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Role of MAPK p38 in the cellular responses to pore-forming toxins

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Abstract

Understanding the mechanism of action of pore-forming toxins (PFT) produced by different bacteria, as well as the host responses to toxin action, would provide ways to deal with these pathogenic bacteria. PFTs affect the permeability of target cells by forming pores in their plasma membrane. Target organisms may overcome these effects by triggering intracellular responses that have evolved as defense mechanisms to PFT. Among them it is well documented that stress-activated protein kinases, and specially MAPK p38 pathway, play a crucial role triggering defense responses to several PFTs in different eukaryotic cells. In this review we describe different intracellular effects induced by PFTs in eukaryotic cells and highlight diverse responses activated by p38 pathway.

1. Introduction

Pore-forming toxins (PFT) produced by bacteria are virulence factors which play a fundamental role in killing eukaryotic cells by forming holes in the cellular membrane of their targets. They represent a large and diverse group of proteins with a wide range of target cells and although their amino acid sequences are not conserved, many of them share several aspects of their mechanism of action. In general, the mechanism of action of PFTs involves receptor recognition, activation by proteases, and aggregation into oligomeric-structures that insert into the membrane to form the ionic pores [30] (Fig 1). On the other side, the target organisms have evolved different mechanisms to counteract PFT action. When PFT are present at high concentrations, cells cannot deal with high pore formation activity in their plasma membrane and die quickly [7] (Fig 1). In contrast, when PFT are present in low sublytic concentrations, cells have different strategies to respond against them promoting cell-survival [7] (Fig 1). It has been extensively shown that the stress-activated protein kinases, named p38 and JNK, are activated as a defense response to different PFT in several eukaryotic cells [7]. In this review we describe the diverse responses activated by stress signaling pathways with special description of MAPK p38 and the intracellular effects induced by some PFT in eukaryotic cells.

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2. Mitogen-activated protein kinases (MAPK) family

MAPK family is a group of highly conserved proline-directed, protein-serine/threonine kinases, involved in intracellular cell regulation in response to extracellular signals or physical stresses, such as radiation, osmotic shock and ischemic injury, allowing eukaryotes cells to respond coordinately to multiple inputs. These MAPK families are considered as stress-activated protein kinases controlling host defense systems involved in cell survival and adaptation [25,31]. The transmission of the extracellular signals to their intracellular targets, is mediated by a network of interacting proteins, governing a large number of cellular processes, such as embryogenesis, cell differentiation, proliferation, surviving, immune responses and death [15,27]. The MAPK act in a cascade module called “the central three-tiered core” composed of three kinases: MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK [41]. The final control of gene expression by MAPK pathways is performed by phosphorylation of transcription factors, but they can also target coactivators and corepressors, leading to the activation or deactivation of a particular signal transduction pathway [41]. The ability of MAPK to transmit different, even opposing signals, in the same cells depends on the selection of binding motifs and scaffold proteins that guarantees the selective and accurate activation of a specific response to a particular signal [8,14].

To date, over 50 different protein kinases have been described as contributors to the MAPK pathways in mammals. They have been characterized in six groups: 1) extracellular signal-regulated kinase (ERK) ERK1/2, 2) Jun NH2 terminal kinases (JNK1/2/3), 3) p38 (p38 a/b/g/d), 4) ERK7/8, 5) ERK3/4 and 6) ERK5. The most extensively studied groups are ERK1/2, JNK and p38 MAPK. One distinctive feature of these MAPK is their activation by phosphorylation since each of these three families revealed a particular signature motif: The ERK are phosphorylated in the threonine and tyrosine residues that are flanking a glutamic acid residue, forming a TEY motif. The intervening residue in the JNKs is a proline residue, forming a TPY motif and in p38 MAPK the residues that are phosphorylated are separated by a glycine residue (TGY motif) [24].

ERK1/2 are proteins ubiquitously expressed, but their relative abundance in mammal tissues is variable. The phosphorylation cascade of ERK1/2 has been extensively studied and is probably the best understood MAPK pathway [23]. The ERK1/2 phosphorylation cascade is initiated by the activation of Ras (a G-protein) by insulin or other mitogens (growth factors) and followed by the sequential activation of Raf1 (MAPKKK), MEK, and ERK. It has been shown that ERK1/2 are able to phosphorylate a large number of substrates in all cellular compartments, including various cytosolic and membrane proteins, as well as nuclear substrates and cytoskeletal proteins. Upon stimulation, a significant proportion of ERK1/2 accumulates in the nucleus [10], and it has been suggested that this pathway participates in cell proliferation [5].

