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Review

MACPF/CDC proteins in development: Insights from *Drosophila* torso-like

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ABSTRACT

The Membrane Attack Complex Perforin-like/Cholesterol-Dependent Cytolysin (MACPF) superfamily is an ancient and biologically diverse group of proteins that are best known for pore-forming roles in mammalian immunity and bacterial pathogenesis. Intriguingly, however, some eukaryotic proteins which contain the MACPF domain that defines this family do not act in attack or defence, and instead have distinct developmental functions. It remains unclear whether these proteins function via pore formation or have a different mechanism of action. Of these, by far the best characterised is Torso-like (Tsl), the only MACPF member that has been identified in the fruit fly, *Drosophila melanogaster*. While it has long been known to have a role in embryonic patterning, recent studies have shown that Tsl in fact has multiple roles in development. As such, it presents an excellent opportunity to investigate how the MACPF domain functions in a developmental context. Here, we review what is known about Tsl in *Drosophila* and other insects, and discuss the potential molecular mechanism by which Tsl and thus other developmental MACPF proteins may function.

Contents

Abbreviations: ASTN, Astrotactin; BRINP, Bone Morphogenic Protein/Retinoic Acid Inducible Neural-Specific Protein; C6-C9, Complement components 6–9; CDC, cholesterol dependent cytolysin; EM, electron microscopy; fs, female sterile; IL-12, Interleukin-12; InR, Insulin-like receptor; MACPF, Membrane Attack Complex Perforin-like; MAPK, mitogen activated protein kinase; mRNA, messenger RN; PG, prothoracic gland; PTTH, Prothoracicotropic hormone; Pvr, platelet-derived growth factor and vascular endothelial growth factor receptor; PVS, perivitelline space; RTK, receptor tyrosine kinase; SLO, Streptolysin O; SMase, Acid sphingomyelinase; Spz, Spätzle; TMH, transmembrane helicies; TNF-α, Tumour necrosis factor-alpha; Tor, Torso; Trk, Trunk; Tsl, Torso-like; VEGF, vascular endothelial growth factor; VM, vitelline membrane.

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1. Introduction

The MACPF domain was originally characterised as an extensive region common to the pore-forming human immunity proteins Perforin and Complement components 6–9 (C6-C9) of the membrane attack complex (MAC) [1]. More recently, structural studies have revealed striking homology between proteins containing the MACPF domain and the cholesterol dependent cytolysin (CDC) family of bacterial pore-forming virulence factors [2,3]. Since this discovery, electron microscopy (EM) and biochemical data from studies of MACPF proteins suggest that pore formation occurs in an analogous fashion to the CDC family [4–7]. We now understand that in order to achieve a pore, MACPF proteins must be able to bind to the surface of their target membrane via interactions with lipids or lipid-binding partner proteins, and oligomerise to form a large supramolecular ring. Through major conformational changes, the MACPF domain within each monomer is then triggered to deploy a pair of membrane-spanning beta-hairpins (transmembrane helices, TMH1 and 2) to form a giant beta-barrel pore (reviewed in [8]).

The benefit of this remarkable molecular ability is suggested by the fact that more than 1000 MACPF proteins have been identified across most forms of life [9]. Of the functionally characterised MACPF proteins, most can be assigned to either immune defence processes or pathogenesis. For instance, protozoan parasites such as *Plasmodium* spp. or *Toxoplasma* spp. utilise MACPF proteins in host invasion or in vacuole escape (reviewed in [10]), whereas mammalian Perforin and Complement C6-C9 facilitate the elimination of transformed (or viral-infected) cells and pathogens respectively (reviewed in [11–13]). Studies of these immune/defence proteins thus suggest that the ability to form large cytolytic pores on target lipid membranes is a universal property of the MACPF domain.

There are, however, a small number of eukaryotic MACPF superfamily members that challenge this view. This is because these proteins perform crucial roles in animal development and neurobiology, for which a role in membrane disruption and cell-killing is difficult to reconcile. For example, the vertebrate MACPF protein Astrotactin-1 (ASTN-1) and its paralogue ASTN-2 have been shown to play an important role in mediating neuronal-glial interactions [14,15]. Similarly, three other mammalian MACPF proteins named Bone Morphogenic Protein/Retinoic Acid Inducible Neural-Specific Protein (BRINP)-1, BRINP-2 and BRINP-3 are also widely expressed in neural tissue [16]. While BRINP-1 has recently been implicated in neuronal differentiation and neurogenesis [17], overall very little functional characterisation of these mammalian proteins has been performed. Consequently the purpose of their MACPF domain remains unclear.

