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The roles of serpins in mosquito immunology and physiology

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ABSTRACT

In vector-borne diseases, the complex interplay between pathogen and its vector's immune system determines the outcome of infection and therefore disease transmission. Serpins have been shown in many animals to be key regulators of innate immune reactions. Their control over regulatory proteolytic cascades ultimately decides whether the recognition of a pathogen will lead to an appropriate immune response. In mosquitoes, serpins (SRPNs) regulate the activation of prophenoloxidase and thus melanization, contribute to malaria parasite lysis, and likely Toll pathway activation. Additionally, in culicine mosquitoes, SRPNs are able to regulate hemostasis in the vertebrate host, suggesting a crucial role during bloodfeeding. This review summarizes the annotation, transcriptional regulation, and current knowledge of SRPN function in the three mosquito species for which the complete genome sequence is available. Additionally, we give a brief overview of how SRPNs may be used to prevent transmission of vector-borne diseases.

1. Introduction

Proteolytic cascades take center stage in many biological processes, because they provide rapid response to danger signals. Well-known examples are the extracellular blood-clotting system in humans, the intracellular caspase cascade leading to the onset of apoptosis, and initiation of Toll signal-transduction pathway in arthropods. Due to the dramatic amplification of the initial signal by the proteolytic cascades it is not surprising that serine proteinase inhibitors associated with these cascades have a critical role. These inhibitors control accidental triggering of the cascades and regulate the spread and shutdown of the signal once the cascades are activated. At least 23 different families of serine proteinase inhibitors are known, and at least twelve have been found in insects (Gubb et al., 2010; Kanost, 1999). Among these are the Kazal, Kunitz, alpha-macroglobulin, and serpin families.

Serpins are the largest family of serine proteinase inhibitors and are found in all higher eukaryotes as well as bacteria and viruses (most recently reviewed by (Olson and Gettins, 2011). Serpins are considered the most important proteinase inhibitor family in higher eukaryotes, and hold a wide range of biological functions. Evidence for about 10,000 serpin sequences can be found in public databases, and this number will no doubt rise as more genomes are sequenced. Serpins are metastable proteins that function as structurally conserved suicide substrates (Huber and Carrell, 1989; Huntington et al., 2000). They can be found intra- as well as extracellularly, and are usually 350–400 amino acid residues long with a reactive center loop (RCL) that is located 30–40 residues from the C-terminal end. Their RCL binds to the active site of the specific target proteinase similar to the binding of a substrate. Upon cleavage of the serpin at its so-called scissile bond (designated P1–P1′), the serpin undergoes a substantial conformational change, which covalently traps the target proteinase (Dunstone and Whisstock, 2011; Huntington et al., 2000). Most serpins inhibit serine proteinases of the chymotrypsin type, but some are cross-class inhibitors that can also target cysteine proteinases (Kantyka and Potempa, 2011; Schick et al., 1997). Additionally, some serpins no longer function as proteinase inhibitors but have adopted other roles including hormone transport (Flink et al., 1986), blood pressure regulation (Doolittle, 1983), and storage (Hunt and Dayhoff, 1980).

This review outlines the diverse roles of serpins in arthropod biology and summarizes our current understanding of serpin biology in mosquitoes, emphasizing their roles in immune response regulation. Additionally, we provide an example how these molecules could be employed in control strategies of vector borne diseases.

2. The diverse functions of serpins in arthopods

The first serpins from invertebrates were isolated from the hemolymph of the silkworm *Bombyx mori* (Sasaki and Kobayashi, 1984) and cloned from the genome of *Manduca sexta* (Kanost et al., 1989). Since their initial description, arthropod serpins have been identified to regulate a variety of biological functions, including reproduction, developmental processes, hematophagy, cellular

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secretion, and immunity. Serpins are found in male accessory glands in a growing number of insects (Coleman et al., 1995; Dottorini et al., 2007; Sirot et al., 2008, 2011). At least some of them are transferred during mating to the female, suggesting a role in reproductive biology. However, their exact functions have thus far been explored only to a limited degree (Clendening et al., 2001; Ram and Wolfner, 2007). *Drosophila melanogaster* Spn27A is required for dorsal-ventral axis formation by inhibiting the Toll pathway during early embryonic development (Hashimoto et al., 2003; Ligoxygakis et al., 2003), while Spn88Ea is required for wing expansion. Several serpins have been identified in the saliva of hematophagous arthropods including ticks and mosquitoes (Francischetti et al., 2009; Stark and James, 1995). Their molecular functions range from inhibiting blood coagulation to host inflammation and platelet aggregation, and are likely to be crucial for blood feeding (Chmelar et al., 2011; Stark and James, 1995). The function of intracellular serpins in arthropods is little understood. The notable exception is *D. melanogaster* Spn4A. This Spn4 isoform inhibits furin, suggesting a role in regulating cellular secretion (Bruning et al., 2007; Osterwalder et al., 2004).