Regarding the JNK and p38 MAPK pathways, it has been suggested that they mainly respond to stress-associated stimuli, and they are collectively termed stress-activated protein kinases to distinguish them from the archetypal mitogen-activated protein kinases [12,26].

The JNK pathways are involved in phosphorylation and activation of the transcription factor c-jun. JNK pathway is activated in response to cytokines, UV irradiation, growth factor deprivation, and DNA damaging agents [36]. Most of the targets for JNKs are nuclear hormone receptors and nuclear transcriptional regulators including ATF2, NF-AT4 and p53. The phosphorylation status of these proteins is important to determine their fate, since transcription factors lacking phosphorylation may be targeted for degradation [36]. JNK pathway is also activated in innate immune response after activation of various members of

Toll-like receptor family [13]. JNKs may localize from the cytoplasm to the nucleus following activation [28]. Many mitochondrial proteins, such as the Bcl-2 family proteins (Bcl-2, Bcl-xl, Bad, Bim and Bax), which have a role in apoptosis, have also been shown to be targets of JNK [28].

MAPK p38 homologues have been identified from yeast to higher eukaryotes. They are also activated by environmental stress and inflammatory cytokines, and could participate in cell proliferation or apoptosis, including cell cycle, immune function, inflammatory responses, such as production of interleukins (IL) and tumor necrosis factor (TNF) or, induction of cyclooxygenase-2 and nitric oxide synthase. Four isoforms of p38 kinases (α, β, γ and δ) have been identified in mammalian cells [33]. These closely related p38 isoforms are not simply redundant proteins, but have specific functions that are determined by the specificity of their upstream activators and the identities and functions of their downstream substrates. Low molecular weight GTP binding proteins (such as Ras), Rho family, chemokines and platelet activating factor have been shown to play role in p38 activation. p38 was shown to be present in both the nucleus and cytoplasm of quiescent cells and directly mediates the activation of several transcription factors, such as ATF2, MEF2C, CHOP, and the members of the TCF family Elk1 and SAP1. p38 also phosphorylates protein kinases that play a role in the intracellular amplification of signals, including the MAPKAPK2 and MAPKAPK3 kinases, which in turn phosphorylate the small heat-shock protein HSP27 or transcription factors such as ATF1 or SRF, MNK [44].

3. MAPK pathway as a response to PFT action

3.1 Role of p38 and JNK in the nematode *Caenorhabditis elegans* as a response to the *Bacillus thuringiensis* Cry toxins (Cry) that are PFT

C. elegans activated both JNK and p38 pathways as a defense mechanism to low doses of Cry5B toxin, a PFT produced by the Gram positive bacterium *Bacillus thuringiensis*. A genomic study performed by microarrays analysis showed that Cry5B toxin up-regulates mRNA accumulation of p38 (PMK-1), SEK-1 (a MAPKK immediately upstream of p38) and JNK kinases [21]. The relevance of SEK-1 and PMK-1 in the defense pathway was demonstrated by feeding *C. elegans* *sek-1* or *pmk-1* mutants with Cry5B; at low doses wild type were healthier than *sek-1* or *pmk-1* mutated animals, which were severely intoxicated. The authors concluded that SEK-1 and PMK-1 function is to protect the nematodes against Cry5B toxin action. The p38 pathway also protected against Cry21A toxin, but not to infection with other Gram-positive bacteria, *B. subtilis*, suggesting that p38 pathway may represent a specific response to PFT action [21]. In contrast, JNK mutated animals were hypersensitive to low doses of Cry5B, Cry21A toxins, *B. subtilis* intoxication, or Cd, suggesting that JNK pathway has a broader role than p38 pathway responding against other stress agents [21]. However, other reports showed that p38 pathway is activated in *C. elegans* as a response to *Pseudomonas aeruginosa* bacterial infection [39], where PMK-1 activated expression of genes codifying for antimicrobials compounds, C-type lectins, lysozymes and neuropeptides. Then, p38 pathway has been considered as an immunity pathway in the nematode involved in a general defense mechanism against different pathogen elicitors including pathogen associated molecular patterns or PFT [39].

