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Roles of small RNAs in the immune defense mechanisms of crustaceans

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ABSTRACT

Small RNAs, 21–24 nucleotides in length, are non-coding RNAs found in most multicellular organisms, as well as in some viruses. There are three main types of small RNAs including microRNA (miRNA), small-interfering RNA (siRNA), and piwi-interacting RNA (piRNA). Small RNAs play key roles in the genetic regulation of eukaryotes; at least 50% of all eukaryote genes are the targets of small RNAs. In recent years, studies have shown that some unique small RNAs are involved in the immune response of crustaceans, leading to lower or higher immune responses to infections and diseases. SiRNAs could be used as therapy for virus infection. In this review, we provide an overview of the diverse roles of small RNAs in the immune defense mechanisms of crustaceans.

1. Introduction

RNA interference (RNAi) is a widespread phenomenon in plants and animals. Small RNAs, such as microRNA (miRNA), small interfering RNA (siRNA), and piwi-interacting RNA (piRNA), are an abundant class of endogenous or exogenous non-coding RNAs with lengths of approximately 21–24 nucleotides that regulate gene expression (Reinhart et al., 2000; Bartel, 2004; Lu et al., 2005; Bushati and Cohen, 2007; Cai et al., 2009; Hammond, 2005). Small RNAs combine with specific Argonaute piwi proteins, which leads to the degradation or translation inhibition of target mRNAs(Ding and Voinnet, 2007; Myles et al., 2008; Hain et al., 2010; Mueller et al., 2010; Bronkhorst et al., 2013). This type of post-transcriptional regulation is involved in nearly all aspects of the life process. However, the diverse functions of individual small RNAs are still largely unknown.

Crustaceans, which make up a very large group of arthropods (e.g., Daphnia, barnacles, chiggers, crab, lobster and shrimp), have great economic value. Crustaceans have different shapes (Kuris, 1990), and individual sizes range from plankton that are visible only under a microscope to lobster 75 cm in body length. A hardened carapace is their typical characteristic and the head-chest is usually a single back cover (Anger, 2006). The vast majority of crustaceans are aquatic, and they are present in both freshwater and marine habitats worldwide. Some crustaceans have adapted to survive on land, such as woodlice. While most crustaceans are saprophages, there are also predatory and phytophagous species (Söderhäll and Thörnqvist, 1997).

2. RNAi pathway in crustaceans

Mediated by small RNAs, RNAi is known as a key factor in post-transcriptional gene regulation (Zamore et al., 2000; Zamore, 2007; Flegel and Sritunyalucksana, 2011). There is abundant evidence that inserting synthetic double-strand RNA (dsRNA) or siRNA into shrimp could cause the inhibition of virus infection (Everett and McFadden 1999; Tirasophon et al., 2007; Xu et al., 2007; Yodmuang et al., 2006; Li and Xiang, 2013). Recently, components of the RNAi pathway (e.g., Drosha, Dicer-1 and Dicer-2) are found and cloned (Tomari and Zamore, 2005; Huang et al., 2012b). Drosha is a class 2 RNase III enzyme responsible for initiating the processing of miRNA. A 1081 amino acid polypeptide is encoded by an Drosha gene in *Marsupenaeus japonicus* shrimp (Huang et al., 2012b). Dicer-1 proteins, which are highly homologous in most invertebrate species (Huang and Zhang, 2012a,b; Su et al., 2008; Yao et al., 2010), have been found in different types of shrimp (*M. japonicus, Litopenaeus vannamei*, and *Penaeus monodon*). While Dicer-2 is much less homologous than Dicer-1, Dicer-2 from *L. vannamei, P. monodon*, and *M. japonicus* (LvDcr2 and MjDcr2) have been found to be very similar to the Dicer-2 proteins of insects (Chen et al., 2011; Huang and Zhang, 2012a,b). Different types of Argonaute proteins have recently been characterized as well (Qu et al., 2008; Phetrungnapha

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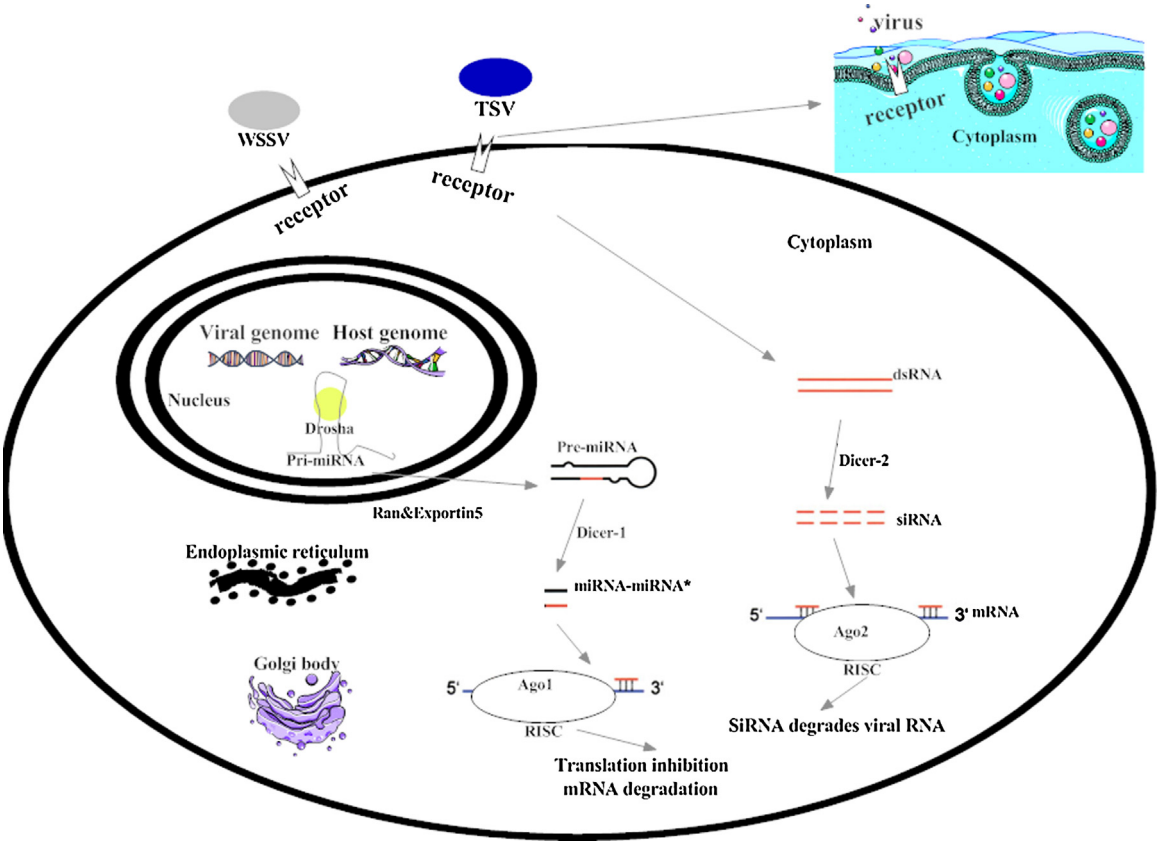

