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Pasteurella multocida Toxin Interaction with Host Cells: Entry and Cellular Effects

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Abstract

The mitogenic dermonecrotic toxin from *Pasteurella multocida* (PMT) is a 1285-residue multipartite protein that belongs to the A-B family of bacterial protein toxins. Through its G-protein-deamidating activity on the α subunits of heterotrimeric G_q-, G_i- and $G_{12/13}$-proteins, PMT potently stimulates downstream mitogenic, calcium, and cytoskeletal signaling pathways. These activities lead to pleiotropic effects in different cell types, which ultimately result in cellular proliferation, while inhibiting cellular differentiation, and account for the myriad of physiological outcomes observed during infection with toxinogenic strains of *P. multocida*.

1 Introduction

Toxinogenic strains of *Pasteurella multocida*, mostly serogroup D and some A, are associated with a number of epizootic and zoonotic diseases, including dermonecrosis, pasteurellosis, and atrophic rhinitis, in domestic and wild animals (DiGiacomo et al. 1991b; Foged 1992; Frymus et al. 1991; Kielstein 1986; Wilson and Ho 2006) and in humans who come in close contact with infected animals (Arashima and Kumasaka 2005; Donnio et al. 1991; Donnio et al. 2004; Garcia 1997; Iaria and Cascio 2007; Kobayaa et al. 2009; Wilson and Ho 2006). The major virulence factor responsible for the symptoms manifested during infection with these *P. multocida* strains is a 1285-residue (146-kDa) protein toxin (PMT), which belongs to the large, prominent group of intracellularly acting, multipartite A-B toxins that modify eukaryotic G-proteins (Wilson and Ho 2010). A-B toxins bind to host cell receptors through their binding B domains and facilitate the cellular uptake and delivery (translocation) of their toxic activity A domains into the host cell cytosol, where the A domains then interact with and modify their cellular G-protein targets to cause cellular toxicity.

The G-protein targets of A-B toxins are GTPases that regulate various cellular signal transduction pathways by cycling between an inactive GDP-bound form and an active GTP-bound form. PMT selectively deamidates a key active site Gln residue of the α subunit of its

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heterotrimeric G-protein targets, G_q, G_i, and $G_{12/13}$ (Orth et al. 2009). This modification locks the GTPase activity of the α subunit into an active state, resulting in persistent stimulation of downstream signaling pathways modulated by the G-protein targets [reviewed in (Wilson and Ho 2010, 2011)]. While we are beginning to have a clearer picture of the molecular basis for the biochemical activity of PMT, much less is known about the molecular mechanisms of cellular intoxication or how the selective deamidation of its G-protein targets leads to the myriad of cellular outcomes observed. In this review, we focus on our current understanding of how PMT interacts with host cells to gain entry and elicit various cellular effects through its G-protein deamidase activity.

2 PMT Structure and Function

PMT is a member of the dermonecrotic toxin family, which includes the cytotoxic necrotizing factors from *E. coli* (CNF1, CNF2, and CNF3) and *Yersinia pseudotuberculosis* (CNFy) and the dermonecrotic toxin from *Bordetella* species (DNT) (Aktories and Barbieri 2005; Hoffmann and Schmidt 2004; Wilson and Ho 2010). Members of this family of A-B toxins share with each other sequence and structural features that enable them to enter host cells and then gain access to their G-protein targets and modify them. The N-terminus of PMT (PMT-N) has significant sequence similarity with the N-termini of the CNFs (Buys et al. 1990; Falbo et al. 1993; Kamps et al. 1990; Lockman et al. 2002; Oswald et al. 1994; Petersen and Foged 1989; Stoll et al. 2009) and to a lesser extent that of DNT (Pullinger et al. 1996). Although there is no crystal structure available for any of the full-length dermonecrotic toxins such that the actual domains responsible for receptor binding and translocation have not yet been clearly defined, there is some biochemical evidence that the N-termini of these proteins are indeed important for toxin binding and translocation (Baldwin et al. 2004; Blumenthal et al. 2007; Brothers et al. 2011; Chung et al. 2003; Kim et al. 2005; Lemichez et al. 1997; Pullinger et al. 2001).