3.2 Role of MAPK p38 in response to PFT in mammalian cells

Several members of the cholesterol binding cytolysin (CDC) PFT family, such as streptolysin O (SLO) produced by *Streptococcus aureus*; the vanguardin (VLY) produced by *Gardnerella vaginalis*; anthrolysin O (AnLO) produced by *B. anthracis* and pneumolysin (PLY) from *S. pneumoniae*, as well as other PFT like α-hemolysin from *Staphylococcus aureus* and proaerolysin from the human pathogen *Aeromonas hydrophila*, also induced

MAPK p38 phosphorylation at subcytolytic concentrations [17,18,21,34,37]. When CDC toxins bind cholesterol in the cell membrane of eukaryotic targets, the toxin monomers form oligomeric structures that insert into the bilayer forming transmembrane pores. At large toxin doses the eukaryotic cells died rapidly as a consequence of osmotic shock. However, at low doses the membrane could be repaired and cells survived (Fig 1). Activation of p38 and JNK kinases occurs rapidly and transiently. The p38 activation depends on their pore formation activity, since a PLY point mutant affected pore formation is unable to activate p38 [34] and VLY mutants in domain 4, affected pore formation, are not toxic and neither induced the fast activation of p38 pathway [18]. Finally, inhibition of p38 in hamster kidney cells, with its specific inhibitor SB203580, caused hypersensitivity to proaerolysin [21].

Osmotic stress produced as consequence of disruption of membrane integrity has been proposed as the main effect that triggers activation of p38. It was shown that Dextran or cellulose could block membrane disruption caused by PLY or α -hemolysin on epithelial cells and these treatments also inhibited p38 phosphorylation [34]. It is proposed that epithelial cells perception of osmotic stress via MAPK p38 activation acts as an early stress response to PFT.

The α -toxin produced by *S. aureus* also induces MAPK p38 phosphorylation in immortalized keratinocyte cells (HaCaT). A role of K^+ efflux as an inductor of p38 phosphorylation was proposed, since HaCaT cells treated with nigericin, a specific K^+ ionophore, resulted in a drop of K^+ concentration simultaneously with p38 phosphorylation [22]. In addition, a high concentration of extracellular K^+ blocked the p38 activation induced by different PTF such as α -toxin, *Vibrio cholerae* cytotoxin, SLO or *Escherichia coli* hemolysin (HlyA) [22], suggesting that K^+ efflux throughout the toxin pore might activate the p38 defense response. It was also reported that activation of p38 by PFT is non-dependent of TLR4 immune receptor since HlyA or SLO toxins are sufficient to activate p38 in epithelial cells or human embryonic kidney cells (HEK-293) lacking CD14, a coreceptor of TLR4 [22].

Overall, these results indicate that p38 pathway is activated in mammalian cells and in nematodes to protect them against different PFTs, and that their pore formation activity is important to trigger this response.

3.3 Role of p38 as a response to Cry PFT in insects

In insects, similar responses were observed, since larvae of two different insect orders, Lepidoptera and Diptera, also activated p38 phosphorylation after treatment with Cry toxins [6]. Cry1Ab is highly toxic against the lepidopteran *Manduca sexta* larvae, while Cry11Aa is active against the dipteran larvae of *Aedes aegypti*. Phosphorylation of p38 was observed rapidly when *M. sexta* or *A. aegypti* larvae were treated with the medium lethal concentration dose of Cry1Ab or Cry11Aa, respectively. It is important to mention that the non-toxic Cry1Ab- or Cry11Aa-mutants affected in pore formation activity were unable to activate p38 in both insect larvae. These observations support the proposition that pore formation into the cell membrane is indispensable for p38 activation [6]. The role of p38 in defense mechanism of *M. sexta* and *A. aegypti* against Cry toxins was confirmed using iRNA-mediated knockout of p38 protein. Both insect larvae lacking this kinase became hypersensitive to Cry toxin intoxication, supporting again that p38 pathway has a protective function against PFT in insects.

