Toxines et Signalisation – Toxins and Signalling
Rencontres en Toxinologie – Meeting on Toxinology, 2009
Editions de la SFET – SFET Editions
Accès libre en ligne sur le site – Free access on line on the site : http://www.sfet.asso.fr

Activation of heterotrimeric G proteins by Pasteurella multocida *toxin*

Joachim H.C. ORTH, Klaus AKTORIES*

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs Universität Freiburg, Albertstraße 25, 79104 Freiburg, Germany.

** Corresponding author ; Tel : +49 (0)761-2035301 ; Fax : +49 (0)761-2035311 ;*
E-mail: Klaus.Aktories@pharmakol.uni-freiburg.de

Abstract

Pasteurella multocida *toxin (PMT) is a 146 kDa protein toxin, which is a major virulence factor of the facultative pathogen bacteria. PMT acts as a deamidase to activate heterotrimeric G proteins. The toxin causes deamidation of an essential glutamine residue of Gα subunits resulting in glutamic acid. This exchange blocks the intrinsic GTPases activity and causes persistent activation of the G protein.*

Activation des protéines G hétérotrimériques par la toxine de Pasteurella multocida

La toxine de Pasteurella multocida *(PMT) est une protéine de 146 kDa, qui constitue le facteur de virulence majeur des bactéries pathogènes facultatives. PMT agit avec une activité déamidase pour activer une protéine G hétérotrimérique. La toxine cause une déamidation d'un résidu glutamine essentiel de la sous-unité Gα ainsi transformé en acide glutamique. Cette modification bloque l'activité GTPasique intrinsèque de la protéine G et induit ainsi son activation permanente.*

Keywords : *Bacterial protein toxin, deamidation, Gα, Gβγ, GTPase.*

Introduction

Pasteurella multocida is a gram-negative coccobacillus colonizing the gastrointestinal tract and nasopharynx of wild and domesticated animals including cats and dogs. The bacteria are facultative pathogens, which cause bite wound infections, pneumonia, endocarditis and septicemia in men. In pigs, *Pasteurella multocida* induces atrophic rhinitis, which is characterized by a loss of nasal turbinate bone (Kamp and Kimman, 1988). Major virulence factor of the pathogen is the 146 kDa protein toxin *Pasteurella multocida* toxin (PMT). This toxin is the causative agent of atrophic rhinitis and was shown to be responsible for the osteolytic activity of bacteria (Chanter, 1990;Kamp *et al.*, 1988;Lax and Chanter, 1990;Lax and Grigoriadis, 2001). Recently the molecular mechanism of the toxin has been elucidated (Orth *et al.*, 2009).

The structure of PMT

PMT consists of 1285 amino acid residues and is structured according to an AB toxin. Whereas the biologically active domain is located in the C-terminal part of the protein (Busch *et al.*, 2001;Pullinger *et al.*, 2001), the N-terminal part is involved in receptor binding and translocation into the cytosol of target cells. The latter part of the toxin exhibits significant sequence similarity with the N-terminal part of the cytotoxic necrotizing factor of *E. coli* that is also involved in binding and translocation.

Recently, the crystal structure of the C-terminal fragment of PMT, covering amino acids 569 to 1285, was solved (Kitadokoro *et al.*, 2007), showing 3 domains. Whereas the C1 domain (residues 569-719) should contribute to intracellular localization of the toxin, the function of the C2 domain (residues 720-1104) remains enigmatic. Most exciting is the C-terminal C3 domain (residues 1105-1285), which resembles a papain-like fold. This domain harbors a catalytic triad characteristic of thiol proteases, harboring the essential amino acids cysteine-1165 (Busch *et al.*, 2001;Ward *et al.*, 1998), histidine-1205 (Orth *et al.*, 2003) and aspartic acid-1220 (*Figure 1*).