The CNFs and DNT share over 50% sequence similarity in their C-terminal domains (residues 720–1014 in the CNFs, 1176–1464 in DNT), which have deamidase and/or transglutaminase activity (Hoffmann and Schmidt 2004). Their common G-protein targets belong to the Rho family of small GTPases, such as RhoA, Rac1, and Cdc42, involved in regulation of cytoskeletal function (Aktories and Barbieri 2005). The G-protein deamidase activity of PMT responsible for activation of mitogenic and calcium signaling pathways also resides within the C-terminal 700 amino acids of PMT (PMT-C) (Baldwin et al. 2004; Busch et al. 2001; Orth et al. 2003; Orth et al. 2009; Pullinger and Lax 2007; Pullinger et al. 2001). The crystal structures of PMT-C [PDB 2EBF] (Kitadokoro et al. 2007) and the C-terminal deamidase domain (residues 720–1014) of CNF1 [PDB 1HQ0] (Buetow et al. 2001) are available. The crystal structure of PMT-C (Kitadokoro et al. 2007) revealed three distinct domains (Fig. 1): a C1 domain (residues 575–719) that has sequence and structural homology with the membrane-targeting domains found in a number of large protein toxins (Geissler et al. 2010); a C2 domain (residues 720–1104) that is as-of-yet unknown function; and a C3 domain (residues 1105–1285) that harbors the minimal domain responsible for intracellular activity (Aminova et al. 2008). The C3 domain contains the active site Cys-His-Asp triad that is important for deamidase activity (Busch et al. 2001; Kitadokoro et al. 2007; Orth et al. 2003; Orth et al. 2009; Pullinger and Lax 2007) and has a papain-like cysteine

protease structural fold that most closely resembles that of certain protein transglutaminases (Kitadokoro et al. 2007; Wilson and Ho 2010). Of particular note, however, was the surprising finding that PMT and CNF1 catalyze the same enzymatic reaction on a functionally equivalent Gln residue at the active site of their respective substrates (Gln-61 in Rac1 and Cdc42; Gln-63 in RhoA; Gln-205 in $G\alpha_i$; Gln-209 in $G\alpha_q$; Gln-229 in $G\alpha_{12/13}$) (Flatau et al. 1997; Orth et al. 2009; Schmidt et al. 1997) and have essential active site catalytic His and Cys residues (Buetow et al. 2001; Busch et al. 2001; Kitadokoro et al. 2007; Orth et al. 2003; Schmidt et al. 1998). Yet, there is no discernable sequence or structural similarity between PMT-C3 and the catalytic domain of CNF1 (Wilson and Ho 2010).

3 Cellular Uptake of PMT

Little is known about the cellular intoxication mechanisms of PMT. As is the case for the other dermonecrotic toxin family members (Hoffmann and Schmidt 2004), PMT-N contains the functional receptor-binding and translocation domains. Although the precise boundaries for the binding and translocation domains have not yet been defined, PMT-N appears to be sufficient for mediating cell binding, uptake, and subsequent delivery of PMT-C into the cytosol (Baldwin et al. 2004; Brothers et al. 2011; Pullinger et al. 2001). Indeed, a fusion protein of PMT-N with GFP at the C-terminus also binds and enters cells (Repella et al. 2011). We will now consider what is known about each of these steps in PMT intoxication.

3.1 Binding to Host Cells

A-B toxins are known to employ different types of receptors to intoxicate host cells. For example, cholera and pertussis toxins utilize gangliosides alone (Merritt et al. 1994; Stein et al. 1994), diphtheria toxin uses proteins alone (Naglich et al. 1992), and botulinum and tetanus neurotoxins use both proteins and gangliosides as co-receptors (Binz and Rummel 2009; Dong et al. 2006). Earlier reports suggested that PMT utilized gangliosides, namely GM_1, GM_2 and GM_3, as receptors (Dudet et al. 1996; Pettit et al. 1993a). However, a more recent study using several different methods, including PMT binding to membrane lipid components using TLC-overlay, liposome-pulldown, and surface plasmon resonance (SPR) experiments, showed that PMT and PMT-N do not bind gangliosides (GM_1, GM_2 or GM_3), but instead both bound well to asialogangliosides, such as lactosylceramide, and in particular sphingomyelin (SM) and positively charged membrane phospholipids, such as phosphatidylcholine (PC) and to some extent phosphatidylethanolamine (PE) and some other lipid components (Brothers et al. 2011).

