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Mini Review

Current activities of the *Yersinia* effector protein YopM

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ABSTRACT

Yersinia outer protein M (YopM) belongs to the group of Yop effector proteins, which are highly conserved among pathogenic *Yersinia* species. During infection, the effectors are delivered into the host cell cytoplasm via the type 3 secretion system to subvert the host immune response and support the survival of *Yersinia*. In contrast to the other Yop effectors, YopM does not possess a known enzymatic activity and its molecular mechanism(s) of action remain(s) poorly understood. However, YopM was shown to promote colonization and dissemination of *Yersinia*, thus being crucial for the pathogen's virulence *in vivo*. Moreover, YopM interacts with several host cell proteins and might utilize them to execute its anti-inflammatory activities. The results obtained so far indicate that YopM is a multifunctional protein that counteracts the host immune defense by multiple activities, which are at least partially independent of each other. Finally, its functions seem to be also influenced by differences between the specific YopM isoforms expressed by *Yersinia* subspecies.

In this review, we focus on the global as well as more specific contribution of YopM to virulence of *Yersinia* during infection and point out the various extra- and intracellular molecular functions of YopM. In addition, the novel cell-penetrating ability of recombinant YopM and its potential applications as a self-delivering immunomodulatory therapeutic will be discussed.

Introduction

The *Yersinia* outer protein M (YopM) is one of six secreted effector proteins termed *Yersinia* outer proteins (Yops) of human-pathogenic *Yersinia* (Viboud and Bliska, 2005). *Yersinia* is a genus of Gram-negative bacteria comprising at present 17 different species. Three of those are pathogenic for humans: *Yersinia pestis*, the causative agent of bubonic/pneumonic plaque, *Yersinia pseudotuberculosis*, and *Yersinia enterocolitica*, both causing gastrointestinal disorders. As many other Gram-negative pathogens, *Yersinia* utilizes a type 3 secretion system (T3SS) for the translocation of Yop effectors into the host cell cytoplasm (Cornelis, 2002). Preferentially the Yops are directly injected into immune cells such as macrophages, neutrophils, lymphocytes, and dendritic cells (Köberle et al., 2009; Marketon et al., 2005). The components of the T3SS and the associated effector proteins are encoded on a virulence plasmid which is highly conserved among human-pathogenic *Yersinia* (Cornelis et al., 1998). During infection, the Yops, namely YopE, YopH, YopJ/YopP, YopM, YopO/YpkA, and YopT, modulate several eukaryotic signaling pathways by different mechanisms (Viboud and Bliska, 2005) to subvert the host innate or adaptive immune responses to *Yersinia*. YopM does not exhibit any enzymatic activity and the exact molecular mechanism of the function of YopM has remained enigmatic. However, YopM was shown to be required for full virulence of *Yersinia* and to have an impact on cytokine expression within the host cell (Kerschen et al., 2004; Leung et al., 1990). Interestingly, recombinant YopM derived from *Y. enterocolitica* O:8 JB580v (pYV8081) was found to enter cells autonomously and independently of the T3SS, acting as a so-called cell-penetrating effector protein (CPE) (Rüter et al., 2010; Rüter and Hardwidge, 2014). In the following, the structural features of YopM and its functions during infection as well as extra- and

Abbreviations: CPE, cell-penetrating effector protein; CPP, cell-penetrating peptide/protein; Egr1, early growth response transcription factor 1; iDC, inflammatory dendritic cell; IFN, interferon; IL, interleukin; LRR, leucine-rich repeat; MAPK, mitogen-activated protein kinase; MLN, mesenteric lymph node; NEL, novel E3 ubiquitin ligase; NEMO, NF-κB essential modulator; NLS, nuclear localization signal; NK cells, natural killer cells; PMN, polymorphonuclear leukocyte; PTD, protein transduction domain; PRK, protein kinase C-related kinase; RSK, ribosomal S6 protein kinase; T3SS, type 3 secretion system; TNF-α, tumor necrosis factor α; Yop, *Yersinia* outer protein; YopM, *Yersinia* outer protein M.