4. Downstream targets of MAPK p38 pathway specifically activated in response to PFT

Regarding the identification of the key downstream targets of the p38 pathway that are specifically activated in response to PFTs, it was shown by comparison of microarray analysis of *C. elegans* wild type or p38 silenced animals, that treatment with Cry5B resulted in activation of two p38-dependent genes named *ttn-1* and *ttn-2* [21]. Analysis of animals affected in the expression of these genes by iRNA knockout, showed hypersensitivity to Cry5B and to Cd confirming that they are targets of p38 involved in a defense response. The gene *ttn-1* was further characterized, showing homology to the human zinc transporter ZnT-3, suggesting a possible role in removing cytotoxic cations as a defense mechanism [21] (Fig 1).

4.1 The unfolded protein response

A systematic genetic analysis performed to identify genes involved in resistance to Cry5B toxin in the nematode *C. elegans*, allowed the identification of additional genes as targets of p38 in the defense mechanism [4]. The stress response to the unfolded proteins of the endoplasmic reticulum (UPR) was reported to be involved in the defense to PFT in this animal. Specifically the *ire-1-xbp-1* arm of UPR is responsible of the survival response in the nematode since mutations in these genes resulted in worms that were hypersensitive to the pore-forming toxin Cry5B (13 fold higher susceptibility) but not to the treatments with CuSO₄ or H₂O₂ [4]. The activation of *ire-1-xbp-1* pathway depends of p38, since its activation did not occur in worms mutated in the *sek-1* or *pmk-1* p38 genes. However it is important to take into account that the defense response induced by p38 in the nematode is quite complex, since mutations in *sek-1* resulted in a 170-fold increase in the susceptibility to Cry5B [4]. The role of UPR as a defense mechanism seems to be general since IRE-1 was also activated in HeLa cell line after treatment with aerolysin [4]. It has been suggested that the IRE-1 pathway could influence immunity response by its association with TRAF-2, which in turns regulates the transcription factor NF- κ B [43] (Fig 1). It may be also relevant that IRE-1 pathway leads to phospholipid biogenesis, which may be linked to its protective role against PFT.

4.2 Activation or inhibition of MAPK inflammatory responses

Several PFT activate p38-dependent inflammatory responses in their target cells. The VLY and PLY toxins activate p38 pathway and consequently induces up regulation of mRNA levels for IL-8, the major neutrophil chemokine, and treatment with the specific p38 inhibitor SB203580 abolished this response [18,34] (Fig 1).

Low doses of SLO in mast cells produced TNF- α and other cytokines such as IL-13, IL-4 and IL-6 (Fig 1). In addition, granulocyte macrophage-colony stimulated factor and monocyte chemoattractant protein-1 are also accumulated in response to SLO. SLO oligomerization and pore formation are indispensable for this response since a SLO mutant that binds to the membrane but suppresses the oligomerization and pore formation of the toxin was unable to produce TNF- α . Inhibition of p38 with SB203580 strongly prevents TNF- α production, without affecting degranulation [37].

The anthrax lethal toxin (LeTx) from *B. anthracis* is composed of the protective antigen and the lethal factor. The protective antigen is the cell binding element and the PFT component of the toxin that delivers the lethal factor into the mammalian cell cytosol where its specific proteolytic activity cleaves members of the MAPKKs family. The LeTx severely affects the immune response by down-regulating the expression of cytokines such as IL-8 [11].

The adenylyl cyclase toxin (CyaA) produced by *Bordetella pertussis* is a particular case since it is a PFT that affects the plasma membrane of macrophages and dendritic cells (DC) harboring the receptor CD11b/CD13 $\alpha\beta$ 2 integrin. CyaA has a pore-forming region composed of four hydrophobic segments that makes pores in black lipid bilayers [3]. However, it also has a high adenylyl cyclase (AC) catalytic activity that increased intracellular levels of cyclic AMP [16] and inhibits the expression of pro-inflammatory cytokines such as TNF α and IL-12p70 in DC and affects their maturation to Th helper cells. These effects depend on its AC activity and on p38 induction [16]. The down regulation on DC functions exerted by CyaA toxin has been considered as a mechanism adopted by the bacterium to hamper the host immune response [16].

4.3 Shedding of proteins

Another response modulated by ERK1/2 and p38 pathways is the shedding of proteins such as the ectodomains of Syndecan-1 and E-cadherin from cell surface observed after treatment with α -toxin [29], AnLO or LeTx [32] (Fig 1). Syndecans are involved in modulation of cell spreading, adhesion, motility and maintenance of intercellular contacts [32]. It was proposed that the shedding could help to enhance host colonization by altering the morphology of cells and compromising the integrity of protective barriers of epithelial tissue. Shedding of aminopeptidase-N was also observed in the insect *Lymantria dispar* in response to Cry1Ab toxin [40], suggesting that this response after PFT treatment could be rather general.

