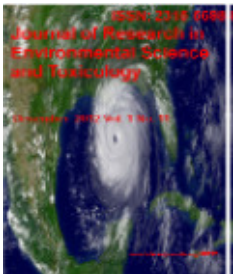

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Review

Ribonucleic Acid Interference (RNAi) technology: promising solution to shrimp viral diseases

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Ribonucleic Acid interference (RNAi) is a natural process that regulates gene expression by a highly precise mechanism of sequence-directed gene silencing at the stage of translating by degrading specific messenger RNAs (mRNAs) or blocking translating. Viral infections are one of the major reasons for the huge economic losses in shrimp farming since the early 1990s. Best management practice only prevents viral infections to some extent. The control of viral diseases in shrimp remains a serious challenge for shrimp culture with major pathogens such as LSNV, IHHNV, WSSV, YHV, TSV, HPV, MBV and GAV. Shrimps lack true adaptive immune response system hence RNAi is adopted as an alternative and more specific method to combat viral diseases. This review focuses on the current knowledge of RNAi technology as a promising therapeutic and efficient control measure for cultured shrimp viral diseases. Initial studies of RNAi focused on cellular mRNA targets but resent studies are on targeting sequence-specific viral RNAs. The gene constructs (sequence-specific and non-specific) used in shrimp RNAi technology to induce an antiviral response in shrimp are exogenously synthetic long double-stranded RNAs (dsRNAs) or short interfering RNAs (siRNAs). They have been used extensively in *Penaeus monodon* and *Litopenaeus vannamei* against WSSV and YHV. Double-stranded RNAs can be administered by injection or orally for sequence-specific viral RNAs. Degree of protection varies with different viral target genes. Short interfering RNA-mediated gene silencing in shrimp is more effective in terms of concentration and number of doses than dsRNA. Gene silencing leads to shut-down of protein synthesis of the viruses hence the viral diseases are treated and/or controlled. Injection of dsRNA is practical impossible in shrimp farming at the field level therefore simple and effective routes for dsRNA and siRNA delivery into shrimp are developed such as production of pellet feed using edible dsRNA producing bacteria.

Keywords: Ribonucleic acid (RNA), gene silencing, mechanism, viral diseases, shrimp aquaculture.

INTRODUCTION

Ribonucleic Acid interference (RNAi) is a molecular-based gene transfer technology. RNA mechanism is initiated by double stranded RNA (dsRNA) that is triggered by DNA construct inserted into a cell. In addition, RNAi is a natural process that regulates gene expression by a highly precise mechanism of sequence-directed gene silencing at the stage of translating by degrading specific mRNAs or blocking translating (Lopez-Fraga *et al*., 2009). RNA interference (RNAi) or Post-Transcriptional Gene Silencing (PTGS) is a conserved biological response to double-stranded RNA that mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes (NCBI, 2011). RNAi technology is an important potential tool in viral disease

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Prevention in shrimps that do not have true adaptive immune response system.

Viral infections are one of the major reasons for the huge economic losses in shrimp farming since the early 1990s. Best management practice only prevents viral infections to some extent. The control of viral diseases in shrimp remains a serious challenge for shrimp culture with major pathogens that are divided into RNA and DNA viruses namely Laem-Singh virus (LSNV), Taura syndrome virus (TSV), gill-associated virus (GAV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), white spot syndrome virus (WSSV), hepatopancreatic parvovirus (HPV) and monodon baculovirus (MBV). RNAi technology shows a promising therapeutic and efficient control measure for shrimp viral diseases as shrimps like other invertebrates do not have true adaptive immunity (Shekhar and Lu, 2009). Gene silencing leads to shut-down of protein synthesis hence the viral diseases are treated and/or prevented. RNAi technology is for therapeutic intervention (treatment of viral infections, dominant disorders, neurological disorders, and many types of cancers (*in vivo* inactivation of gene products linked to human disease progression and pathology), functional genomics (systematic analysis of loss-of-function phenotypes induced by RNAi triggers), practical applications in agriculture and other areas (NCBI, 2011).

Background history of RNAi technology

Recent discoveries show that a class of RNA molecules (small RNAs) operates many of the cell's controls (BENITEC, 2007). They can turn the tables on DNA, shut down genes or alter their levels of expression (Couzin, 2002). Napoli *et al*. (1990) were the first to report RNAi in plants. Petunia pigment genes were shut down when they inserted extra copies of the genes in attempt to deepen the purple colour. Fire *et al*. (1998) reported that the double –stranded RNA (dsRNAs) injected into the nematode (*Caenorhabditis elegans*) silenced the corresponding genes containing complementary sequences. This is sequence-specific degradation of target RNA. Graham (2000) demonstrated the universality of RNAi and invented DNA constructs to trigger the RNAi process in human and mammalian cells. Elbashir *et al.* (2001a) provided a biochemical understanding of RNAi pathway. That study showed that the functional units of RNAi are likely represented by dsRNAs shorter than 30 base pairs. McCaffrey et al. (2002) published the first in vivo evidence of RNAi in adult mice. This gave more insight on gene therapy research. The RNAi pathway is present in every cell of almost every multicellular organisms for as innate mechanism for cellular defense against dsRNA viruses and interferon-regulated antiviral pathways.

A simplified model for the RNAi pathway

This includes two steps involving ribonuclease enzymes (NCBI, 2011). In step one, the trigger RNA (either dsRNA or miRNA primary transcript) is processed into a short, interfering RNA (siRNA) by the Rnase III enzymes: Dicer and Drosha. The step two involves the siRNAs being loaded into the effector complex RNA-induced silencing complex (RISC). The siRNA is unwound during RISC assembly and the single-stranded RNA hybridizes with mRNA target, Gene silencing is a result of nucleolytic degradation of the targeted mRNA by the Rnase H enzyme Argonaute (Slicer). If the siRNA/mRNA duplex contains mismatches the mRNA is not cleaved rather, gene silencing is a result of translational inhibition.