Fig. 1. RNAi pathway in crustaceans. In the siRNA mediated antiviral response, dsRNAs can be generated from the replication intermediate of DNA (e.g., WSSV) or RNA viruses (e.g., TSV). Host Dicer-2 recognizes and cuts the dsRNA into 22–24 nt siRNA. Then, siRNAs are sent to an RISC (containing Ago2) and degrade viral RNAs. MiRNAs are first transcribed into long pri-mRNAs from host or viral DNA genome. The pri-miRNA is cut into the pre-miRNA by Drosha in the nucleus. Then pre-miRNA is exported to the cytoplasm by Ran and Exportin5 for further processing by Dicer-1. The miRNA:miRNA* duplex intermediate is sent into RISC (containing Ago1), and one chain stays in the RISC to bind target mRNAs. At last, target mRNAs are degraded or the translation is inhibited.

et al., 2013; Labreuche and Warr, 2013). Although an increasing number of RNAi-related proteins have been found, our understanding of the RNAi system of crustaceans is still quite poor. Fig. 1 shows the different RNAi pathways in crustaceans under DNA or RNA virus invasion.

2.1. Biogenesis of siRNA

SiRNA, which is an intermediate product in the RNAi pathway, plays a key role in the RNA silencing pathway. RNAi mediated by siRNA is composed mainly of Dicer and RNAi gene defect-1 (Rde-1) regulation. Dicer is an RNase III endonuclease with four domains—PAZ domain, Argonaute family of RNA enzyme activity area, type III dsRNA binding region, and DEAH/DEXHRNA helicase activity area—and Rde-1 distinguishes exogenous dsRNA. Due to RNA virus intrusion, reverse transcription, and genome transgenic transposon repeat sequence transcription, dsRNA appears in cells, and when it reaches a certain amount, Rde-1 and Dicer combine, forming an enzyme–dsRNA complex. Dicer cuts the siRNA, and an RNA-induced silencing complex (RISC) is assembled. SiRNA is transported into the RISC and pairs completely with the target gene coding region area or untranslated region (UTR). SiRNA only degrades the complementary sequence.

2.2. Biogenesis of miRNA

MiRNA is usually the product of RNA polymerase II in the nucleus. Initially, pri-miRNA has a cap with a large structure (7MGpppG) and a poly-A tail (AAAAA). Pri-miRNA is processed into 70 nucleotides (pre-miRNA) in the nuclease by Drosha and its cofactor Pasha. Under the action of Ran-GTP and exportin-5, pre-miRNA is transported to the cytoplasm(Denli et al., 2004). Subsequently, another nuclease, Dicer, shears the pre-miRNA into double-stranded miRNA:miRNA* (about 22 nucleotides in length), and the double chain is soon guided into the RISC (Chendrimada et al., 2007). Mature miRNA binds to its complementary mRNA site by base pairing. MiRNA inhibits the target's expression in the protein translation level when it is incompletely complementary to the target mRNA. However, there is evidence that miRNA could also affect the stability of mRNA. Using this mechanism, the binding sites of miRNA are usually the mRNA 3′UTR (seed sequence).

3. SiRNA-related antiviral response in crustaceans

RNAi mediated by siRNA is an evolutionarily highly conserved antiviral pathway in nearly all eukaryotes (Xia et al., 2002). Gene expression knockdown by siRNA has been widely applied, and both dsRNA and siRNA block virus replication in shrimp (Kawai and Akira, 2006; Labreuche et al., 2010). Research has shown that viral infection can be inhibited via injection of the dsRNA or siRNA of viral genes (Jayachandran et al., 2012; Wang et al., 2012), including white spot syndrome virus (WSSV), Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV), yellow head virus (YHV), and *Penaeus monodon* densovirus (pmDNV) (Yodmuang et al., 2006; Tirasophon et al., 2007; Xu et al., 2007; Attasart et al., 2011; Loy et al., 2012; Chiang et al., 2013). Replications of WSSV and TSV are shown to be inhibited by injection of dsRNA in L. *vannamei* (Robalino et al., 2004). In addition, synthesized dsRNA confers pro-