4.4 Death responses activated by p38 MAPK and PFT

In the case of PLY it was demonstrated that activation of p38 and JNK pathways induces apoptosis in human endothelial and neuroblastoma cells by activation of caspases -6, -9 and a late activation of caspase-3. DNA fragmentation and chromatin condensation were also observed [19]. The extracellular Ca^{2+} influx by PLY action and p38 phosphorylation correlated with this response, since inhibition of Ca^{2+} influx in a Ca^{2+} free bathing solution or inhibition of p38 activity with selective inhibitors such as SB203580, strongly reduced caspases activation, and apoptosis response. Thus, neuronal cells could be rescued from death, suggesting that Ca^{2+} pores induced by PLY, activate MAPK p38 response and correlated with apoptosis [38,19].

5. Other bacterial toxins (non-PFT) that affect MAPK p38 pathway

The shiga toxin (STx) is a member of the AB5 family which is characterized by a bipartite structure consisting of a pentameric B5 moiety involved in receptor binding and the catalytic A subunit, which is a N-glycosidase that inactivates the protein synthesis machinery. Both subunits are not covalently linked. The B5 subunit binds Gb3 glycolipids and follows a retrograde trafficking pathway that facilitates toxin access to the cytosol. The STx activates p38 pathway, which in turn activates an inflammatory response inducing expression of IL-6 and TNF α . It was shown that inhibition of p38 with the selective inhibitor SB202190 blocks MAPK p38 activation and the inflammatory response [35].

6. Additional responses involved in defense to PFT but not related to MAPK p38 pathway

6.1 Hypoxia response

Up regulation of low oxygen response (also described as hypoxia response) pathway confers resistance to Cry21A in the nematode *C. elegans* and to VCC in mammalian cells [2]. Elimination of the hypoxia inducible factor 1, that is one of the main effectors of hypoxia pathway, leads to hypersensitivity to PFT; besides, the exposure of the nematodes to low oxygen confers protection against PFT. It was reported that hypoxia functions upstream

activating the XBP-1 arm of the UPR that, as mentioned above, is involved in defense to PFT action [43].

6.2 ERK pathway response

An additional pathway that confers resistance to PFT in *C. elegans* is a special branch of the DAF-2 Insulin-like signaling network involving WWP-1 protein [9]. This pathway regulates development, metabolism, and longevity in the nematode. The main branch includes DAF-2, which is the homolog of the insulin/IGF-1 receptor in mammals, and activation of this receptor resulted in the activation of the serine/threonine kinases AKT/PKB. It was shown that a mutant defective of DAF-2 is ten times more resistant to Cry5B toxin. The DAF-2 network includes a putative E3 ubiquitin ligase, WWP-1, involved in promoting innate immunity against pathogenic bacteria and in promoting life span [9]. Mutations in *wwp-1* gene become hypersensitive (six fold) to Cry5B toxin, suggesting that this protein is involved in the nematode defense to PFT [9].

6.3 AKT pathway response

Sublytic concentrations of different PFT such as HlyA, aerolysin, α -toxin and leukotoxin in leukocytes and epithelial cells are able to stimulate specific host phosphatases that inactivate AKT [1,42]. AKT is also known as protein kinase B or PKB, it is a key regulator of host cell survival playing an important role in controlling cell cycle, vesicular trafficking, apoptosis inhibition, and inflammatory responses to bacterial infections. The inactivation of AKT response by PFT depends on their pore formation activity and influx of Ca^{2+} ions. This inactivation of AKT by PFT adversely affects host inflammatory responses and induces apoptotic response at sublytic concentrations [42].

6.4 Activation of the central regulator of lipid metabolism

Chinese hamster ovary cells and HeLa cells treated with aerolysin or α -toxin triggers the K^{+} efflux that is linked to the activation of the master regulator of lipid metabolism (SREBP) [20]. The activated SREBP function as transcription factor that promoted the synthesis of genes involved in membrane biogenesis, leading into a defense response against to PFT damage. Pore formation activity is linked to this response since toxin mutants affected in membrane insertion or in oligomer formation that lack pore formation activity did not activate SREBP pathway [20].