The most extensively studied developmental MACPF protein is Torso-like (Tsl) from the fruit fly *Drosophila melanogaster* [3,18]. Tsl was first identified in the 1980s as a maternally provided protein essential for patterning the ends of the embryo, or termini [19–22]. Terminal patterning is the result of an extracellular signal that is generated locally by Tsl via an unknown mechanism and then transduced through a receptor tyrosine kinase (RTK) pathway [21,23–25]. Our extensive understanding of the molecular and genetic control of *Drosophila* development and the genetic tractability of this organism makes Tsl an excellent option for investigating what are now central questions for the MACPF field: how do MACPF proteins function in development, and does this involve pore formation?

Towards answering these questions, recent work has identified several new and important developmental roles for Tsl in insects, and provided additional insights into the function of Tsl in embryonic patterning. Here we review these findings and draw together the available knowledge on Tsl function. We then explore functional aspects of characterised MACPF proteins to generate hypotheses for the molecular function of Tsl in cell signalling.

2. Torso-like in *Drosophila* embryonic terminal patterning

2.1. Torso-like is the localising cue for terminal patterning

The *tsl* gene was discovered in pioneering genetic screens that identified many of the genes that govern the major patterning systems of the *Drosophila* embryo [20,22,26,27]. This important work determined that early embryonic patterning is achieved by the action of genes expressed in the mother (maternal genes) prior to fertilisation [28]. Maternal gene products are provided to the developing oocyte by two groups of cells, the germ line-derived nurse cells and the somatic follicle cells (Fig. 1A). The nurse cells provide the oocyte with messenger RNAs (mRNAs) and proteins that are essential for embryonic development. The follicle cells envelope the oocyte and secrete key patterning factors and structural components of the eggshell, including the vitelline membrane (VM); a heavily glycosylated proteinaceous matrix that lies in close proximity to the embryo surface (reviewed in [29]). The follicle cells degenerate at the end of oogenesis and are thus no longer present in the early embryo. Importantly, both components of the VM and gene products secreted into the perivitelline space (PVS, the extracellular space between the embryo and the VM) control when and where key signalling events for embryonic patterning occur (Fig. 1B) [30,31].

Tsl was identified as one of five maternally provided proteins required for cell fate specification of the unsegmented structures at the embryo termini [20,22,26,27]. Embryos laid by females homozygous for mutations in any of these terminal class genes (*tsl, torso (tor), trunk (trk), female sterile (fs) nasrat, fs(1)polehole*) show an identical mutant phenotype – the loss of structures posterior to abdominal segment seven and defects in the anterior head skeleton (Fig. 1C) [19,21,22]. Through a significant body of work, we now understand that terminal patterning is governed by the localised activation of Tor, an RTK (reviewed in [32]). This signal is transduced via activation of the highly conserved Ras/mitogen activated protein kinase (MAPK) phosphorylation cascade and transcriptional derepression of key zygotic terminal cell fate determinants [23,25,33].

The proposed ligand for Tor is Trk, a member of the Noggin-like branch of the cysteine knot-like growth factor superfamily [34,35]. The mRNAs for *tor* and *trk* are homogeneously distributed in the embryo, and several lines of genetic evidence suggest that the Tor receptor is present ubiquitously on the embryo plasma membrane [23,36,37]. As *trk* encodes a secreted growth factor, it has long been presumed to be secreted ubiquitously into the PVS [37]. However, despite the ubiquitous expression of both Tor and Trk, Tor signalling is only activated at the embryo termini.

The only known localised factor in the terminal patterning pathway is Tsl. Expression of *tsl* is limited to a sub-population of follicle cells at each end of the developing oocyte [19,21], and Tsl protein is present at both ends of the embryo following fertilisation [19]. Genetic analyses indicate that Tsl functions upstream of Trk and Tor [38], and in the absence of Tsl, Tor signalling is not activated [25]. Importantly, ectopic expression of Tsl from all follicle cells causes ubiquitous Tor signalling, resulting in the "spliced" cuticle phenotype in which the terminal regions are expanded at the expense of the central segments [19,21]. Taken together, these findings demonstrate that Tsl is the key to localised activation of Tor signalling.

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