Results from this study also implicated the potential involvement of a protein co-receptor in PMT binding to cells (Brothers et al. 2011), since no single treatment with sphingomyelinase, phospholipase D, or trypsin completely abolished PMT binding to cell membranes, and instead each treatment reduced cellular binding of PMT by about one-third. Indeed, trypsin treatment of cell membranes had minimal effect on membranes already depleted of choline or phosphocholine head groups, indicating that protein binding is only important for PMT binding when PMT is also interacting with SM and/or PC in the membrane. Based on the observed binding kinetics from the SPR studies under the different treatment conditions, a model for PMT interaction with host cells was proposed, whereby

PMT initially binds nonspecifically and with low affinity to the more abundant membrane lipid components such as PC at the surface, but this is then followed by a more specific, tight-binding interaction with SM and possibly other membrane components, including a putative protein co-receptor(s).

3.2 Trafficking in Cells

Once PMT binds to host cells, it is internalized through receptor-mediated endocytosis and then trafficked to acidic endosomes, where it is translocated across the vesicle membrane into the host cell cytosol (Baldwin et al. 2004; Repella et al. 2011; Rozengurt et al. 1990). Although knowledge of the detailed mechanism of this process is lacking, we are beginning to gain some insights. A recent study showed that receptor-mediated endocytosis and initial trafficking of PMT are dependent on the small regulatory G-protein Arf6 (Repella et al. 2011). PMT is initially internalized and trafficked to Arf6-containing vesicles, where it co-localizes with cholera toxin and transferrin, but transferrin is subsequently trafficked to recycling endosomes and cholera toxin is trafficked retrograde to the endoplasmic reticulum, while PMT-containing early endosomes are diverted to late endosomes.

Disassembly of microtubules important for trafficking from early to late endosomes by treatment with nocodazole or disruption of actin polymerization by treatment with cytochalasin D also block PMT activation of mitogenic signaling (Repella et al. 2011), suggesting that membrane translocation and cytotoxicity of PMT are dependent on trafficking to late acidic endosomes. Additional evidence that PMT trafficking to late acidic endosomes is important for PMT action comes from the finding that treatment with brefeldin A, which disrupts Golgi-endoplasmic reticulum trafficking, enhances PMT activity by over 20-fold, presumably by preventing PMT trafficking through nonproductive pathways that do not lead to translocation (Repella et al. 2011). Based on these results, a model was proposed for PMT intoxication, as depicted in Fig. 2.

3.3 Translocation Across Cell Membranes

The above results showing that PMT activity depends on trafficking to late acidic endosomes, where presumably translocation into the cytosol occurs, confirmed earlier findings that weak bases, such as ammonium chloride, chloroquine, or methylamine, which buffer acidification of endosomes, inhibit PMT effects on cells (Rozengurt et al. 1990). Moreover, bafilomycin A1, a potent and specific inhibitor of the vacuolar H^+-ATPase pump that is responsible for acidifying early to late endosomes, likewise inhibits PMT action (Baldwin et al. 2004; Repella et al. 2011). Further evidence for a low pH-dependent membrane translocation event in PMT action was provided by experiments that showed cell surface-bound PMT could directly enter cells, even in the presence of bafilomycin A1, through a low pH pulse at 4°C, which normally blocks endocytosis (Baldwin et al. 2004).

A predicted helix-loop-helix motif, which corresponded to a similar helix-loop-helix in CNF1 (Pei et al. 2001), was identified in PMT (Baldwin et al. 2004). This motif contained two hydrophobic helices (residues 402–423 and 437–457) linked by a hydrophilic loop (residues 424–436) and was proposed to be involved in the pH-sensitive membrane translocation step (Baldwin et al. 2004). Mutation of acidic residues (Asp-373 and Asp-379)

in the loop region of the helix-loop-helix motif in CNF1 resulted in complete loss of biological activity (Pei et al. 2001). However, mutation of analogous residues in the loop region of PMT (Asp-425, Asp-432, Glu-434) resulted in only partial reduction of toxin activity, whereas mutation of an acidic residue (Asp-401) just outside of the predicted motif completely abolished PMT activity (Baldwin et al. 2004). Nevertheless, these results support a model where this helix-loop-helix region of PMT is part of a putative translocation domain.