7. Final Remarks

The analysis of cellular responses to PFT has revealed conserved responses in different organisms. Among these, activation of protein kinase p38 is a conserved response in nematodes, insects and mammals. This signal transduction pathway is triggered by low doses of PFT promoting in most of the cases the cell survival. It has been extensively shown that activation by phosphorylation of this stress-activated p38 pathway depends on K^{+} efflux or Ca^{2+} influx induced by PFT. Different isoforms of p38 kinases (a, b, g and d) have been identified in mammalian cells [33], and each one of these proteins has specific functions determined by their upstream activators and their downstream substrates resulting in initiation of a wide variety of secondary responses. The secondary consequences of p38 activation will depend on the type of PFT and on the target cell, resulting in initiation of different mechanisms involved in protection against PFT, and in few cases in activation of programmed death responses.

The identification of the different scaffold proteins that are responsible of the selective and accurate responses activated by p38 after PFT intoxication in different organisms could provide ways to counter act the action of bacterial pathogens or to enhance the activity of

biotechnological important PFT as Cry toxins produced *B. thuringiensis* that have insecticidal activity.

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Abbreviations

PFT	pore-forming toxin(s)
MAPK	mitogen-activated protein kinase(s)
ERK	extracellular signal-regulated kinase(s)
JNK	Jun NH2 terminal kinase(s)
IL	interleukin
TNF	tumor necrosis factor
Cry	insecticidal Cry toxin(s)
CDC	cholesterol binding cytolysin
SLO	streptolysin O
VLY	vanginolysin
AnlO	anthrolysin O
PLY	pneumolysin
HlyA	<i>Escherichia coli</i> hemolysin
UPR	unfolded proteins response of the endoplasmic reticulum
CyaA	adenylate cyclase toxin
DC	dendritic cells
AC	adenylate cyclase
LeTx	lethal toxin
STx	shiga toxin
SREBP	master regulator of lipid metabolism

References

1. Atapattu DN, Czuprynski CJ. *Mannehemia haemolytica* leukotoxin induces apoptosis of bovine lymphoblastoid cells BL-3 via caspase-9 dependent mitochondrial pathway. *Infect Immunol* 2005;3:5504–13. [PubMed: 16113266]
2. Bellier A, Chen Ch-S, Kao Ch-Y, Cinar HN, Aroian RV. Hypoxia and the hypoxic response pathway protect against pore-forming toxins in *C. elegans*. *PLoS Pathogen* 2009;5(12):e1000689. [PubMed: 20011506]
3. Benz R, Maier E, Landant D, Ullmann A, Sebo P. Adenylate cyclase toxin (CyaA) of *Bordetella pertussis*. Evidence for the formation of small ion permeable channels and comparison with HlyA of *Escherichia coli*. *J Biol Chem* 1994;269:27231–9. [PubMed: 7525549]
4. Bischof LJ, Kao Ch-Y, Los FCO, Gonzalez MR, Shen Z, Briggs SP, et al. Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin *in vivo*. *PLoS Pathogens* 2008;4:e1000176. [PubMed: 18846208]