PMT activates various cellular signal pathways

PMT is an extremely potent mitogen and stimulates DNA synthesis and proliferation of several cell lines (Dudet *et al.*, 1996;Higgins *et al.*, 1992;Mullan and Lax, 1996;Rozengurt *et al.*, 1990). The mitogenic action of PMT depends on the stimulation of the MAP-kinase ERK (extracellular signal regulated kinase) (Seo *et al.*, 2000). PMT was shown to stimulate phospholipase Cβ1 (PLC-β1). This causes calcium mobilization, accumulation of diacylglycerol and activation of protein kinase C (Staddon *et al.*, 1991). The toxin activates

PLC-β1 by an action on the heterotrimeric G_q protein (Wilson *et al.*, 1997). Heterotrimeric G proteins are grouped in at least 4 families: the G_s-, $G_{i/o}$-, $G_{q/11}$- and $G_{12/13}$- families. It was shown by gene deletion of the α-subunits of G_q and G_{11} that PMT acts on PLC-β *via* $G\alpha_q$ but not *via* $G\alpha_{11}$ (Zywietz *et al.*, 2001). This is remarkable, because $G\alpha_q$ and $G\alpha_{11}$ share 89% of their amino acid residues. The helical domain of $G\alpha_q$ was identified to be essential for activation of PLCβ by PMT (Orth *et al.*, 2004).

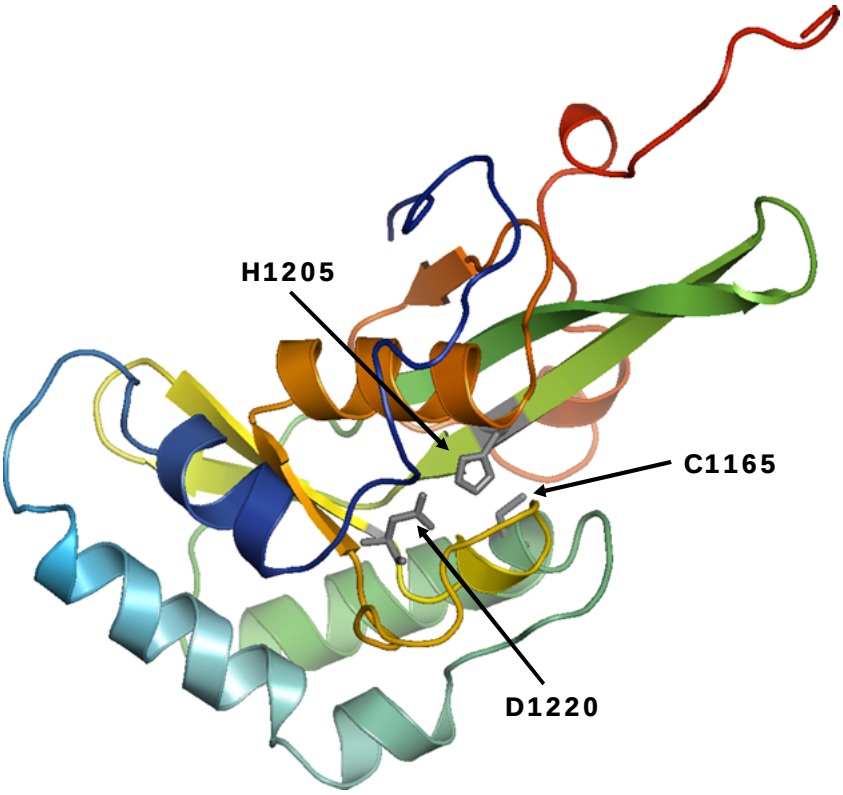

Fig. 1. Structure of the very C-terminal C3 domain harboring the biological activity of PMT. The catalytic triad cysteine-1165, histidine-1205 and aspartic acid-1220 is highlighted in the folds. Image was generated using PyMOL and PDB data file 2EBF.

Fig. 1. *Structure du domaine C-terminal C3 porteur de l'activité biologique de la PMT. La triade catalytique Cys1165, His1205 et Asp1220 est indiquée sur la structure. L'image est générée à l'aide de PyMOL et la référence PDB est 2EBF.*

In addition, PMT activates the small GTPase RhoA, thereby inducing formation of stress fibers, focal adhesions and tyrosine phosphorylation of focal adhesion kinase and paxillin (Dudet *et al.*, 1996;Lacerda *et al.*, 1996). The activation of Rho, ERK and Jun kinase depends not only on G_q (Zywietz *et al.*, 2001). In addition to $G\alpha_q$, PMT activates $G\alpha_{13}$ of the $G_{12/13}$ family, which activates the small GTPase RhoA and causes formation of stress fibers (Orth *et al.*, 2005). More recently, it was observed that PMT is also a potent activator of $G\alpha_i$. PMT inhibits beta-adrenoceptor- or forskolin-induced activation of adenylyl cyclase in cell membrane preparations and in intact cells (Orth *et al.*, 2008). Moreover, G_i activation by PMT results in release of βγ-subunits and activation of PI3-kinase γ (Preuss *et al.*, 2009) (*Figure 2*).