The majority of characterized arthropod serpins regulate innate immune responses. Proteolytic cascades take a central role in many immune reactions as they amplify the invasion signal and activate various lines of attack against the pathogen (Fig. 1). Serpins inhibit many of these cascades, including the hemolymph coagulation cascade in horseshoe crabs (Iwanaga et al., 1998), proteolytic activation of the Toll pathway (Ahmad et al., 2009; An et al., 2011b; Fullaondo et al., 2011; Jiang et al., 2009; Levashina et al., 1999; Zou and Jiang, 2005); and proteolytic activation of pro-phenoloxidase (proPO) and as a consequence melanization (De Gregorio et al., 2002; Jiang et al., 2003; Ligoxygakis et al., 2002; Scherfer et al., 2008; Tang et al., 2006; Tong and Kanost, 2005). Additionally, activation of the complement-like system in insects requires proteolytic cleavage of thioester-containing proteins at the center of this immune response. The proteinases involved in this process await identification and are likely to be tightly regulated by inhibitors.

Arthropod serpins thus function in a wide variety of physiological processes with important implications for pathogen transmission. It is thus not surprising that since the publication of the *Anopheles gambiae* genome ten years ago, this protein family has been studied increasingly in mosquitoes that vector human pathogens. The following sections will summarize our current understanding of serpin biology in mosquitoes and how these proteins partake in disease transmission.

3. Serpins encoded in mosquito genomes

The overall number of serpins per genome varies widely among arthropod species. The number of serpins per mosquito genome is significantly lower compared to the 35 serpins described in *D. melanogaster* (Reichhart et al., 2011), while the *Ixodes scapularis* genome encodes at least 45 serpins (Mulenga et al., 2009). The genome of *An. gambiae*, *Aedes aegypti*, and *Culex quinquefasciatus*, the three mosquito genomes sequenced so far, contain 18, 23, and 31 serpin (*SRPN*) genes, respectively (Table S1). Numbering of mosquito serpins is arbitrary, but indicates orthology. Alternative splicing mainly at the 3'end of the serpin coding sequence is commonly observed in invertebrates leading to a diversification of RCL sequences and target specificities (Bartholomay et al., 2010; Bruning et al., 2007; Danielli et al., 2003; Jiang et al., 1996; Kruger et al., 2002). Due to alternative splicing, the number of distinct SRPN proteins is substantially higher, increasing to 23, 26 and 39.

In *An. gambiae*, all *SRPN*s are located on chromosomes 2 and 3, with twelve SRPNs clustering in four regions suggesting that genes within clusters arose from recent duplication events. Most

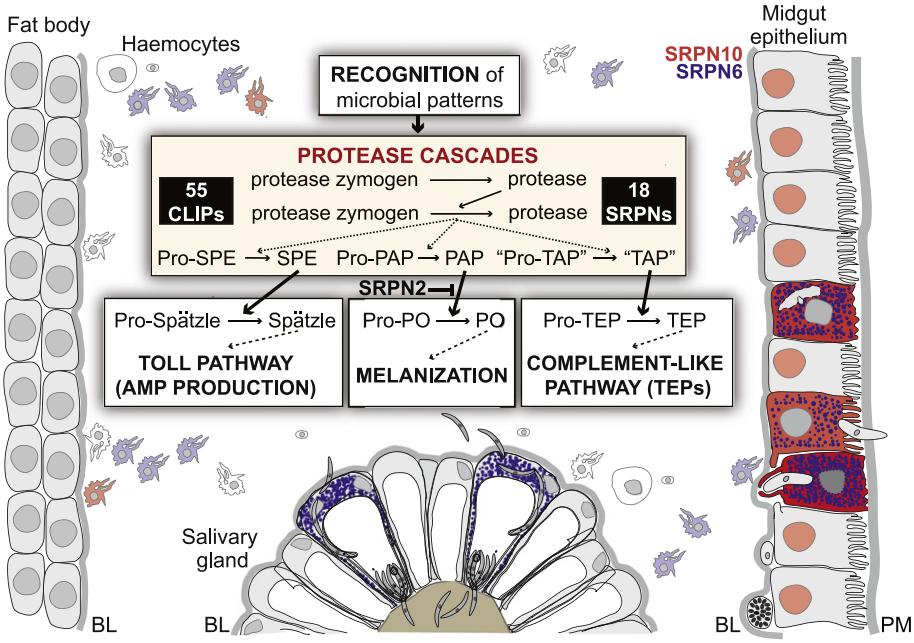

Fig. 1. Overview of the mosquito immune system and its regulation by serpins. At least four tissues contribute to immune responses against human pathogens, including fat body, hemocytes, and salivary gland and midgut epithelia. Upon recognition of the pathogen, proteinase cascades are initiated, which activate a number of downstream immune reactions. These include (i) the Toll pathway leading to antimicrobial peptide production, (ii) activation of phenoloxidases resulting in melanin production, and (iii) activation of thioester proteins as part of the complement pathway. Two mosquito serpins (SRPNs) act as acute-phase response molecules: SRPN6 (blue dots) and 10 (red stain) are upregulated in epithelial midgut cells upon malaria parasite invasion. SRPN6 protein is also detectable upon malaria parasite invasion of the salivary gland epithelium. The only mosquito serpin with known molecular function is SRPN2, which regulates melanization by inhibiting the serine proteinase that activates prophenoloxidase. BL, basal lamina; CLIP, clip-domain serine proteinase; PAP, phenoloxidase-activating protease; PM; peritrophic matrix; PO, phenoloxidase; SPE, spaetzle-processing enzyme; SRPN, serpin; TAP, thioester protein-activation protease; TEP, thioester protein.