5. Boultron TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenhesser SD, et al. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 1991;65:663–75. [PubMed: 2032290]
6. Cancino-Rodezno A, Alexander C, Villasenor R, Pacheco S, Porta H, Pauchet Y, et al. The mitogen-activated protein kinase p38 is involved in insect defense against Cry toxins from *Bacillus thuringiensis*. *Insect Biochem Mol Biol* 2009;40:58–63. [PubMed: 20040372]
7. Cancino-Rodezno A, Porta H, Soberón M, Bravo A. Defense and death responses to pore forming toxins. *Biotechnol Gen Eng Rev* 2009;26:65–94.
8. Chang L, Karin M. Mammalian MAP kinase signalling cascades. *Nature* 2001;410:211–8.
9. Chen Cha S, Bellier A, Kao Ch-Y, Yang Y-L, Chen H-D, Los FCO, et al. WWP-1 is a novel modulator of the DAF-2 insulin-like signaling network involved in pore-forming toxin cellular defenses in *Caenorhabditis elegans*. *PLoS One* 2010;5:e9494. [PubMed: 20209166]
10. Chen RH, Sarnecki C, Blenis J. Nuclear localization and regulation of Erk- and Rsk-encoded protein kinases. *Mol Cell Biol* 1992;12:915–27. [PubMed: 1545823]
11. Chow EMC, Batty S, Modridge J. Anthrax lethal toxin promotes dephosphorylation of TTP and formation of processing bodies. *Cell Microbiol* 2010;12:557–68. [PubMed: 19995385]
12. Dérjard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, et al. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 1994;76:1025–37. [PubMed: 8137421]
13. Dong C, Davis RJ, Flavell RA. MAP kinases in the immune response. *Annu Rev Immunol* 2002;20:55–72. [PubMed: 11861597]
14. Ebisuya M, Kondoh K, Nishida E. The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms for providing signaling specificity. *J Cell Sci* 2005;118:2997–3002. [PubMed: 16014377]
15. Errede B, Cade RM, Yashar BM, Kamada Y, Levin DE, Irie K, et al. Dynamics and organization of MAP kinase signal pathways. *Mol Rep Develop* 1995;42:477–85.
16. Fedele G, Spensieri F, Palazzo R, Nasso M, Cheung GY, Coote JG, et al. *Bordetella pertussis* commits human dendritic cells to promote Th1/Th17 response through the activity of adenylate cyclase toxin and MAPK- pathway. *PLoS One* 2010;15(5):e8734. [PubMed: 20090944]
17. Fickl H, Cockeran R, Steel HC, Feldman C, Cowan G, Mitchell TJ, et al. Pneumolysin-mediated activation of NFκB in human neutrophils is antagonized by docosahexaenoic acid. *Clin Exp Immunol* 2005;140:274–81. [PubMed: 15807851]
18. Gelber SE, Aguilar JL, Lewis KLT, Ratner AJ. Functional and phylogenetic characterization of vaginolysin the human specific cytolysin from *Gardnerella vaginalis*. *J Bacteriol* 2008;190:3896–903. [PubMed: 18390664]
19. N'Guessan PD, Schmeck B, Ayim A, Hocke AC, Brell B, Hammerschmidt S, et al. *Streptococcus pneumoniae* R6x induced p38 and JNK-mediated caspase-dependent apoptosis in human endothelial cells. *Thromb Haemost* 2005;94:295–303. [PubMed: 16113818]
20. Gurcel L, Abrami L, Girardin S, Tschopp J, van der Goot FG. Caspase-1 activation of lipid metabolic pathways in response to bacterial pore-forming toxins promotes cell survival. *Cell* 2006;126:1135–45. [PubMed: 16990137]
21. Huffman DL, Abrami L, Sasik Corbeil J, van der Goot FG, Aroian RV. Mitogen-activated protein kinase pathways defend against bacterial pore-forming toxins. *Proc Natl Acad Sci USA* 2004;101:10995–11000. [PubMed: 15256590]
22. Kloft N, Busch T, Neukirch C, Weis S, Boukhallouk F, Bobkiewicz W, et al. Pore-forming toxins activate MAPK p38 by causing loss of cellular potassium. *Biochem Biophys Res Commun* 2009;385:503–6. [PubMed: 19497299]
23. Kolch W. Coordinating ERK/MAPK signalling through scaffolds and inhibitors. *Nature Rev Mol Cell Biol* 2005;6:827–37. [PubMed: 16227978]
24. Kosako H, Gotoh Y, Matsuda S, Ishikawa M, Nishida E. *Xenopus* MAP kinase activator is a serine/threonine/tyrosine kinase activated by threonine phosphorylation. *EMBO J* 1992;11:2903–8. [PubMed: 1322292]
25. Krishna M, Narang H. The complexity of mitogen-activated protein kinases (MAPK) made simple. *Cell Mol Life Sci* 2008;65:3525–44. [PubMed: 18668205]