Regulation of heterotrimeric G proteins

Heterotrimeric G proteins consist of the GTP-binding Gα-, the Gβ- and the Gγ-subunits. The G proteins are regulated by a GTPase cycle and are inactive in the heterotrimeric complex with GDP-bound at the α-subunit. Activation starts with the release of GDP. This process is greatly facilitated by interaction with GPCRs, which function as GEF proteins (guanine nucleotide exchange factors). Subsequent binding of GTP causes conformational changes, which promote separation of the α-subunit from the βγ-subunit. Both, the α-subunit and the βγ-subunit activate effector proteins (*e.g.*, enzymes or channels) (Cabrera-Vera *et al.*, 2003). The activated state is terminated by hydrolysis of bound GTP catalyzed by the intrinsic GTPase activity of the α-subunit following re-association to form the inactive heterotrimeric complex. GTP-hydrolysis can be accelerated by GTPase-activating proteins called RGS proteins (regulators of G protein signaling), which speed up inactivation of the G proteins.

The molecular mechanism of PMT

Until recently the molecular mechanism of PMT was enigmatic. Four scientific steps were of major importance to elucidate the mode of action of PMT. First, it was observed that activation of $G\alpha_q$ by PMT is independent of G protein-coupled membrane receptors (GPCR). This was shown by using $G\alpha_q$ subunits, which were C-

terminally deleted in 5 amino acid residues (Orth *et al.*, 2007). Without the C-terminus, the interaction of the G protein with the GPCR is blocked. Second, it was shown that the activation of G_q by PMT is persistent and does not depend on the permanent presence of the toxin (Orth *et al.*, 2007). Third, the finding that G_i is a substrate of the toxin was of special importance for analysis of the molecular mechanism of PMT, because G_i proteins are readily accessible for analyses and recombinant expression. Moreover, G_i is a substrate for ADP-ribosylation by pertussis toxin but activation of G_i by PMT turns the G protein into a pertussis toxin-insensitive state (Orth *et al.*, 2008).

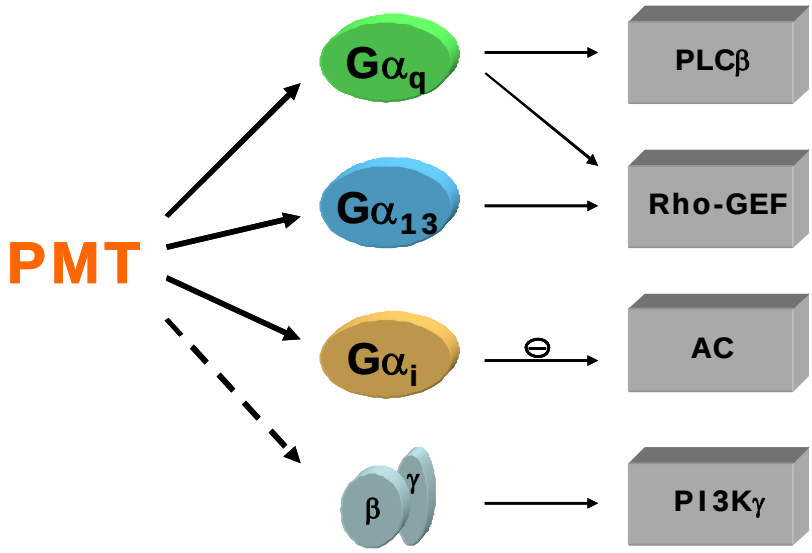

Fig. 2. Overview on PMT-activated G proteins and subsequent signal transduction pathways. PMT activates $G\alpha_q$ to induce stimulation of phospholipase Cβ (PLCβ) and activation of RhoA *via* Rho guanine nucleotide exchange factors (Rho-GEF). Activation of $G\alpha_{12/13}$ leads also to Rho-GEF-dependent RhoA activation. Toxin-induced activation of $G\alpha_i$ inhibits adenylyl cyclase (AC) activity. By activation of the α-subunits, Gβγ-subunits are released, which stimulate *e.g.* phosphoinositide-3-kinase γ (PI3Kγ) activity.

