The FEBS Journal

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COMMENTARY

Matrix metalloproteinases and their inhibitors – pleiotropic functions in insect immunity and metamorphosis

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Keywords
Bombyx mori; innate immunity; insects; matrix metalloproteinases; metamorphosis; tissue inhibitors of metalloproteinases

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(Received 11 November 2021, accepted 3 December 2021)

doi:10.1111/febs.16314

Matrix metalloproteinases (MMPs) enable tissue remodeling and immune responses by degrading extracellular matrix proteins. They are regulated by tissue inhibitors of metalloproteinases (TIMPs). Mammals produce more than 20 MMPs but insects produce fewer than 3, at odds with the extensive tissue remodeling required during metamorphosis and inflammation. Addressing this apparent paradox, Liu *et al.* demonstrate the pleiotropic functions of silkworm MMPs and TIMP. They measured expression levels during pupation and during a response to viral infection in transgenic overexpression and knockout lines for selected MMP/TIMP genes. This confirmed the multiple roles of these key enzymes in insect immunity and metamorphosis.

Comment on https://doi.org/10.1111/febs.16313

Introduction

Matrix metalloproteinases (MMPs) are evolutionarily conserved and multifunctional enzymes that degrade extracellular matrix proteins such as collagens, elastin, proteoglycans and laminins, which are normally resistant to hydrolysis by other types of proteinases. More than 20 MMPs with distinct and overlapping functions are produced by humans, and they have attracted interest because they are the key enzymes not only required for tissue remodeling during development, wound healing and inflammation but also play a role in diseases such as cancer, rheumatism and osteoarthritis [1–3]. MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs). Humans produce four TIMPs with multifaceted roles in developmental, physiological and pathological processes.

The repertoire of MMPs in insects is much smaller, with a maximum of three genes found in the species investigated thus far. This is at odds with the complete metamorphosis observed in holometabolous insects such as beetles (Coleoptera), flies (Diptera) and butterflies (Lepidoptera). During this complex process, larval organs are completely remodeled and replaced by those of the imagines, which requires the orchestrated activity of MMPs to achieve the controlled breakdown of the larval extracellular matrix. Previous studies provided evidence that MMPs play a role in tissue remodeling in all of these taxa [4–6]. In their work published in *The FEBS Journal*, Liu *et al.* used CRISPR/Cas9 technology to achieve the transgenic overexpression or suppression of MMP and TIMP genes in the silkworm

Abbreviations
AMP, antimicrobial peptides; CRISPR, clustered regularly interspaced short palindromic repeats; IMPI, insect metalloproteinase inhibitor; MMP, matrix metalloproteinase; RNAi, RNA Interference; TIMP, tissue inhibitors of metalloproteinases.

Bombyx mori, one of the economically most important insect species due to its role in global silk production [7]. Their results confirm that MMPs and TIMP display pleiotropic functions in insect immunity and metamorphosis.

Pleiotropic functions of MMPs and TIMPs in insects

To investigate the potential dual functions of MMPs and TIMP in *B. mori*, Liu *et al.* analyzed the expression of MMP/TMP genes during metamorphosis and viral infection. In agreement with earlier studies [5–6], they found that MMP genes were upregulated during metamorphosis. They also found that the silencing of MMP genes using CRISPR/Cas9 technology increased mortality, in line with the arrested development and developmental defects previously reported following RNAi-mediated silencing [6]. Liu *et al.* also found that MMP genes were induced by viral infection [7], confirming their postulated role in insect immunity as previously demonstrated in response to bacterial or fungal infection. The silencing of an MMP gene by RNAi in *Tribolium castaneum* also enhanced the susceptibility of this model beetle to the fungal pathogen *Beauveria bassiana* [6].

The results presented by Liu *et al.* add a formerly missing piece to the puzzle of the evolutionary origin and selective pressures that maintain the pleiotropic roles of MMPs. Using another lepidopteran model, the greater wax moth *Galleria mellonella*, it was shown that the transient upregulation of an MMP during metamorphosis is accompanied by the simultaneous induction of antimicrobial peptides (AMPs), providing evidence of a development-related immune response [5,8]. The MMP-mediated degradation of extracellular matrix proteins such as collagen type IV (the only

