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Rac1 mediates cytokine-stimulated hemocyte spreading via prostaglandin biosynthesis in the beet armyworm, *Spodoptera exigua*

Jiyeong Park [a], David Stanley [b], Yonggyun Kim [a,*]

[a] Department of Bioresource Sciences, Andong National University, Andong 760-749, Republic of Korea
[b] Biological Control of Insects Research Laboratory, USDA/Agricultural Research Service, 1503 Providence Rd., Columbia, MO 65203, USA

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ABSTRACT

Cell spreading is an integral component of insect hemocytic immune reactions to infections and invasions. Cell spreading is accomplished by cytoskeleton rearrangement, which is activated by three major immune mediators, biogenic monoamines, plasmatocyte-spreading peptide (PSP), and eicosanoids, particularly prostaglandin E_2 (PGE_2). However, little is known about how these immune mediators activate hemocyte spreading at the intra-cellular level. A small G protein, Rac1, acts in cytoskeleton arrangements in mammalian cells. Based on this information, we identified a Rac1 transcript (*SeRac1*) in hemocytes prepared from *Spodoptera exigua*. *SeRac1* was expressed in most developmental stages and in the two main immunity-conferring tissues, hemocytes and fat body, in larvae. In response to bacterial challenge, its expression was up-regulated by >37-fold at 2 h post-injection and returned to a basal level about 2 h later. Silencing *SeRac1* expression inhibited hemocyte spreading in response to three immune mediators, octopamine, 5-hydroxytryptamine, and PSP. Addition of PGE_2 to *SeRac1*-silenced larvae rescued the influence of these three mediators on hemocyte spreading. These compounds also increased phospholipase A_2 activity via SeRac1, which leads to prostaglandin biosynthesis. We infer that SeRac1 transduces OA, 5-HT, and PSP signaling via activating biosynthesis of prostaglandins and possibly other eicosanoids.

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1. Introduction

Insect immunity is assorted into two broad categories, cellular and humoral innate responses, although these categories overlap considerably (Beckage, 2008). Immune reactions are launched after pattern recognition molecules recognize microbial pathogens and activate appropriate immune signals (Lemaitre and Hoffmann, 2007). The recognition signals are propagated by immune mediators, including eicosanoids, biogenic monoamines, and the cytokine, plasmatocyte-spreading peptide (PSP) (Gillespie et al., 1997). Ultimately, immune signaling activates hemocytes and fat body as the major immune effector tissues responsible for protecting insects from infections and invasions. Cellular immune responses, phagocytosis, nodulation, and encapsulation, are acute and activated within the first minute after challenge (Strand, 2008). Humoral immune responses include production of antimicrobial peptides, which appear in hemolymph about 6–12 h following infection. Haine et al. (2008) argued that humoral reactions are especially effective to clear invaders remaining after the acute immune responses. More recently, An et al. (2012) reported that the tanning hormone, bursicon, acts in a novel homodimeric configuration to induce humoral immunity in a prophylactic function during the vulnerable molting periods.

Immune mediators activate effectors by amplifying nonself recognition signals (Stanley and Kim, 2011). PSP, the first identified insect cytokine, activates hemocyte spreading in several lepidopteran species (Clark et al., 1997; Jung and Kim, 2006; Matsumoto et al., 2012). Octopamine (OA) and 5-hydroxytryptamine (5-HT) also activate hemocyte spreading along with increasing circulating populations of hemocytes (Kim and Kim, 2010). Eicosanoids, including prostaglandins (PGs), are derived from any of three C20 polyunsaturated fatty acids, usually arachidonic acid, and these compounds mediate cellular and humoral defense reactions (Stanley, 2000, 2006a). PSP and biogenic monoamines activate hemocyte spreading via eicosanoids (Kim and Kim, 2010; Srikanth et al., 2011). Eicosanoids also act in other areas of insect cellular immunity, including release of prophenoloxidase from hemocytes into circulating hemolymph, prior to proteolytic activation (Kanost, 1999; Shrestha and Kim, 2008). Thus there is functional cross-talk between immune mediators (Stanley and Kim, 2011).

Immune signaling mediators activate hemocyte spreading via eicosanoids in the beet armyworm, *Spodoptera exigua*, which may represent most insect species in this regard (Kim and Kim, 2010; Srikanth et al., 2011). However, little is known about the cellular

* Corresponding author. Tel.: +82 521 820 5638; fax: +82 521 820 6320.
E-mail address: hosanna@andong.ac.kr (Y. Kim).

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signaling processes between immune mediators of hemocyte spreading. In general, at least two molecular events are required for hemocyte spreading. One is to activate a cell membrane protein integrin (α and β), a heterodimer that mediates cell-to-cell or cell-to-extracellular matrix interactions (Hughes, 2001). Silencing expression of the gene encoding the integrin β1 subunit impairs hemocyte spreading in *S. exigua* (Surakasi et al., 2011). The other molecular event is reorganization of the actin cytoskeleton by polymerization from G-actin to F-actin (Nalini and Kim, 2007; Xu et al., 2012). F-actin formation requires a small G protein mediator, Rac1, because a specific Rac1 inhibitor significantly antagonizes *S. exigua* hemocyte spreading in response to two biogenic monoamines (Kim and Kim, 2010). In *Drosophila melanogaster*, Rac1 is required for integrin localization toward pathogens on hemocyte membranes for effective cellular immunity (Xavier and Williams, 2011). Based on this information, we posed the hypothesis that Rac1 acts in hemocyte spreading by increasing eicosanoid biosynthesis. This study reports a hemocyte Rac1 gene, which mediates hemocyte spreading in *S. exigua*.