Fig. 2. *Revue des protéines G activées par la PMT et des cascades de signalisation impliquées. PMT active $G\alpha_q$ et induit la stimulation de la phospholipase Cβ (PLCβ) et l'activation de RhoA* via *le facteur d'échange de nucléotide guanine de Rho (Rho-GEF). L'activation de $G\alpha_{12/13}$ conduit aussi à l'activation de RhoA dépendant de Rho-GEF. L'activation de $G\alpha_i$ par la toxine inhibe l'activité de l'adénylate cyclase (AC). En activant la sous-unité α, les sous-unités Gβγ sont relarguées, stimulant ainsi l'activité phosphoinositide-3-kinase γ (PI3Kγ).*

Therefore, ADP-ribosylation by pertussis toxin can be used to monitor PMT effects. However, although having recombinant G_i protein and PMT in hand, initial studies did not show any G protein activation by incubation of purified proteins *in vitro*. The crucial step was the coexpression of $G\alpha_i$ and PMT in *E. coli*. Subsequent *in vitro*-ADP-ribosylation of $G\alpha_i$ by pertussis toxin (to monitor the activation state of the G protein) indicated that wild-type PMT activated $G\alpha_i$, whereas an inactive PMT mutant was without effect. To analyze the reason for the activation of the G-protein, the inherent GTPase activity of the G protein was studied showing that PMT causes inhibition of the hydrolysis of bound GTP. Moreover, RGS proteins were not able to activate the GTPase activity. With the purified PMT-activated $G\alpha_i$ proteins it was possible to perform mass spectrometric analyses. These studies revealed that PMT-treated $G\alpha_i$ has glutamic acid instead of glutamine in position 205 (Orth *et al.*, 2009). These data indicated that PMT causes deamidation of a specific glutamine residue and acts like a deamidase (*Figure 3*).

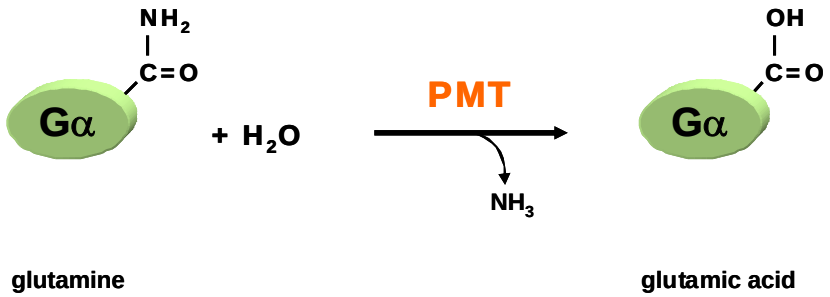

Fig. 3. Scheme of deamidation of PMT. The toxin deamidates a specific glutamine residue essential for GTPase activity of the α-subunit. As compared to transglutaminases PMT utilizes a water molecule as cosubstrate instead of an amine. During the reaction the NH_2-group of the glutamine is released as ammonia and a carboxyl group is formed resulting in a glutamic acid residue.

Fig. 3. *Schéma de l'activité déamidase de la PMT. La toxine déamide le résidu glutamine essentiel à l'activité GTPase de la sous-unité α. Comparé à une activité transglutaminase, la PMT utilise une molécule d'eau comme co-substrat plutôt qu'une amine. Au cours de la réaction, le groupement amine de la glutamine est substitué par le groupement carboxyl de l'acide glutamique.*

The data obtained with recombinant $G\alpha_i$ protein were corroborated with native G_i protein from PMT-pretreated intact cells. Moreover, it was also shown that PMT causes deamidation of glutamine 209 of G_q. This residue of G_q is equivalent to glutamine-205 of $G\alpha_i$. By contrast $G\alpha_{11}$ is not deamidated by PMT, a finding which is in full agreement with the reported insensitivity of $G\alpha_{11}$ towards the toxin (Orth *et al.*, 2009).