4 Effects on Cell Signaling

PMT causes a number of pleiotropic effects on targeted host cells. Most notably, PMT induces strong mitogenic (Aminova et al. 2008; Dudet et al. 1996; Mullan and Lax 1996, 1998; Rozengurt et al. 1990; Seo et al. 2000; Wilson et al. 2000; Zywietz et al. 2001) and anti-apoptotic (Aminova and Wilson 2007; Orth et al. 2007a; Preuss et al. 2010; Sabri et al. 2002) signaling in various cell lines, while simultaneously downregulating signaling pathways involved in cellular differentiation, including osteogenesis (Harmey et al. 2004; Mullan and Lax 1998; Sterner-Kock et al. 1995), adipogenesis (Aminova and Wilson 2007), and immune cell differentiation (Bagley et al. 2005; Blocker et al. 2006; Jordan et al. 2003; van Diemen et al. 1994, 1996). The cellular outcomes of these effects of PMT are manifested in the various observed disease symptoms at the different sites of infection.

Exposure to PMT through respiratory infection results in bone resorption of nasal turbinates in progressive atrophic rhinitis (DiGiacomo et al. 1991b; Foged 1992; Frymus et al. 1991; Lax and Chanter 1990; Magyar 1989; Wilson and Ho 2006) and pneumonia (a.k.a, pasteurellosis in rabbits or bovine respiratory distress in cattle) (Chrisp and Foged 1991; DiGiacomo et al. 1991a; Frymus et al. 1991; Kielstein 1986; Klein and Cunha 1997). Chronic or systemic infections also lead to testicular and splenic atrophy (Chrisp and Foged 1991; Nakai et al. 1984), liver necrosis (Cheville and Rimler 1989; Cheville et al. 1988; Chrisp and Foged 1991), kidney or bladder impairment (Hoskins et al. 1997), overall reduced weight and body fat (Cheville and Rimler 1989; Thurston et al. 1992), and growth retardation (Ackermann et al. 1996; Ackermann et al. 1995; Al-Haddawi et al. 2001). Infections in humans usually result from close contact with infected animals, particularly through respiratory exposure or bite wounds and have similar disease manifestations as those observed in animals (Arashima and Kumasaka 2005; Donnio et al. 1999; Donnio et al. 1991; Donnio et al. 2004; Frederiksen 1993; Garcia 1997; Griego et al. 1995; Henderson et al. 2010; Holst et al. 1992; Iaria and Cascio 2007; Kobayaa et al. 2009; Migliore et al. 2009; Satomura et al. 2010; Waldor et al. 1992).

4.1 Cellular Responses

Cellular responses to PMT are induced by activation of at least three different heterotrimeric G-protein families, G_q, G_i, and $G_{12/13}$ (Fig. 3). PMT-mediated activation of G_q and $G_{12/13}$ signaling leads to stimulation of mitogenic responses through increased intracellular calcium and inositol phosphate levels as a result of activation of phospholipase Cβ (PLCβ) by G_q (Aminova et al. 2008; Aminova and Wilson 2007; Luo et al. 2008; Murphy and Rozengurt 1992; Staddon et al. 1991; Wilson et al. 1997) and cytoskeletal changes through activation of Rho-dependent actin signaling by G_q and $G_{12/13}$ (Aepfelbacher and Essler 2001; Lacerda

et al. 1996; Orth et al. 2005; Sagi et al. 2001). Simultaneous activation of G_i signaling by PMT leads to blockade of G_s-regulated adenylyl cyclase (AC) activity (Orth et al. 2008), which in turn downregulates cAMP-dependent signaling pathways involved in cellular differentiation processes. In the following sections we will summarize the known cellular effects mediated through signaling pathways activated by PMT action on its G-protein targets. It should also be noted that in addition to release of the activated Gα subunits, PMT action also releases the cognate G$\beta\gamma$ subunits from the heterotrimeric complexes, which concomitantly modulate other signaling pathways such as the phosphoinositide 3-kinase γ (PI3 Kγ) pathway (Preuss et al. 2009) and perhaps ion channels (Bunemann et al. 2000; Meyer et al. 2001).