26. Kyriakis JM, Bnerjee P, Nikolakaki E, Dai T, Ribie EA, Ahmad MF, et al. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 1994;369:156–60. [PubMed: 8177321]
27. Mizukami Y, Yoshida KI. Mitogen-activated protein kinase translocates to the nucleus during ischemia and is activated during reperfusion. *Biochem J* 1997;323:785–90. [PubMed: 9169613]
28. Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. *Cancer Res* 1998;58:3163–72. [PubMed: 9679985]
29. Park PW, Foster TJ, Nishi E, Duncan SJ, Klagsbrun M, Chen Y. Activation of syndecan-1 ectodomain shedding by *Staphylococcus aureus* α -toxin and β toxin. *J Biol Chem* 2004;279:251–9. [PubMed: 14573623]
30. Parker MW, Feil SC. Pore forming proteins toxins: from structure to function. *Progress Biophys Mol Biol* 2005;88:91–142.
31. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, et al. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. *Endocr Rev* 2001;22:153–218. [PubMed: 11294822]
32. Popova TG, Millis B, Bradburne Ch, Nazarenko S, Bailey Ch, Chandhoke V, et al. Acceleration of epithelial cell syndecan-1 shedding by anthrax hemolytic virulence factors. *BMC Microbiol* 2006;6:8. [PubMed: 16464252]
33. Raingeaud J, Gupta S, Rogers JS, Dickens M, Han J, Ulevitch RJ, et al. Pro-inflammatory cytokines and environmental stress cause p38 mitogen-activated protein kinase activation by dual phosphorylation on tyrosine and threonine. *J Biol Chem* 1995;270:7420–6. [PubMed: 7535770]
34. Ratner AJ, Hippe KR, Aguilar JL, Bender MH, Nelson AL, Weiser JN. Epithelial cells are sensitive detectors of bacterial pore-forming toxins. *J Biol Chem* 2006;281:12994–8. [PubMed: 16520379]
35. Saenz JB, Li J, Hasiam DB. The MAP kinase-activated protein kinase 2(MK2) contributes to the shiga toxin-induced inflammatory response. *Cell Microbiol* 2010;12:516–29. [PubMed: 19951368]
36. Sanchez-Prieto R, Rojas JM, Taya Y, Gutkind JS. A role for the p38 mitogen-activated protein kinase pathway in the transcriptional activation of p53 on genotoxic stress by chemotherapeutic agents. *Cancer Res* 2000;60:2464–72. [PubMed: 10811125]
37. Stassen M, Muller C, Richter C, Neudorfl C, Hultner L, Bhakdi S, et al. The streptococcal exotoxin streptolysin O activates mast cells to produce tumor necrosis factor alpha by p38 mitogen-activated protein kinase- and protein kinase C-dependent pathways. *Infect Immun* 2003;71:6171–7. [PubMed: 14573633]
38. Stringaris AK, Geisenhainer J, Bergmann F, Balshusemann C, Lee U, Zysk G, et al. Neurotoxicity of pneumolysin, a major pneumococcal virulence factor, involves calcium influx and depends on activation of p38 mitogen-activated protein kinase. *Neurobiol Dis* 2002;11:355–68. [PubMed: 12586546]
39. Troemel ER, Chu SW, Reinke V, Lee SS, Ausubel FM, Kim DH. p38 MAPK regulates expression of immune response genes and contributes to longevity in *C. elegans*. *PLoS Genet* 2006;2:e183. [PubMed: 17096597]
40. Valaitis A. *Bacillus thuringiensis* pore-forming toxins trigger massive shedding of GPI-anchored aminopeptidase N from gypsy moth midgut epithelial cells. *Insect Biochem Mol Biol* 2008;38:611–8. [PubMed: 18510972]
41. Widmann CS, Gibson MB, Jarpe MB, Johnson GL. MAPK pathways: conservation of a three-kinase module from yeast to man. *Physiol Rev* 1999;79:143–80. [PubMed: 9922370]
42. Wiles TJ, Dhakal BK, Eto DS, Mulvey MA. Inactivation of host Akt/protein kinase B signaling by bacterial pore-forming toxins. *Mol Biol Cell* 2008;19:1427–38. [PubMed: 18234841]
43. Xu C, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 2005;115:2656–64. [PubMed: 16200199]
44. Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res* 2005;15:11–18. [PubMed: 15686620]

Figure 1.

Role of MAPK p38 in activating responses to PFT action.

The mechanism of action of PFTs involves receptor recognition (1), activation by proteases (2), and aggregation into oligomeric structures that insert into the membrane to form the ionic pores (3). When PFT are present at high concentrations, cells cannot deal with high pore formation activity in their plasma membrane and die quickly (4). But, when PFT are present in low sublytic concentrations, activation of p38 pathway may trigger different strategies in the cells to respond against PFT action.