Application of dsRNAi in Shrimp Farming

dsRNAi is being used in combating viral diseases in shrimps. RNAi mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein-coding genes. The initial studies of RNAi focused on cellular mRNA targets (Randall *et al*., 2003) but present studies are on targeting sequence-specific viral RNAs. Robalino *et al*. (2004 and 2005), Tirasophon *et al*. (2005), Westenberg *et al*. (2005) and Xu *et al*. (2007) reported the use of both exogenously synthetic long dsRNAs or siRNAs to induce an antiviral response in shrimp. The various gene constructs used in shrimp RNAi technology that result in RNA interference are shown in Table 1.

Importance of dsRNA and siRNA-mediated gene silencing in shrimp

RNAi technology is a cellular defence against viral infections and gene silencing leads to shut-down of protein synthesis of the viruses hence the viral diseases are treated and/or controlled. Double –stranded RNA is 2ecognized by Toll pathway in shrimp antiviral immunity in a sequence-independent manner (Arts *et al*., 2007). It can be administered by injection or orally for sequence-specific. Degree of protection varies with different viral target genes. Non-specific response is induced by dsRNA by non-specific dsRNA. Short- stranded RNA is more effective than dsRNA in terms of concentration and number of doses.

Counter defence mechanism to RNAi in shrimp

Inhibition of gene silencing mechanism due to virus-induced phenomenon (Robalino *et al*., 2007), for example, WSSV-mediated silencing of hemocyanin and

Table 1. Gene constructs used in shrimp RNAi technology resulting in RNA interference

Shrimp/cells	Gene construct	Protection studies against (virus)	Gene	Reference
Sequence-specific gene constructs				
L. vannamei	dsRNA	WSSV	DNA pol	Robalino *et al*. 2005
	dsRNA	WSSV	Rr2	
	dsRNA	WSSV	ORF252	
	dsRNA	WSSV	Vp28	
	dsRNA	WSSV	Vp19	
P. monodon	siRNA	WSSV	Vp28	Westenberg *et al*. 2005
	siRNA	WSSV	Vp15	
P. monodon	dsRNA	YHV	Protease	Yodmuang *et al*. 2006
M. japonicus	siRNA	WSSV	Vp28	Xu *et al*. 2007
L. vannamei	siRNA	WSSV	DNA pol	Wu *et al*. 2007
	siRNA	WSSV	Rr2	
	siRNA	WSSV	Tk–tmk	
	siRNA	WSSV	vp24	
	siRNA	WSSV	Vp28	
P. chinensis	dsRNA	WSSV	Vp28	Kim *et al*. 2007
	dsRNA	WSSV	Vp281	
	dsRNA	WSSV	Protein kinase	
P. monodon	dsRNA	YHV	Protease	Tirasophon *et al*. 2007
L. vannamei	siRNA	WSSV	Tk–tmk	Wu *et al*. 2007
	siRNA	WSSV	Vp24	
	dsRNA	WSSV	Vp28	Sarathi *et al*. 2008a
P. monodon				
P. monodon	dsRNA	WSSV	vp28	Sarathi *et al*. 2008b
Non-specific gene constructs				
L. vannamei	dsRNA	WSSV	Duck Immunoglobulin	
	dsRNA	WSSV	Genomic non Coding region of catfish	Robalino *et al*. 2004
	dsRNA	WSSV		
	dsRNA	WSSV		
	dsRNA analogue	WSSV		
P. monodon	siRNA	WSSV	Gfp	Westenberg et al. 2005
P. monodon	dsRNA	YHV	Gfp	Yodmuang *et al*. 2006
P. chinensis	dsRNA	WSSV	Gfp	Kim *et al*. 2007
Cell culture				
Oka	dsRNA	YHV	Protease	Tirasophon *et al*. 2005
	dsRNA	YHV	Polymerase	
	dsRNA	YHV	Helicase	
	dsRNA	YHV	Gp65	
	dsRNA	YHV	gp116	
Sf21	siRNA	WSSV	vp28	Westenberg *et al*. 2005
SISK				
Endogenous genes				
L. vannamei	dsRNA		Hemocyanin	Robalino *et al*. 2005
	dsRNA		CDP	

rr2 Ribonucleotide reductase small subunit, tk–tmk thymidine kinase–thymidylate kinase, gfp green fluorescent protein; DNA pol = DNA polymerase, CDP CUB (complement subcomponents C1r-C1s/sea urchin protein Uegf/bone morphogenetic protein 1) domain protein Source: Shekhar and Lu (2009)

homologue of the signal transducer and activator of transcription (STAT) is tissue-specific (hepatopancreas).

Limitation and future prospects of RNAi technology in shrimp farming

Injection of dsRNA is practical impossible in shrimp farming at the field level. There is need to develop simple and effective routes for dsRNA and siRNA delivery into shrimp. Alternative approaches are use of edible dsRNA producing bacteria. Sarathi *et al*. (2008a) fed shrimp with pellet feed coated with inactivated vp28-dsRNA-induced bacteria and a vp28-dsRNA-chitosan nanoparticle complex for delivering of the WSSV-vp28-dsRNA.

CONCLUSION AND RECOMMENDATION

RNAi represents a type of sequence-directed immunity and exists in shrimp to counteract viral diseases. The specific dsRNA and siRNAs as a dose- and sequence-dependent phenomenon offer protection and treatment in shrimp against viral diseases. Injection of dsRNA is practical impossible in shrimp farming at the field level therefore simple and effective routes for dsRNA and siRNA delivery into shrimp are developed such as production of pellet feed using edible dsRNA producing bacteria.

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