4.2 Calcium Signaling

PMT strongly stimulates phospholipase Cβ1 and to a lesser extent PLCβ3, but not PLCβ2 (Wilson et al. 1997), through its selective activation of $G\alpha_q$ protein and not the closely related $G\alpha_{11}$ protein (Staddon et al. 1991; Wilson et al. 1997; Zywietz et al. 2001). Although the mechanism whereby PMT discriminates between $G\alpha_q$ and $G\alpha_{11}$ is not clear, this preference appears to occur through selective recognition of the helical domain of the α subunits, and not the highly conserved regions flanking the target Gln-209 residue (Orth et al. 2004). Dissociation of the $G\alpha_q$ subunit from the G$\alpha\beta\gamma$ complex, through treatment with anti-Gβ antibodies to release the $G\alpha_q$ subunit or by treatment with pertussis toxin to sequester the G$\beta\gamma$ subunits away from the $G\alpha_q$ subunit, potentiates the PMT-induced PLCβ response (Wilson et al. 1997). Overexpression of $G\alpha_q$ protein likewise enhances the PMT-induced response. These results support the monomeric form of $G\alpha_q$ protein as the preferred substrate of PMT. PMT deamidation of $G\alpha_q$ results in constitutive activation (Orth et al. 2009), which is irreversible and persistent (Orth et al. 2007b; Wilson et al. 1997) and independent of interaction with G-protein-coupled receptors (GPCRs) (Orth et al. 2008; Orth et al. 2007b; Orth et al. 2009; Wilson et al. 1997).

Activation of PLCβ1 triggers the hydrolysis of phosphatidylinositol 4,5-bisphosphate to release inositol 1,3,5-trisphosphate (IP_3) and diacylglycerol, which in turn results in mobilization of intracellular calcium pools (Staddon et al. 1991; Wilson et al. 1997) and stimulates calcium signaling pathways (Aminova et al. 2008; Aminova and Wilson 2007; Hennig et al. 2008; Luo et al. 2008) and protein kinase C (PCK)-dependent and -independent phosphorylations (Obreztchikova et al. 2006; Orth et al. 2007a; Ozgen et al. 2008; Sabri et al. 2002; Seo et al. 2000; Staddon et al. 1990; Thomas et al. 2001; Wilson et al. 2000; Zywietz et al. 2001).

4.3 Mitogenic Signaling

PMT elicits a strong mitogenic response in most cultured cell lines that leads to either proliferative or cytopathic effects. In fibroblasts (Dudet et al. 1996; Rozengurt et al. 1990; Sabri et al. 2002; Seo et al. 2000; Wilson et al. 2000; Zywietz et al. 2001), preadipocytes (Aminova and Wilson 2007), and osteoclasts (Felix et al. 1992; Gwaltney et al. 1997; Hildebrand et al. 2010a; Jutras and Martineau-Doize 1996; Martineau-Doize et al. 1993; Mullan and Lax 1996, 1998), PMT potently stimulates DNA synthesis and proliferation. PMT also strongly stimulates anti-apoptotic signaling pathways (Hildebrand et al. 2010b;

Orth et al. 2007a; Ozgen et al. 2008; Preuss et al. 2010; Staddon et al. 1992). In other cells, such as embryonic bovine lung cells (Rutter and Luther 1984), Vero cells (Pennings and Storm 1984; Wilson et al. 2000), cardiomyocytes (Obreztchikova et al. 2006; Sabri et al. 2000; Sabri et al. 2002), osteoblasts, and osteosarcoma cells (Gwaltney et al. 1997; Harmey et al. 2004; Pettit et al. 1993a; Sterner-Kock et al. 1995), PMT elicits a cytopathic response characterized by stress responses, actin rearrangements and other morphological changes (see below). However, after the strong initial PMT-induced cellular response, no further stimulation occurs upon additional treatment with PMT, indicating that an uncoupling of the G-protein signaling pathways occurs (Orth et al. 2008; Wilson et al. 2000; Wilson et al. 1997).