Functional consequences of PMT-induced deamidation

Glutamine-205 of $G\alpha_i$ is known to be essential for GTPase activity. This amino acid is not only conserved in heterotrimeric G proteins but also in the superfamily of GTPases including small GTPases like Ras and Rho. The residue has been frequently exchanged for leucine to construct constitutively active G-proteins (De Vivo *et al.*, 1992;Majumdar *et al.*, 2006). Glutamine-205 of $G\alpha_i$ is reportedly a key catalytic residue of the endogenous GTPase activity of $G\alpha_i$ (Coleman *et al.*, 1994;Sprang, 1997;Tesmer *et al.*, 1997). It stabilizes the pentavalent transition state of GTP hydrolyses and is essential for orientation of the incoming water nucleophile. Therefore, the deamidation of this glutamine residue to glutamic acid prevents GTP hydrolysis and turns the $G\alpha$ subunit into a persistently active state. Glutamine-209 of $G\alpha_q$, which is activated by PMT, has the same function in GTP hydrolysis and in regulation of the activity state as glutamine-205 in $G\alpha_i$.

PMT as a member of a family of deamidating toxins

Deamidation of target proteins by bacterial protein toxins is not a unique mechanism of PMT. The cytotoxic necrotizing factors CNF, which are produced by *E. coli* and *Yersinia* strains cause deamidation of Rho proteins at glutamine-61(63), which is functionally equivalent to glutamine-205 of $G\alpha_i$. (Flatau *et al.*, 1997;Schmidt *et al.*, 1997). In addition dermonecrotic toxin (DNT) from *Bordetella pertussis* and *B. bronchiseptica* belongs to the family of deamidating toxins. DNT deamidates Rho proteins at glutamine-61(63) like CNF. Moreover, DNT can act as a transglutaminase on Rho proteins (Horiguchi *et al.*, 1997;Masuda *et al.*, 2000). Notably, PMT has sequence similarity with CNF and DNT in the N-terminal binding and translocation domains. By contrast, PMT has no obvious structural similarity with CNFs or DNT at the C-terminal catalytic domain (Buetow *et al.*, 2001;Kitadokoro *et al.*, 2007). Both PMT and CNF1 share the catalytic residues cysteine and histidine. A third catalytic residue is aspartate in PMT and valine in CNF1 (*Figure 4A*). Accordingly, the 3D-structure of the catalytic domain of PMT is different from that of CNF1. The active site of DNT is similar to PMT and CNF in respect to the catalytic cysteine and histidine. The supposed third catalytic active amino acid residue is not known in the case of DNT. Together with the lack of a crystal structure of DNT, it is not possible to answer the question, whether the catalytic site of PMT is structurally related to the active site of the deamidating and transglutaminating toxin DNT.

