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Review

Drosophila melanogaster clip-domain serine proteases: Structure, function and regulation

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ABSTRACT

Mammalian chymotrypsin-like serine proteases (SPs) are one of the best-studied family of enzymes with roles in a wide range of physiological processes, including digestion, blood coagulation, fibrinolysis and humoral immunity. Extracellular SPs can form cascades, in which one protease activates the zymogen of the next protease in the chain, to amplify physiological or pathological signals. These extracellular SPs are generally multi-domain proteins, with pro-domains that are involved in protein–protein interactions critical for the sequential organization of the cascades, the control of their intensity and their proper localization. Far less is known about invertebrate SPs than their mammalian counterparts. In insect genomes, SPs and their proteolytically inactive homologs (SPHs) constitute large protein families. In addition to the chymotrypsin fold, many of these proteins contain additional structural domains, often with conserved mammalian orthologues. However, the largest group of arthropod SP regulatory modules is the clip domains family, which has only been identified in arthropods. The clip-domain SPs are extracellular and have roles in the immune response and embryonic development. The powerful reverse-genetics tools in *Drosophila melanogaster* have been essential to identify the functions of clip-SPs and their organization in sequential cascades. This review focuses on the current knowledge of *Drosophila* clip-SPs and presents, when necessary, data obtained in other insect models. We will first cover the biochemical and structural features of clip domain SPs and SPHs. Clip-SPs are implicated in three main biological processes: the control of the dorso-ventral patterning during embryonic development; the activation of the Toll-mediated response to microbial infections and the prophenoloxydase cascade, which triggers melanization. Finally, we review the regulation of SPs and SPHs, from specificity of activation to inhibition by endogenous or pathogen-encoded inhibitors.

Contents

Abbreviations: AMP, antimicrobial peptide; CCP domain, complement control protein domain; Clip-SP, Clip-domain serine protease; Clip-SPH, Clip-domain serine protease homolog; *Dm*, *Drosophila melanogaster*; Ea, easter; Gd, gastrulation defective; GNBP, β-glucan binding proteins; *Hd*, *Holotrichia diomphalia*; Imd, immune-deficiency; LDLa, low-density lipoprotein receptor class A repeats; MASPs, MBL-associated serine protease; ModSP/MSP, modular serine protease; *Ms*, *Manduca sexta*; Ndl, nudel; nec, necrotic; NF-kB, nuclear factor kB; PAE, prophenoloxydase activating enzyme; PAP, prophenoloxydase activating protein; PGRP, peptidoglycan recognition proteins; PO, phenoloxydase; PPAF, prophenoloxydase activating factor; PPO, prophenoloxydase; PRR, pattern recognition receptor; Psh, persephone; Snk, snake; SP, serine protease; SPE, spaezle processing enzyme; SPH, serine protease homolog; Spz, spaetzle; TEP, thioester containing protein.

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1. Introduction

The chymotrypsin-like serine proteases (SPs) family (or S1 family) is one of the best-characterized families of enzymes [1,2]. SPs have essential roles in digestion, blood coagulation, fibrinolysis, cellular and humoral immunity, fertilization and embryonic development. SPs are commonly synthesized as inactive zymogens, which need to be proteolytically cleaved to obtain the catalytic domain under its active conformation. Extracellular SPs form cascades in which each protease activates the subsequent zymogen to amplify physiological or pathological signals. While zymogens of digestive SPs such as trypsin or chymotrypsin carry a short N-terminal peptide connected to the catalytic domain, SPs involved in more complex processes are generally multi-domain proteins. During the activation of extracellular cascades, N-terminal pro-regions allow protein–protein interactions critical for the sequential organization of the cascades, the control of their intensity and their proper localization. The blood clotting and the complement system, activated in response to tissue damage and microbial infection, respectively, are two examples of mammalian extracellular SP cascades.

Extracellular multi-domain SPs are also present in arthropods. Indeed, in known insect genomes, SPs and proteolytically inactive SP homologs (SPHs), constitute a large family of protease folds, with 50–300 members [3–6]. Many of these SPs contain structural modules important for protein–protein interactions, already identified in mammals, such as the low-density lipoprotein receptor class A repeats (LDLa), the scavenger receptor (SR), the Sushi, or Wonton domains, etc… (Complete list in Ref. [7]). The largest group of regulatory modules in arthropod SPs is the clip domain family, first identified in the horseshoe crab pro-clotting enzyme [8,9]. Clip-domain Serine Proteases (clip-SPs) have since been identified in other arthropods, where they participate in embryonic development and defense responses, including hemolymph coagulation, melanotic encapsulation, induction of antimicrobial peptide synthesis and activation of cytokines [10,11].

Drosophila melanogaster (the fruit fly) is a popular model organism due to the high accessibility of reverse-genetics tools and the relationship between the *Drosophila* and human genomes [12,13]. It is also a popular model to decipher arthropod immune responses, with the goal of understanding insect vector-borne diseases and controlling pest species. In this review, we will focus on the current knowledge on *Drosophila* clip-SPs, from their biochemical and structural properties to their physiological functions and regulation.

2. Sequence, folding, catalytic mechanism, substrate specificity and classification of Clip-SPs and SPHs

2.1. Sequence and domain organization

Serine proteases (SPs) and Serine proteases homologs (SPHs) constitute the second largest family of genes in the *Drosophila melanogaster* genome [4]. Among the 147 SPs and 57 SPHs identified, 28 SPs and 14 SPHs contain regulatory clip domains (Table 1). Of note, the Clip domain of 4 SPs and 1 SPH were originally described as only partial due to the lack of the full protein sequences [4]. Most of theses protease folds are associated with a single clip domain, but two SPs contains two clip domains (CG1299 and CG10232), while several SPHs carry two (CG8586 and CG8738), three (CG11066) or even five (CG15002) clip domains. These multiple clip domains are separated by a fairly long linker sequences (>30 residues), in contrast to the lepidopteran prophenoloxydase activating proteins (PAPs) that have only one to seven residues connecting adjacent clip domains [4,14]. Clip domains are connected to the chymotrypsin fold by a 23–92 residues long linker containing at least one cysteine, which is involved in a disulfide bond with a cysteine within the catalytic domain. The zymogen forms of these proteases are converted into their activated forms by cleavage at a residue corresponding to Arg15 of chymotrypsinogen. Owing to the disulfide linkage, the N-terminal clip domain remains covalently attached to the catalytic domain after zymogens activation. Remarkably, the clip-SPs never possess additional regulatory modules of other types (Table 1).

2.2. Clip domain properties

2.2.1. Generalities and sequences

Clip domains are usually 30 to 63 residues long and are characterized by six strictly conserved cysteine residues, which form 3

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