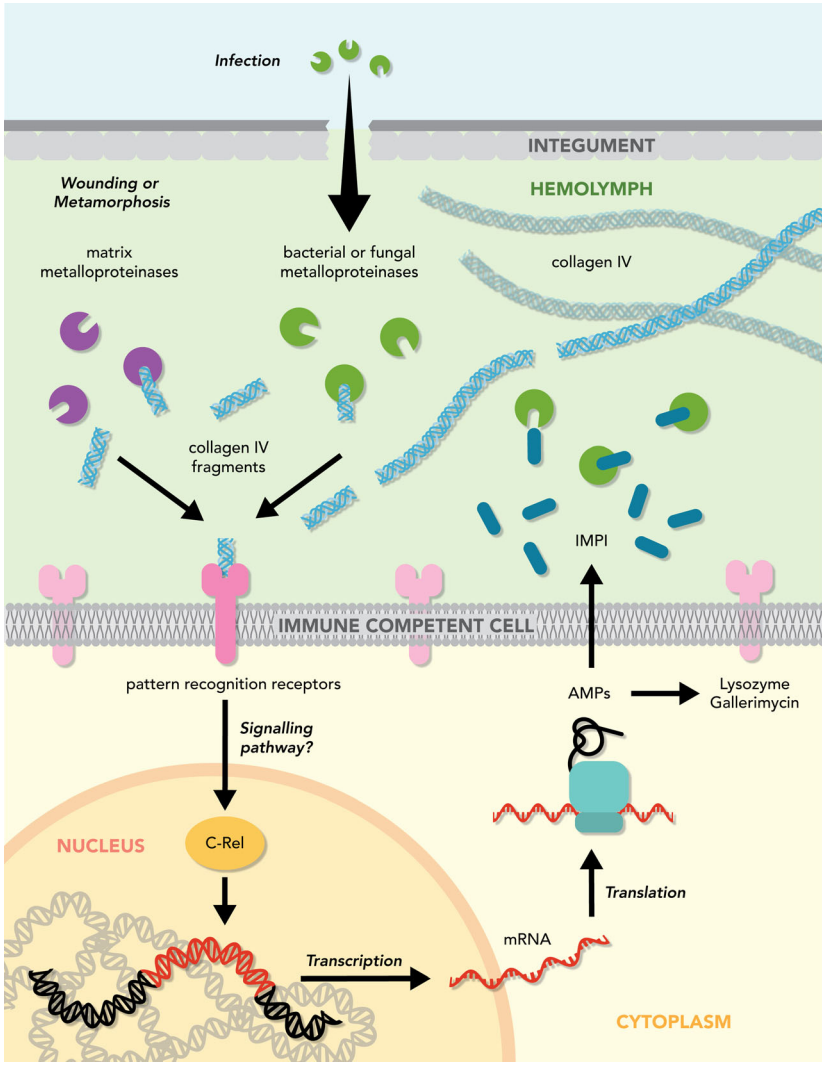

Fig. 1. Model illustrating metalloproteinase-mediated immunity-related signaling in Lepidoptera. Endogenous MMPs and microbial metalloproteinases associated with invading bacterial or fungal pathogens degrade extracellular matrix proteins such as collagen type IV. Microbial and endogenous metalloproteinases degrade collagen IV at similar cleavage sites, resulting in the formation of peptide danger signals. These in turn elicit innate immune responses, for example, by inducing the synthesis of antimicrobial peptides such as lysozyme and the antifungal peptide gallerimycin. They also induce the insect metalloproteinase inhibitor (IMPI) gene which encodes two inhibitors: a N-terminal IMPI peptide (a specific inhibitor of microbial metalloproteinases) and a C-terminal inhibitor of MMPs. When released into the hemolymph, both act as feedback regulators, inhibiting microbial and endogenous MMPs, respectively, thereby limiting the production of further collagen IV fragments.

The FEBS Journal **289** (2022) 2805–2808 © 2021 The Authors. The *FEBS Journal* published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies.

collagen present in insects) is thought to generate peptide fragments that function as danger signals to elicit immune responses [8,9]. Entomopathogenic viruses, bacteria and fungi are all equipped with metalloproteinases (particularly thermolysin-like metalloproteinases representing the M4 family) that operate as virulence factors, breaking down the extracellular matrix within the infected host [10]. These microbial metalloproteinases generate collagen IV fragments during infection, similar to those produced by the MMP-mediated degradation of the extracellular matrix during metamorphosis [11]. The insect immune system cannot distinguish between the collagen IV-derived danger signals generated by either microbial metalloproteinases during infection or endogenous MMPs during metamorphosis [8,12]. In the Lepidoptera at least, this dilemma resulted in the evolution of a novel regulatory mechanism that acts on both virulence-associated metalloproteinases and endogenous MMPs.

The insect metalloproteinase inhibitor (IMPI) is a unique peptide-based inhibitor stabilized by five internal disulfide bonds, which was discovered among other immune-inducible AMPs in greater wax moth larvae [13]. IMPI is transcribed from a gene encoding two distinct inhibitors, one with specific activity against microbial metalloproteinases operating as virulence factors [14] and the other with activity against MMPs [15]. The IMPI protein may, therefore, help insect TIMPs to regulate MMPs [16]. The feedback loop enabling the regulation of endogenous MMPs and microbial metalloproteinases is shown in Fig. 1. The *B. mori* genome also contains an IMPI gene. It would be interesting to test whether this is also induced by viral infection, and to determine which MMPs are inhibited by TIMP and/or IMPI.

Conclusion and future prospects

The MMPs and TIMPs display evolutionarily conserved pleiotropic functions in mammals and insects allowing the controlled degradation of extracellular matrix proteins during development. The metamorphosis of holometabolous insects involves complete tissue and organ remodeling during the transformation of larvae into imagines. The prominent role of MMPs in insect metamorphosis has been confirmed by gene knockdown experiments (RNAi and CRISPR/Cas9). The role of insect MMPs in immunity is not fully understood, but their ability to generate peptide danger signals may facilitate the amplification of AMP synthesis elicited by infections. TIMPs regulate endogenous MMPs in mammals and in insects. However, at least the Lepidoptera have evolved IMPI, a highly specific inhibitor of microbial metalloproteinases that operate as virulence factors, which is currently under development as a virulence blocker for the treatment of human patients infected with multidrug-resistant pathogens [17].

Acknowledgement

The author thanks Mona Liu for drafting the figure and Richard M Twyman for editing the manuscript. Open access funding enabled and organized by Projekt DEAL.

Conflict of interest

The author declare no conflict of interest.

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