4.4 Cytoskeletal Signaling

PMT initiates RhoA-dependent cytoskeletal signaling, including actin rearrangements, stress fiber formation, and focal adhesion assembly (Aepfelbacher and Essler 2001; Blocker et al. 2006; Dudet et al. 1996; Lacerda et al. 1996; Ohnishi et al. 1998; Orth et al. 2005; Sabri et al. 2002; Sagi et al. 2001; Thomas et al. 2001). However, PMT does not directly modify RhoA (Horiguchi 2001; Lacerda et al. 1996; Ohnishi et al. 1998); instead, PMT activation of RhoA occurs indirectly through activation of $G\alpha_{12/13}$ (Orth et al. 2005) and to some extent $G\alpha_q$ (Sagi et al. 2001). PMT-induced RhoA activation leads to activation of Rho kinase and phosphorylation of focal adhesion kinase (Lacerda et al. 1996; Thomas et al. 2001) and myosin light chain (Aepfelbacher and Essler 2001; Essler et al. 1998), which in turn regulates the actin cytoskeleton, stress fiber formation, focal adhesion assembly, and endothelial cell barrier permeability. PMT disturbance of endothelial barrier function has been attributed as the cause of the observed vascular effects in dermonecrotic lesions from PMT-infected bite wounds (Aepfelbacher and Essler 2001; Elling et al. 1988).

4.5 cAMP Signaling

While potently inducing mitogenic and cytoskeletal signaling through activation of $G\alpha_q$ and $G\alpha_{12/13}$ signaling, PMT simultaneously inhibits AC activity and downstream cAMP-mediated processes through activation of $G\alpha_i$ signaling (Orth et al. 2008). PMT-induced $G\alpha_i$ activation locks the $G\alpha_i$ subunit in its monomeric active form and overrides isoproterenol stimulation of AC by G_s-protein-coupled receptors (Orth et al. 2008). In addition, PMT deamidation of $G\alpha_i$ interferes with the interaction of the $G\alpha_i$ subunit and its cognate $G\beta\gamma$ subunits, and thereby converts the $G\alpha_i$ protein into a pertussis toxin-insensitive form (Orth et al. 2009), since pertussis toxin prefers to ADP-ribosylate the heterotrimeric $G\alpha_i\beta\gamma$ complex and not the monomeric $G\alpha_i$ protein (Katada et al. 1986). Thus, PMT treatment effectively shifts the equilibrium to dissociate the heterotrimeric complex and releases $G\alpha_i$, which blocks AC and cAMP accumulation (Orth et al. 2008), and $G\beta\gamma$ subunits, which can interact with their cognate downstream effectors, such as PI3 Kγ (Preuss et al. 2009), which leads to inhibition of apoptotic signaling pathways (Preuss et al. 2010).

4.6 Adipogenic Signaling

PMT treatment was shown to prevent adipocyte differentiation and block adipogenesis in cultured 3T3-L1 cells under differentiation-inducing conditions (Aminova and Wilson

2007). In this study, PMT prevented expression of key adipocyte-specific markers, C/EBPα and PPARγ, in 3T3-L1 preadipocytes and downregulated these markers in mature adipocytes. PMT also prevented the downregulation of Pref1 (also called Dlk1), an EGF-like transmembrane protein that is strongly downregulated during adipocyte differentiation (Boney et al. 1996; Garces et al. 1999; Sul 2009). PMT was further shown to completely downregulate Notch1 mRNA and protein expression, while stabilizing β-catenin protein levels (Aminova and Wilson 2007). Notch1 and Wnt/β-catenin signaling pathways are involved in pivotal cell fate decisions (Andersson et al. 2011). Interestingly, the inhibitory effects of PMT on adipocyte differentiation and Notch1 could not be reversed by treatment with cyclosporine A (CsA) (Aminova and Wilson 2007), an inhibitor of calcium-calmodulin-dependent calcineurin signaling that is known to reverse G_q-PLCβ1-mediated inhibition of adipogenesis (Liu and Clipstone 2007; Neal and Clipstone 2002). These results suggest that PMT-induced G_q–PLCβ1 activation of calcium signaling is not the only signaling pathway mediated by PMT to block adipocyte differentiation, which leaves the possibility that PMT blockade of adipogenesis might also be mediated through PMT activation of either G_i or $G_{12/13}$ signaling.