2. Materials and methods

2.1. Insect and bacterial culture

The beet armyworm, *S. exigua*, colony used in this study originated from a Welsh onion (*Allium fistulosum* L.) field in Andong, Korea. Larvae were reared on an artificial diet (Goh et al., 1990) at 25 ± 1 °C. Details of artificial diet preparation were described in an earlier study (Shrestha et al., 2011). *Escherichia coli* Top10 (Invitrogen, Carlsbad, CA, USA) was cultured overnight in Luria–Bertani medium (Difco, Sparks, MD, USA) at 37 °C in a shaking incubator at 270 rpm.

2.2. Chemicals

The eicosanoid biosynthesis inhibitor, dexamethasone (DEX) [(11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-dione], arachidonic acid (AA) [5,8,11,14-eicosatetraenoic acid], dimethyl sulfoxide (DMSO), prostaglandin E_2 (PGE_2)

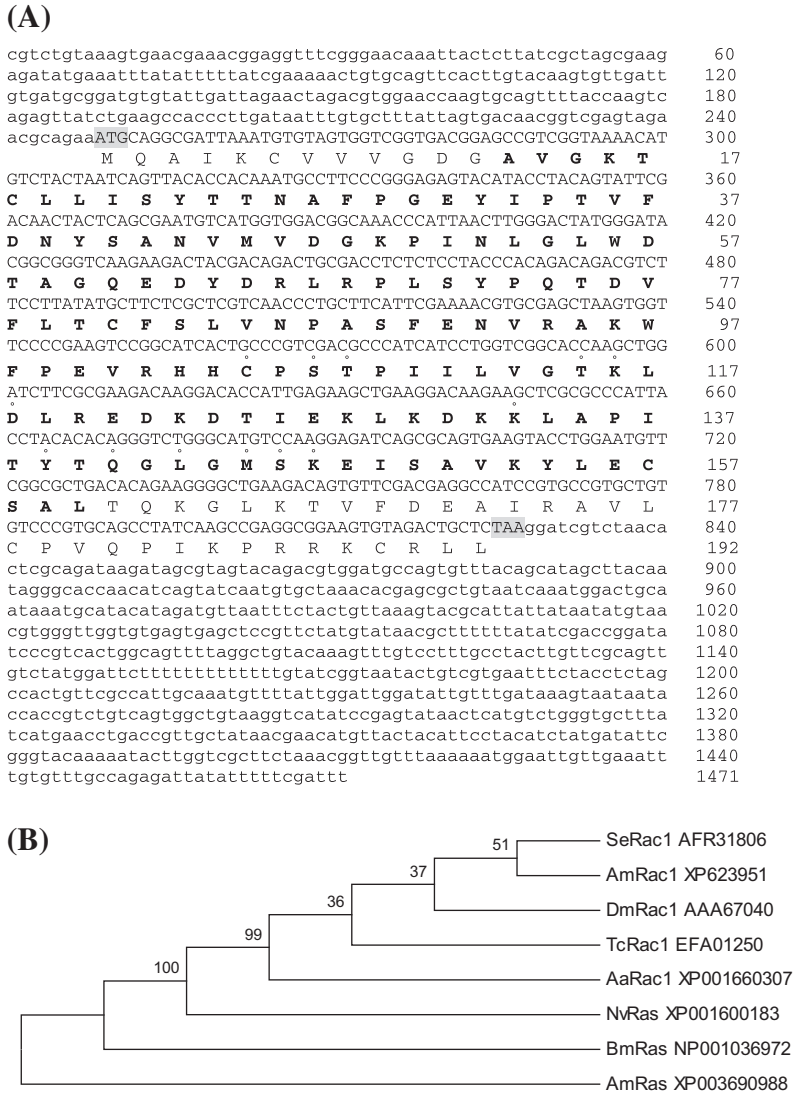

Fig. 1. Cloning a gene encoding a small G protein, Rac1, of *S. exigua* (*SeRac1*). (A) Its cDNA sequence and a predicted amino acid sequence (GenBank accession number: JX 392403). Start and stop codons are shaded. GTP-binding domain is bold-lettered. Mg^{++}-binding sites are denoted with small hollow spots. (B) A phylogenetic tree of some insect Rho family genes. Sequence alignment used Clustal W program. Bootstrap values were obtained with 1000 repetitions. GenBank numbers are added to Rho family genes of different insect species including *Apis mellifera* (Am), *Tribolium castaneum* (Tc), *Aedes aegypti* (Aa), *Drosophila melanogaster* (Dm), *Nasonia vitripennis* (Nv), and *Bombyx mori* (Bm).

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