Developmental and Comparative Immunology 56 (2016) 37–45

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Gasmin (*BV2-5*), a polydnaviral-acquired gene in *Spodoptera exigua*. Trade-off in the defense against bacterial and viral infections

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ARTICLE INFO

Article history:
Received 14 September 2015
Received in revised form
25 November 2015
Accepted 26 November 2015
Available online 30 November 2015

Keywords:
Gasmin
Baculovirus
Immune response
Cytoskeleton
Phagocytosis
Actin
Bacillus thuringiensis

ABSTRACT

Thousands of Hymenopteran endoparasitoids have developed a unique symbiotic relationship with viruses named polydnavirus (PDVs). These viruses immunocompromise the lepidopteran host allowing the survival of the wasp eggs. In a previous work, we have shown the horizontal transfer of some polydnaviral genes into the genome of the Lepidoptera, *Spodoptera exigua*. One of these genes, *BV2-5* (named *gasmin*) interferes with actin polymerization, negatively affecting the multiplication of baculovirus in cell culture. In this work, we have focused in the study of the effect of Gasmin expression on different aspects of the baculovirus production. In addition, and since actin polymerization is crucial for phagocytosis, we have studied the effect of Gasmin expression on the larval interaction with bacterial pathogens. Overexpression of Gasmin on hemocytes significantly reduces their capacity to phagocytize the pathogenic bacteria *Bacillus thuringiensis*. According to these results, *gasmin* domestication negatively affects baculovirus replication, but increases larvae susceptibility to bacterial infections as pay off. Although the effect of Gasmin on the insect interaction with other pathogens or parasitoids remain unknown, the opposite effects described here could shape the biological history of this species based on the abundance of certain type of pathogens as suggested by the presence of truncated forms of this protein in several regions of the world.

1. Introduction

Insects represent a large and vast group of animals distributed worldwide and adapted to different types of environments. Although they lack adaptive immune systems, they have developed an efficient innate immunity that allowed them to face a world surrounded by natural enemies (Beckage, 2008). In response to pathogens, insects activate a large repertoire of cellular and humoral components aimed to block or reduce the progress of the pathogen as well as to minimize the detrimental effects produced by the pathogen (Feldhaar and Gross, 2008).

Before activation of the immune response by the host, pathogens need to overcome the primary line of defense, which consists of the cuticle barrier, gut and trachea. If the invader succeeds to penetrate these barriers, the non-self-recognition is crucial to stimulate the insect immune system. Although insects lack antibody based immunity, it has been shown that they produce a set of proteins that recognize and bind conserved domains on the pathogen surface, named pattern recognition proteins (Gillespie et al., 1997; Jiravanichpaisal et al., 2006). Once pattern recognition proteins bind to the target, a number of signaling pathways leading to humoral and cellular responses take place. In general, humoral immune response refers to the expression of peptides that are produced mainly by fat body, but also by some other tissues such as hemocytes and gut. These peptides target a wide range of microorganisms including bacteria, virus or fungi. Such responses also include the activation of enzymatic cascades regulating hemolymph coagulation and melanisation (Cerenius and Söderhäll, 2004). The cellular defense is mediated by hemocytes through different mechanisms such as phagocytosis and encapsulation of the pathogen (Lavine and Strand, 2002). Phagocytosis consists of the recognition and uptake of microorganisms followed by their destruction (Jiravanichpaisal et al., 2006), whereas encapsulation refers to the binding of hemocytes to larger targets such as parasitoids (Browne et al., 2013).

Parasitic insects rely on the parasitized hosts for the development of their offspring. They have developed efficient strategies in

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http://dx.doi.org/10.1016/j.dci.2015.11.014
0145-305X/

order to survive the host immune defense (Beckage and Gelman, 2004). For instance, members of the hymenopteran wasps *Braconidae* and *Ichneumonidae* have developed unusual symbiotic relationship with a type of viruses named polydnaviruses (PDVs) (Strand and Burke, 2013). PDVs are double stranded DNA viruses residing in the wasp chromosome as a proviral form. Their replication is restricted to specialized calyx cells in the female ovaries and vertically transmitted to descendants (Strand and Burke, 2013). They are injected together with the wasp's eggs into the lepidopteran host hemocoel as segmented circular DNA. The circular form of the virus is unable to replicate since it lacks replication genes. Thus, PDVs do not replicate in the lepidopteran host, but express specific proteins able to interfere with the insect's immune system (Drezen et al., 2014).

In order to survive inside the lepidopteran host, parasitoids alter hemocyte's functions. In general, PDVs efficiently infect lepidopteran hemocytes inducing the expression of their specific immunosuppressive proteins (Asgari et al., 1996; Turnbull et al., 2004). In some host species, hemocytes dysfunction involves a dramatic reduction in the number of functional hemocytes, while in others, PDV proteins alter the behavior of hemocytes that fail to spread over the surface of the parasitoid egg (Amaya et al., 2005). Hemocytes alteration has been described as a result of the disruption of cytoskeleton actin arrangement (Asgari et al., 1997). In addition, it has been described that some PDVs proteins can inhibit the actin expression in the immune cells and so causing their dysfunction, such as the case of the glc1.8 protein expressed by *Microplitis demolitor* bracovirus parasitizing *Pseudoplusia includes* (Strand et al., 2006). In order to provide a sustainable expression of such immunosuppressive proteins, it has been suggested that the PDV integrates part of its genome in the DNA of certain cells of the host (Drezen et al., 2014).