4.7 Osteogenic Signaling

PMT is the primary etiological agent responsible for progressive atrophic rhinitis in pigs, rabbits, and other animals (Deeb et al. 1990; DiGiacomo et al. 1993; Foged 1992; Magyar 1989; Wilson and Ho 2006). Atrophic rhinitis is characterized by destruction of the nasal turbinate bones through disruption of bone biogenesis by osteoblasts and bone degradation processes (resorption) by macrophage-like osteoclasts (Kimman and Kamp 1986; Kimman et al. 1987; Mullan and Lax 1996, 1998). In vivo, PMT treatment appears to promote bone resorption through differentiation of preosteoclasts into osteoclasts, which then proliferate (Jutras and Martineau-Doize 1996; Martineau-Doize et al. 1993). In cell culture, PMT also promotes bone resorption by osteoclasts (Felix et al. 1992; Gwaltney et al. 1997; Mullan and Lax 1996) and inhibits bone biogenesis by inhibiting osteoblast differentiation (Gwaltney et al. 1997; Harmey et al. 2004; Mullan and Lax 1998; Sterner-Kock et al. 1995).

4.8 Immune Signaling

Although immunization with PMT protein toxoid affords protection against atrophic rhinitis (Bourdon et al. 2007; Chanter and Rutter 1990; Foged et al. 1989; Frymus et al. 1989; Pettit et al. 1993b; Suckow 2000; Suckow et al. 1995; Thurston et al. 1992), natural infection with toxinogenic *P. multocida* is characterized by an overall lack of immune response against PMT (Bagley et al. 2005; Hamilton et al. 1998; Jordan et al. 2003; van Diemen et al. 1996). Indeed, in vivo PMT is a poor immunogen and appears to suppress antibody responses to PMT and other antigens (Bagley et al. 2005; Hamilton et al. 1998; Jordan et al. 2003), suggesting a possible in vivo role for PMT as an immunomodulator during infection.

While PMT activates human monocyte-derived and murine bone marrow-derived dendritic cells in vitro, it inhibits migration of the dendritic cells (Bagley et al. 2005; Blocker et al. 2006). PMT treatment results in G_q-dependent phosphorylation and activation of Janus tyrosine kinases (JAK1 and JAK2), which leads to activation of downstream STAT signaling and consequent upregulation of proinflammatory responses (Orth et al. 2007a) and

cytokine signaling (Hildebrand et al. 2010b). Clearly, additional studies are needed to clarify the contrasting effects observed regarding PMT action on immune signaling in vitro versus in vivo.

5 Perspective

Initial studies of how bacterial protein toxins modulate host cells focused primarily on the structural organization and mode of action of the toxins. Recently, the focus of toxin studies has shifted more toward understanding the molecular interactions of the toxins with host cells, specifically how they are taken up, trafficked, and translocated into the cytosol, and how they modulate various cellular signaling pathways that lead to changes in cellular function and physiology. There are still a number of large gaps in our understanding of the intoxication process, not only for PMT but also for many other large AB toxins as well.

Since the active site Gln residue of the Gα-protein targeted by PMT is highly conserved throughout the heterotrimeric Gα-proteins, it remains to be determined which of the other Gα-proteins are also substrates for PMT and what the substrate recognition determinants are that discriminate one substrate from the others. Another area that remains unclear is the consequences to the Gα-protein once it has been modified by PMT, particularly the mechanism of its subsequent downregulation. Defining more clearly the substrate preferences of PMT and the ultimate consequences that toxin modification have on the various Gα-protein targets will assist in deciphering the differential effects on the signaling pathways elicited in different cell types and the overall cellular outcomes. Understanding toxin-induced changes in modulation of various cellular processes, including processes that facilitate actin rearrangements, proliferation, and cellular differentiation, will illuminate the critical role of toxins for the successful survival of the pathogen in the animal host. Certainly, reconciling in vitro observations with in vivo outcomes of toxin exposure is high on the list of tasks for future studies.

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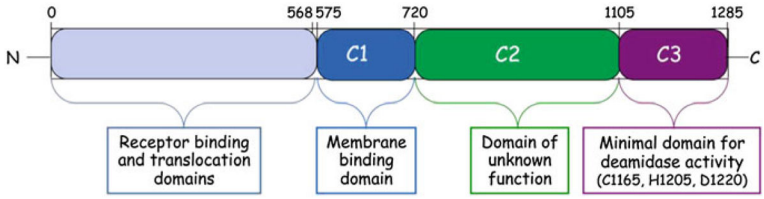

Fig. 1.