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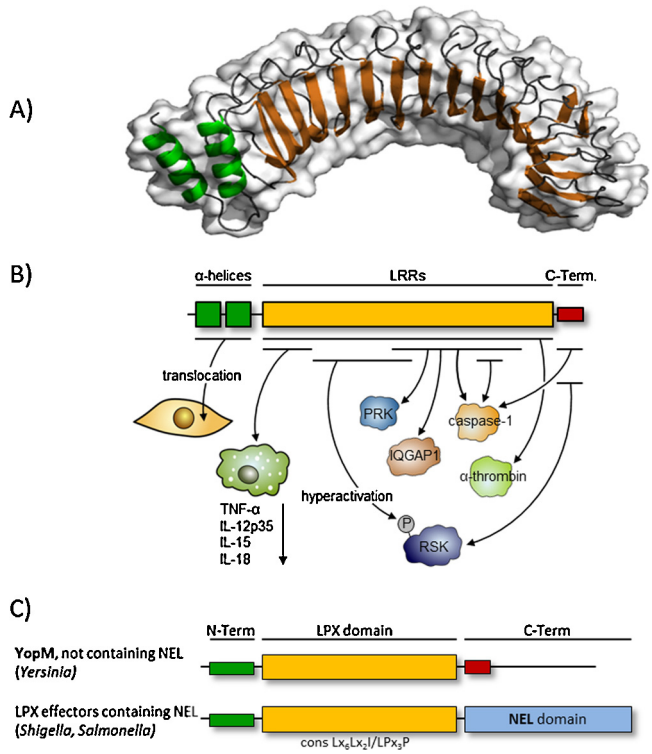

Fig. 1. Schematic representations of the YopM monomer, its functions and comparison to other T3SS effector proteins of the LPX subtype. (A) Structure of YopM. YopM consists of two conserved, antiparallel N-terminal helices (green) followed by a leucine-rich repeat (LRR) region and a short conserved but unstructured C-terminus. Within the YopM LRR region the number and composition of LRRs varies depending on the *Yersinia* strain and serotype. The LRRs form β-strands (orange) which are assembled into a curved parallel β-sheet structure, thus building up 'horseshoe-shaped' proteins of 42 kDa to 57 kDa. PDB: 1JL5; YopM of *Y. pestis*, containing 15 LRRs (Evdokimov et al., 2001). Overlay of the cartoon and the surface display was generated using PyMOL. (B) Domain specific functions of YopM. The two N-terminal α-helices of YopM are responsible for its autonomous translocation into eukaryotic cells (Rüter et al., 2010). While the whole LRR stretch of YopM (pYV8081) together with the C-terminus are necessary for interaction with PRK (Höfling et al., 2014), LRRs 1–3 are sufficient for inhibition for expression of pro-inflammatory cytokines such as TNF-α in lymphocytes (Rüter et al., 2010, Höfling et al., 2014). Hyperactivation of RSK in turn is dependent on LRRs 4–9 plus the C-terminus (Höfling et al., 2014). The 15-LRR isoform of *Y. pseudotuberculosis* YPIII YopM binds pro-caspase-1 and active caspase-1 using a 4 aa long pseudosubstrate motif (YLTD) within LRR 10. Additionally, Chung et al. (2014) showed for YopM proteins lacking this motif, that the LRRs 6 to 15 and the C-terminal tail were required for inhibition of caspase-1. Furthermore, Chung et al. (2014) revealed the binding of the 15-LRR YopM of *Y. pestis* KIM to the scaffolding protein IQ motif-containing Ras-GTPase-activating like protein 1 (IQGAP1) via its LRRs 6–11. (C) Schematic comparison of YopM with LPX effectors. YopM as well as different *Shigella*- and *Salmonella*-derived effector proteins of the LPX subtype share a central domain consisting of a series of LRRs, also referred as LPX domain (yellow). In contrast to YopM, the remaining LPX effector proteins reveal an additional C-terminally located domain encoding a class of novel E3 ligases (NEL) (blue).

intracellular activities will be reviewed. Furthermore, the action of recombinant YopM as a CPE and its potential use as a self-delivering immunomodulatory drug will be discussed.