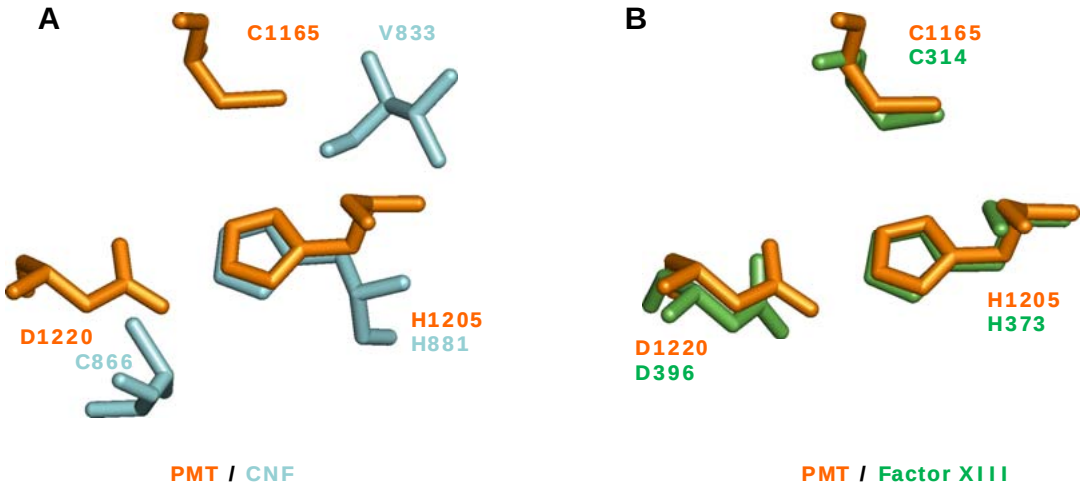

Fig. 4. Differences in catalytic triads of deamidating toxins. (**A**) The catalytic triads of PMT (cysteine-1165, histidine-1205 and aspartic acid-1220) and CNF1 (valine-833, cysteine-866 and histidine-881) are compared after superimposing the catalytic histidines. (**B**) The catalytic triads of PMT (cysteine-1165, histidine-1205 and aspartic acid-1220) and the transglutaminase human factor XIII (cysteine-314, histidine-373 and aspartic acid-396) are compared after superimposing the catalytic histidines. Image was generated using PyMOL and PDB data file 2EBF (PMT), 1HQO (CNF1) and 1GGT (factor XIII).

Fig. 4. *Différences entre les triades catalytiques des toxines à activité déamidase. Les triades catalytiques de la PMT (cystéine-1165, histidine-1205 et acide aspartique-1220) et du CNF1 (valine-833, cystéine-866 et histidine-881) (**A**) ou du facteur XIII* (cystéine-314, histidine-373 et acide aspartique-396) (***B***) *sont comparées après superposition des histidines. L'image est générée par PyMOL et les coordonnées PDB sont 2EBF (PMT), 1HQO (CNF1) et 1GGT (facteur XIII).*

The catalytic triad of PMT is similar to the catalytic triad of the thiol protease papain. This was the reason to suggest that PMT may act as a protease (Kitadokoro *et al.*, 2007). Comparing the structure of thiol proteases with transglutaminases (e.g. human factor XIII) shows high homology. The typ of chemical reaction is comparable: Transglutaminases replace the NH_2-group of an amide with another NH_2-group of an amine. Thiol proteases catalyze the reverse reaction. Deamidases in turn are acting like transglutaminases

using a H_2O instead of an amine. Structural comparison of the active centers of deamidases and thiol proteases with transglutaminases shows similar orientation of the active site. Transglutaminases (e.g. human factor XIII), which possess a similar catalytic triad as PMT proteases, replace the NH_2-group of the amide of glutamine by another amine residue and (Buetow *et al.*, 2001;Chao *et al.*, 2006;Kashiwagi *et al.*, 2002;Noguchi *et al.*, 2001;Pedersen *et al.*, 1994). Although the catalytic triad of PMT perfectly matches the catalytic site of a transglutaminase (*Figure 4B*), until to date no transglutaminating activity could be detected for PMT.

Conclusion

PMT acts as a deamidase to activate heterotrimeric G proteins. The toxin causes deamidation of an essential glutamine residue of Gα subunits resulting in glutamic acid. This exchange blocks the intrinsic GTPases activity and causes persistent activation of the G protein. The mitogenic effect of PMT has been repeatedly discussed as a possible source of cancerogenic activity. Interestingly, it was reported recently that frequent somatic mutations in the gene *Gnaq*, encoding $G\alpha_q$, are found in melanoma of the uvea (46%) and in blue naevi (83%). This mutations results in change of glutamine-209 in a manner similar to the constitutive activation as found for PMT (Van Raamsdonk *et al.*, 2008). It remains to be elucidated whether PMT-induced activation of G proteins by deamidation plays a role in cancer development.

Acknowledgements. *Studies from the laboratory of the authors reported in this review were supported by the Sonderforschungsbereich 746 (project P17).*

References

Buetow L, Flatau G, Chiu K, Boquet P, Ghosh P (2001) Structure of the Rho-activating domain of *Escherichia coli* cytotoxic necrotizing factor 1. *Nature Struct Biol,* **8**: 584-588

Busch C, Orth J, Djouder N, Aktories K (2001) Biological activity of a C-terminal fragment of *Pasteurella multocida* toxin. *Infect Immun,* **69**: 3628-3634

Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE (2003) Insights into G protein structure, function, and regulation. *Endocr Rev,* **24**: 765-781

Chanter N (1990) Molecular aspects of the virulence of *Pasteurella multocida*. *Can J Vet Res,* **54 Suppl**: S45-S47

Chao X, Muff TJ, Park SY, Zhang S, Pollard AM, Ordal GW, Bilwes AM, Crane BR (2006) A receptor-modifying deamidase in complex with a signaling phosphatase reveals reciprocal regulation. *Cell,* **124**: 561-571

Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR (1994) Structures of active conformations of Gi alpha 1 and the mechanism of GTP hydrolysis. *Science,* **265**: 1405-1412

De Vivo M, Chen J, Codina J, Iyengar R (1992) Enhanced phospholipase C stimulation and transformation in NIH-3T3 cells expressing Q209LGq-alpha-subunits. *J Biol Chem,* **267**: 18263-18266

Dudet LI, Chailler P, Dubreuil D, Martineau-Doize B (1996) *Pasteurella multocida* toxin stimulates mitogenesis and cytoskeleton reorganization in swiss 3T3 fibroblasts. *J Cell Physiol,* **168**: 173-182

Flatau G, Lemichez E, Gauthier M, Chardin P, Paris S, Fiorentini C, Boquet P (1997) Toxin-induced activation of the G protein p21 Rho by deamidation of glutamine. *Nature,* **387**: 729-733

Higgins TE, Murphy AC, Staddon JM, Lax AJ, Rozengurt E (1992) *Pasteurella multocida* toxin is a potent inducer of anchorage-independent cell growth. *Proc Natl Acad Sci U S A,* **89**: 4240-4244

Horiguchi Y, Inoue N, Masuda M, Kashimoto T, Katahira J, Sugimoto N, Matsuda M (1997) *Bordetella bronchiseptica* dermonecrotizing toxin induces reorganization of actin stress fibers through deamidation of Gln-63 of the GTP-binding protein Rho. *Proc Natl Acad Sci USA* **94**: 11623-11626

Kamp EM, Kimman TG (1988) Induction of nasal turbinate atrophy in germ-free pigs, using *Pasteurella multocida* as well as bacterium-free crude and purified dermonecrotic toxin of *P multocida*. *Am J Vet Res,* **49**: 1844-1849

Kashiwagi T, Yokoyama K, Ishikawa K, Ono K, Ejima D, Matsui H, Suzuki E (2002) Crystal structure of microbial transglutaminase from *Streptoverticillium mobaraense*. *J Biol Chem,* **277**: 44252-44260

Kitadokoro K, Kamitani S, Miyazawa M, Hanajima-Ozawa M, Fukui A, Miyake M, Horiguchi Y (2007) Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of *Pasteurella multocida* toxin. *Proc Natl Acad Sci U S A,* **104**: 5139-5144

Lacerda HM, Lax AJ, Rozengurt E (1996) *Pasteurella multocida* toxin, a potent intracellularly acting mitogen, induces $p125^{FAK}$ and paxillin tyrosine phosphorylation, actin stress fiber formation, and focal contact assembly in Swiss 3T3 cells. *J Biol Chem,* **271**: 439-445

Lax AJ, Chanter N (1990) Cloning of the toxin gene from *Pasteurella multocida* and its role in atrophic rhinitis. *J Gen Microbiol,* **136**: 81-87

Lax AJ, Grigoriadis AE (2001) *Pasteurella multocida* toxin: the mitogenic toxin that stimulates signalling cascades to regulate growth and differentiation. *Int J Med Microbiol,* **291**: 261-268

Majumdar S, Ramachandran S, Cerione RA (2006) New insights into the role of conserved, essential residues in the GTP binding/GTP hydrolytic cycle of large G proteins. *J Biol Chem,* **281**: 9219-9226

Masuda M, Betancourt L, Matsuzawa T, Kashimoto T, Takao T, Shimonishi Y, Horiguchi Y (2000) Activation of Rho through a cross-link with polyamines catalyzed by *Bordetella* dermonecrotizing toxin. *EMBO J* **19**: 521-530

Mullan PB, Lax AJ (1996) *Pasteurella multocida* toxin is a mitogen for bone cells in primary culture. *Infect Immun,* **64**: 959-965

Noguchi K, Ishikawa K, Yokoyama K, Ohtsuka T, Nio N, Suzuki E (2001) Crystal structure of red sea bream transglutaminase. *J Biol Chem,* **276**: 12055-12059

Orth JH, Blöcker D, Aktories K (2003) His1205 and His 1223 are essential for the activity of the mitogenic *Pasteurella multocida* toxin. *Biochemistry,* **42**: 4971-4977

Orth JH, Fester I, Preuss I, Agnoletto L, Wilson BA, Aktories K (2008) Activation of Galphai and subsequent uncoupling of receptor-Galphai signaling by *Pasteurella multocida* toxin. *J Biol Chem,* **283**: 23288-23294