A schematic diagram of the overall structure of PMT. PMT-N (residues 1–568) and the known C-terminal structural domains (C1–C3) of PMT-C (residues 569–1285) are indicated along with their known or putative functions

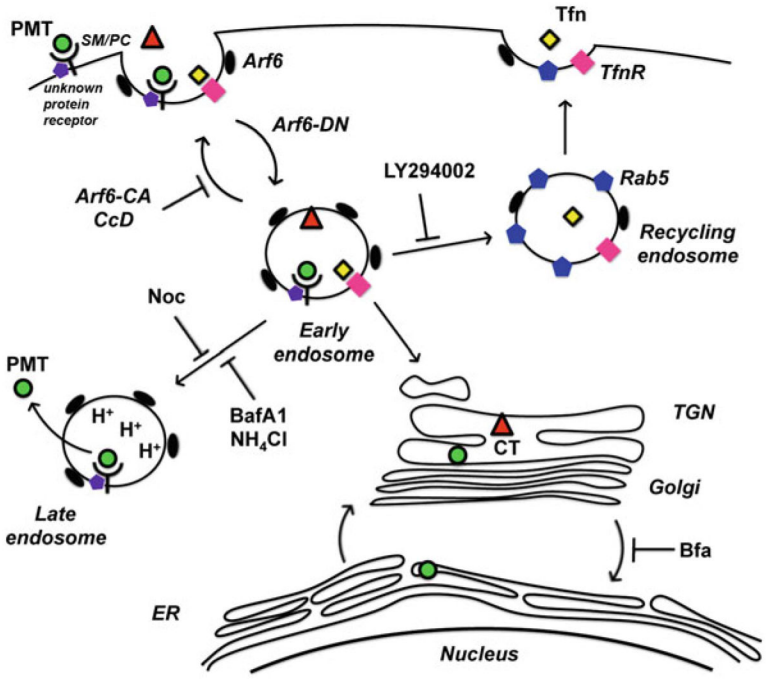

Fig. 2.

Proposed model of PMT entry and trafficking. *Tfn* transferrin, *TfnR* transferrin receptor, *CT* cholera toxin, *Rab5* GTPase marker of recycling endosomes, *TGN* trans-Golgi network, *ER* endoplasmic reticulum, *SM/PC* sphingomyelin/PC receptors of PMT, *Arf6-CA* constitutively active Arf6 GTPase, *Arf6-DN* dominantly negative Arf6, *CcD* cytochalasin D, *LY294002* PI3 K inhibitor of early endosome-recycling endosome fusion, *Noc* nocodazole, *BafA1* bafilomycin A1, *Bfa* brefeldin A. [Adapted from (Repella et al. 2011)]

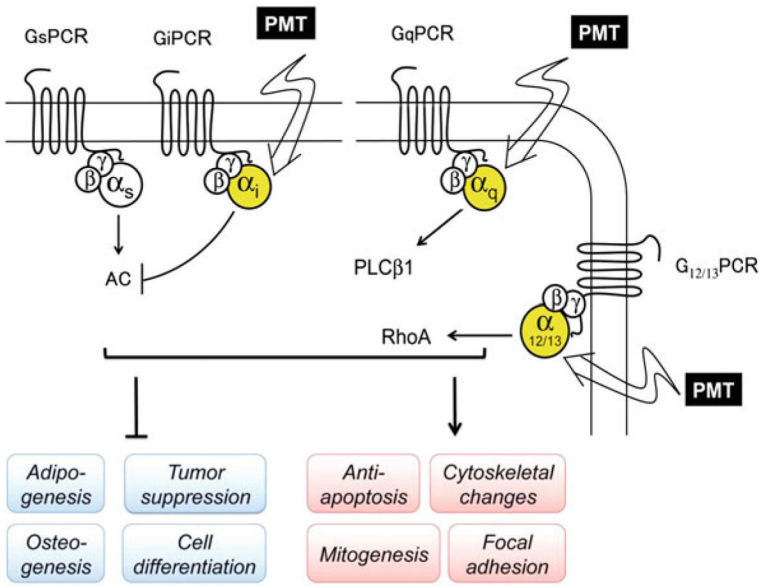

Fig. 3.
Signaling pathways modulated by PMT and their cellular outcomes