Structural features of YopM

YopM consists of two conserved antiparallel α-helices at the N-terminus followed by a leucine-rich repeat (LRR) domain, forming the largest part of the protein, and a short, unstructured but conserved C-terminus (Boland et al., 1998; Evdokimov et al., 2001) (Fig. 1A). Depending on the specific *Yersinia* strain and its serotype, the LRR domain harbors a variable number (13–21) of 20–22 amino acids-containing LRRs. While the LRRs in the N- and C-terminal region are highly homologous (95% and 90% identity), the internal LRRs vary in their sequence composition (Benabdillah et al., 2004). The functional consequences of these variations are not yet fully understood. Furthermore, the LRRs form β-strands assembled into a curved parallel β-sheet structure, thus building up 'horseshoe-shaped' proteins of 42–57 kDa in size. The concave inside of the horseshoe is believed to serve as a 'docking surface' for eukaryotic proteins, as it had been suggested for other LRR containing proteins (Kim et al., 2007; Uff et al., 2002). Due to sharing the N-terminal LRR motifs, YopM is structurally closely related to other effector proteins from different bacterial species such as the IpaH proteins from *Shigella* as well as to *Salmonella*'s SspH1, SspH2, and SlrP (Miao et al., 1999). These T3SS proteins all exhibit a subtype of the LRR motif, also referred to as LPX domain, which was found to be specific for bacterial effectors (Buchanan and Gay, 1996). As already indicated for YopM, the substrate and binding specificity of several LPX effector proteins is mediated by this LRR domain (Huibregtse and Rohde, 2014); equally to YopM, also the SspH1 protein from *Salmonella* interacts with the human serine/threonine kinase protein kinase N1 (PKN1) in a LRR-dependent manner (Haraga and Miller, 2006; Keszei et al., 2014). Furthermore, the IpaH9.8 protein from *Shigella* was shown to recruit its interaction partner 'NF-κB essential modulator' (NEMO, also known as IKKγ) via its LRR domain (Ashida et al., 2010). Apart from YopM which lacks an enzymatic domain, all remaining LPX effector proteins harbor a C-terminally located, highly conserved domain encoding a class of novel E3 ubiquitin ligases (NEL domain; Fig. 1C; Miao et al., 1999; Quezada et al., 2009; Rohde et al., 2007; Zhu et al., 2008).

Crystallographic analysis of the quarternary structure of YopM derived from *Y. pestis* revealed the formation of a superhelix composed of four YopM monomers. The tetramer represents a hollow cylinder with an inner diameter of ~35 Å and is associated with two pairs of calcium ions in the interface of the YopM dimers (Evdokimov et al., 2001). Until now, the biological significance of the YopM tetramer remains unclear. Apart from that, data obtained under more physiological conditions indicate that the tetramer might be an artifact due to the high concentration of calcium needed for crystallization. Still, it is possible that tetramers are formed upon binding of YopM to cellular targets (Evdokimov et al., 2001).

According to its delivery by the T3SS, YopM harbors signals for both secretion and translocation within its N-terminal region (aa 1–40 and aa 41–100) (Boland et al., 1996). To date, no YopM-specific secretion chaperone was identified and experimental data suggest that YopM has no need for such a chaperone to be secreted (Trülzsch et al., 2003). After T3SS-dependent translocation into eukaryotic cells, YopM has been demonstrated to localize to the nucleus – probably by trafficking via a vesicular pathway (Benabdillah et al., 2004; Skrzypek et al., 1998). In addition, YopM was detected in the nucleus upon overexpression in *Saccharomyces cerevisiae* or following autonomous translocation of the recombinant protein, indicating that nuclear trafficking is triggered by an intrinsic signal and is independent of the delivery by the T3SS and other *Yersinia* effectors (Lesser and Miller, 2001; Rüter et al., 2010; Scharnert et al., 2013; Skrzypek et al., 2003). This is further supported by the two nuclear localization signals (NLSs) that were identified within the LRRs 1–3 and the last 32 C-terminal amino acids (Benabdillah et al., 2004; Skrzypek et al., 2003). These sequences do not resemble any known eukaryotic NLS. Moreover, the nuclear localization of YopM was shown to be independent of importin (Benabdillah et al., 2004). Nevertheless, both NLSs conduct nuclear targeting independently of each other in yeast and mammalian cells after delivery of the protein via the T3SS as well as after autonomous translocation (Benabdillah et al., 2004; Höfling et al., 2014). Only a deletion of both NLSs abrogates nuclear targeting. However, it remains still unclear whether these bacteria-borne NLSs are yet unknown