Orth JH, Lang S, Aktories K (2004) Action of *Pasteurella multocida* toxin depends on the helical domain of Galphaq. *J Biol Chem,* **279**: 34150-34155

Orth JH, Lang S, Preuss I, Milligan G, Aktories K (2007) Action of *Pasteurella multocida* toxin on Galpha(q) is persistent and independent of interaction with G-protein-coupled receptors. *Cell Signal,* **19**: 2174-2182

Orth JH, Lang S, Taniguchi M, Aktories K (2005) *Pasteurella multocida* toxin-induced activation of RhoA is mediated *via* two families of G{alpha} proteins, G{alpha}q and G{alpha}12/13. *J Biol Chem,* **280**: 36701-36707

Orth JH, Preuss I, Fester I, Schlosser A, Wilson BA, Aktories K (2009) *Pasteurella multocida* toxin activation of heterotrimeric G proteins by deamidation. *Proc Natl Acad Sci U S A,* **106**: 7179-7184

Pedersen LC, Yee VC, Bishop PD, Trong IL, Teller DC, Stenkamp RE (1994) Transglutaminase factor XIII uses proteinase-like catalytic triad to crosslink macromolecules. *Protein Sci,* **3**: 1131-1135

Preuss I, Kurig B, Nurnberg B, Orth JH, Aktories K (2009) *Pasteurella multocida* toxin activates Gbetagamma dimers of heterotrimeric G proteins. *Cell Signal,* **21**: 551-558

Pullinger GD, Sowdhamini R, Lax AJ (2001) Localization of functional domains of the mitogenic toxin of *Pasteurella multocida*. *Infect Immun,* **69**: 7839-7850

Rozengurt E, Higgins T, Chanter N, Lax AJ, Staddon JM (1990) *Pasteurella multocida* toxin: potent mitogen for cultured fibroblasts. *Proc Natl Acad Sci USA,* **87**: 123-127

Schmidt G, Sehr P, Wilm M, Selzer J, Mann M, Aktories K (1997) Gln63 of Rho is deamidated by *Escherichia coli* cytotoxic necrotizing factor 1. *Nature,* **387**: 725-729

Seo B, Choy EW, Maudsley WE, Miller WE, Wilson BA, Luttrell LM (2000) *Pasteurella multocida* toxin stimulates mitogen-activated protein kinase *via* $G_{q/11}$-dependent transactivation of the epidermal growth factor receptor. *J Biol Chem,* **275**: 2239-2245

Sprang SR (1997) G protein mechanisms: insights from structural analysis. *Annu Rev Biochem,* **66**: 639-678

Staddon JM, Barker CJ, Murphy AC, Chanter N, Lax AJ, Michell RH, Rozengurt E (1991) *Pasteurella multocida* toxin, a potent mitogen, increases inositol 1,4,5-triphosphate and mobilizes Ca^{2+} in swiss 3T3 cells. *J Biol Chem,* **266**: 4840-4847

Tesmer JJ, Berman DM, Gilman AG, Sprang SR (1997) Structure of RGS4 bound to AlF4--activated G(i alpha1): stabilization of the transition state for GTP hydrolysis. *Cell,* **89**: 251-261

Van Raamsdonk CD, Bezrookove V, Green G, Bauer J, Gaugler L, O'Brien JM, Simpson EM, Barsh GS, Bastian BC (2008) Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature,* **457**: 599-602

Ward PN, Miles AJ, Sumner IG, Thomas LH, Lax AJ (1998) Activity of the mitogenic *Pasteurella multocida* toxin requires an essential C-terminal residue. *Infect Immun,* **66**: 5636-5642

Wilson BA, Zhu X, Ho M, Lu L (1997) *Pasteurella multocida* toxin activates the inositol triphosphate signaling pathway in *Xenopus* oocytes *via* G_{qa}-coupled phospholipase C-b1. *J Biol Chem,* **272**: 1268-1275

Zywietz A, Gohla A, Schmelz M, Schultz G, Offermanns S (2001) Pleiotropic effects of *Pasteurella multocida* toxin are mediated by Gq-dependent and -independent mechanisms. Involvement of Gq but not G11. *J Biol Chem,* **276**: 3840-3845