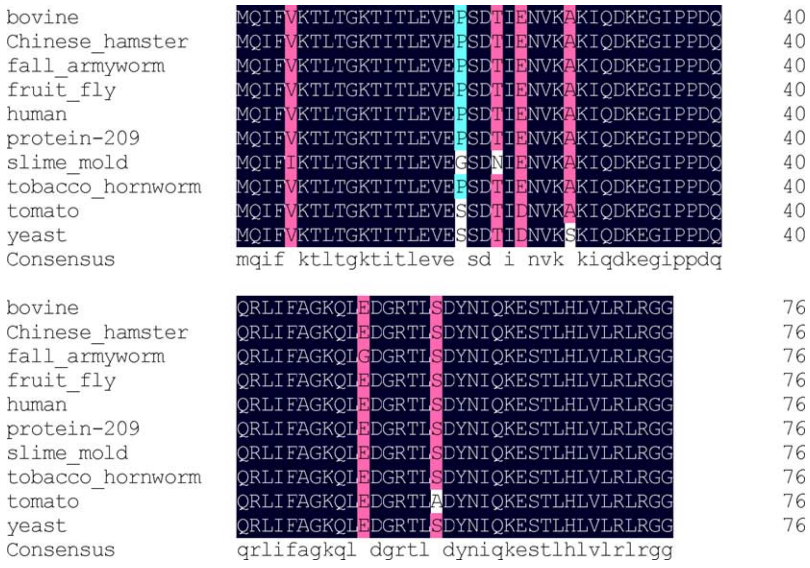

Fig. 5. Multiple alignment of shrimp ubiquitin protein-209 with the homologous genes of bovine, Chinese hamster, fall armyworm, fruit fly, human, slime mold, tobacco hornworm, tomato and yeast. The black shading indicates identical amino acids. The numbers to the left indicate the amino acid position of ubiquitin in the corresponding species. The shrimp ubiquitin sequence has been submitted to GenBank with accession number AY695937.

The values of EC_{50} and CC_{50} were 10 and >200 μg/ml, respectively, suggesting the IntlP protein has non-specific antiviral activity and no cytotoxicity to the tested cells.

4. Discussion

In an outbreak of the WSSV infection in a shrimp pond, there are always a few shrimp (<1%) that survived. Evidently, these shrimp possess stronger defense abilities. So what is the difference between normal and virus-resistant shrimp at gene expression level? In this paper, genes differentially expressed between the normal and virus-resistant shrimp were compared by using the suppression subtractive hybridization technique and the up-regulated, virus-resistant-relevant genes were reported.

Suppression subtractive hybridization is one of the most powerful methods for isolating differential expressed transcripts. SSH, of course, may yield false positives. In order to reduce the false results of SSH, differential hybridization was employed to obtain the more likely up-regulated genes and its effectiveness were further confirmed by virtual Northern hybridization. Many spots detected with both probes (F and R cDNA pools) in strong intensity were excluded before sequencing, which makes the result more convincing. By virtual Northern hybridization, four randomly selected genes were found to be up-regulated in the virus-resistant shrimp. Reasoning from the result, most genes listed in the table are differentially expressed in the virus-resistant shrimp.

After SSH and differential screening, two interesting proteins: the interferon-like protein (Fig. 4) and the (2′–5′) oligo(A) synthetase-like protein (data not shown) were detected. Using FASTA program, it was found that there are some similarities between shrimp IntlP and the mammalian alpha interferon precursor, the shrimp (2′–5′) oligo(A) synthetase-like protein and the mouse (2′–5′) oligo(A) synthetase, respectively (data not shown). The IFN and (2′–5′) oligo(A) synthetase are critical components of the interferon system in vertebrates, which play important roles in the antiviral activities of animals (Sen and Lengyel, 1992). Reasonably, these two proteins identified in our library maybe have the antiviral effects like those in mammalians. Our recent cytotoxicity experiment on IntlP showed that this protein possesses non-specific antiviral ability by inhibiting SGIV (grouper iridovirus) in GP cells (grouper embryo cells) (Fig. 3). But the mechanism of antiviral activities of these proteins remains to be further clarified.

The translationally controlled tumor protein, also known as fortilin and P23, found in the library shares 91 and 58% identity with the one in *Penaeus monodon* and *Drosophila melanogaster*, respectively. It is one of the most abundant genes in the subtracted library (see Table 1). Similar result has been reported by Rojtinnakorn et al. (2002) in WSSV-infected shrimp by EST approach. The rather high expression in virus-resistant shrimp implies that TCTP plays a critical role in the defense process. But its antiviral mechanism still remains to be elucidated.

Another interesting protein is the ubiquitin, which is reported for the first time in shrimp so far even though it distributes widely in eukaryotes. The sequence encodes a 76-amino acid ubiquitin protein. A similarity of 100% was found with the sequences from GenBank of bovine, Chinese hamster, fruit fly, human and tobacco hornworm, 98.7% with that of fall armyworm, 97.4% with those of yeast and tomato, 96.0% with slime mold (Fig. 5). Ubiquitin is widely known as a post-translational tag used to signal a protein's hydrolytic destruction and the ubiquitin-dependent degradation is an important pathway of non-lysosomal protein breakdown

(Finley, 2001). As reported previously, the Epstein–Barr virus latent membrane protein 1 (LMP1) could be degraded by ubiquitin pathway via ubiquination of the N-terminal residue (Aviel et al., 2000) and ubiquitin gene can be transcriptionally induced by lytic infection with herpes simplex virus, causing accumulation of ubiquitin RNA and protein in the infected cell (Latchman et al., 1987). Further study focused on ubiquitin will give a more comprehensive understanding of functions of ubiquitin in innate immunity of shrimp.

An interesting group of proteins which hold certain similarities with the components of the complement and cytokines system were found in our subtractive library, such as granulocyte–macrophage colony-stimulating factor precursor, complement factor B precursor, transforming growth factor beta precursor. Though these sequences are only a part of their full length and the searching results obtained from the Internet may not represent the real proteins themselves, the sequences may give us a clue that the immunity of the shrimp may be as complicated as the vertebrates'.

Besides, some other components such as redox-related factors (NADH dehydrogenase; glutathione peroxidase and AP1), C-type lectins and cytoskeleton-related protein-cofilin were highly expressed in the virus-resistant shrimp (Table 1), suggesting that these proteins may contribute to virus resistance. The protein cofilins, in particular, which are small actin-binding proteins, play an important role in cytokinesis (Abe et al., 1996), endocytosis, as well as in pathological situation (Heyworth et al., 1997).

The sequences identified in the library and their expression profile are significantly different from those found in the microbial-challenge shrimp library (He et al., 2004), indicating that the virus-resistant mechanism greatly differs from the mechanism of antimicrobial infection. The understanding of the immunity of the shrimp is limited at present and the expression profile of virus-resistant relevant genes will no doubt accelerate our overall comprehension of immune system of shrimp, as well as the invertebrates.

Acknowledgements

This work was supported by the Key Program of National Natural Science Foundation of China (30330470), Chinese High Tech "863" Program (2001AA621130).

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