In a previous study (Gasmi et al., 2015), we have reported horizontal gene transfer events that have occurred from a symbiotic bracovirus of an unidentified *Cotesia* sp, to different lepidopteran species. One of these events has resulted in the integration of a *Cotesia* sp bracovirus sequence encoding the *gasmin* (*BV2-5*) gene into the beet armyworm *Spodoptera exigua* germ line. *Gasmin* is a member of a specific gene family of *Cotesia* bracovirus. Chen and colleagues (Chen et al., 2007) have shown that CvBV2 is expressed in parasitized *Plutella xylostella* hemocytes at a very early stage of parasitism and suggested that *BV2* can be implicated in the early protection of the parasitoid egg against encapsulation. Previous studies have shown that Gasmin physically interfere with actin polymerization, thereby negatively affecting the multiplication of baculovirus in cell culture. Cytoskeletal actin is involved in different cellular mechanisms, such as phagocytosis and encapsulation, and it is used by baculovirus to successfully infect the lepidopteran cells (Ohkawa et al., 2010). Moreover, it has been observed that two different alleles in *S. exigua* populations are present in different geographic locations. While European populations carry a truncated and non-functional form of Gasmin, a functional form of this protein has been found in tested North American and Asian populations (Gasmi et al., 2015).

Based on these previous results and in order to increase our knowledge about the trade-offs associated to the recent acquisition of this gene by *S. exigua*, we have investigated how the interference of Gasmin with the cytoskeleton can affect the host interaction with two common pathogens encountered by *S. exigua* in the field: baculovirus and the bacterium, *Bacillus thuringiensis*.

2. Experimental procedures

2.1. Insects and DNA samples

Three different colonies of *S. exigua* were continuously reared on artificial diet (Greene et al., 1976) at 25 ± 3 °C with 70 ± 5% relative humidity and a photoperiod of 16 h light: 8 h dark. The FRA strain was kindly supplied by M. Lopez-Ferber, INRA (St.Christol les Alés, France). The SUI population was provided by Andermatt Biocontrol AG (Grossdietwil, Switzerland). The XEN-R strain was obtained from insects collected from cotton fields in Pattville, (USA) which were later selected for resistance to *B. thuringiensis* (Hernández-Martínez et al., 2010; Park et al., 2014).

2.2. BV2-5 expression analyses by reverse transcriptase quantitative-PCR (RT-qPCR)

To study the change in the expression profile of *gasmin* after intrahemocoelic larval injection with bacteria, *S. exigua* FRA fourth instar larvae were injected with 5 μl of 10^7 cells/ml from either *Micrococcus luteus*, *Escherichia coli* or *B. thuringiensis*. Bacterial preparations were previously inactivated by heat shock treatment (120 °C, 30 min) and suspended in PBS. As control, larvae were injected with 5 μl of PBS (1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 27 mM KCl; pH 7.0). Hemocytes and fat body were collected from bacteria-injected larvae, as well as control larvae, 8 h and 24 h post injection. Total RNA from the treated and control larvae were collected and processed for RT-qPCR. Data are presented as fold change using the method of $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001) and normalized to the internal control gene, *ATP synthase subunit C* (Herrero et al., 2007). The standard deviation of the ΔCt values of treated and control samples was calculated as $(s_1{}^2 + s_2{}^2)^{1/2}$, where s_1 is the standard deviation of the gene of interest and s_2 is the standard deviation of the reference gene. The standard deviation of the ΔCt was then incorporated into the fold change calculation as described in the Applied Biosystem "Guide to Performing Relative Quantitation of Gene Expression Using Real-Time Quantitative PCR".

The effect of baculovirus infection on the expression pattern of *gasmin* was also determined. Third instar larvae (SUI colony) were orally infected with *S. exigua* nucleopolyhedrovirus SeMNPV. Each larva was fed with 10^4 viral occlusion bodies (OBs); non-infected larvae were considered as control larvae. Different tissues (hemocytes, midgut and fat body) of infected and non-infected larvae were collected 72 h post infection (hpi). Total RNA from treated and non-treated larvae were obtained as and reverse-transcribed to obtain correspondent cDNAs. The changes in the expression pattern due to the viral infection were calculates as described previously.

2.3. Generation of recombinant baculovirus

Two types of baculovirus constructs have been employed in this study. Generation of recombinant baculovirus expressing the different forms of Gasmin and forming viral occlusion bodies (by expressing AcMNPV polyhedrin gene downstream the ph promoter) have been previously described (Gasmi et al., 2015).

2.4. Gasmin effect on the baculovirus infectivity and production

In separate T75 flasks, about 10^6 Sf21 cells were infected with the AcMNPV-pH_Gasmin, AcMNPV-pH_Gasmin-t and AcMNPV-pH viruses at MOI 2. In addition, one group of cells was infected with the control virus AcMNPV-pH and treated with 5 μM latrunculin A at 12 hpi. Four days post infection, the percentage of cells containing OBs was manually counted. Then, infected cells were