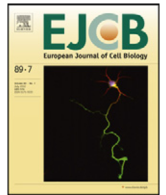
European Journal of Cell Biology 90 (2011) 951–954

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb



Review

Activity modulation of the bacterial Rho GAP YopE: An inspiration for the investigation of mammalian Rho GAPs

Martin Aepfelbacher*, Bernhard Roppenser, Moritz Hentschke, Klaus Ruckdeschel

Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany

ARTICLE INFO

Article history:
Received 15 November 2010
Received in revised form
15 December 2010
Accepted 15 December 2010

Keywords:
Cellular Microbiology
Yersinia
Rho GTP-binding proteins
Rho GAP
RhoG

ABSTRACT

The *Yersinia enterocolitica* Rho GTPase Activating Protein (Rho GAP) YopE belongs to a group of bacterial virulence factors that is translocated into infected target cells by a type three secretion system. Structurally and biochemically YopE resembles eukaryotic Rho GAPs which control various cellular functions by modulating the activity of Rho GTP binding proteins. Here we summarise the published information on cellular effects, Rho protein substrates, compartmentalisation and turnover of YopE. A fascinating picture evolves of how this virulence factor integrates in host cellular regulatory mechanisms to fine tune bacterial pathogenicity.

© 2011 Elsevier GmbH. All rights reserved.

Introduction

Small GTP-binding proteins of the Ras-superfamily have been implicated in essentially all important cell functions known to date. Presumably because Ras-family proteins are master switches in so many cellular pathways they have evolved as major targets of microbial virulence factors. Of particular relevance in this regard is the Rho-subfamily of Ras-like proteins (Aktories and Barbieri, 2005). The 15 Rho-family proteins are best known for regulating the actin cytoskeleton, but also play roles in inflammatory mediator production, vesicle transport, gene transcription and cell cycle control (Jaffe and Hall, 2005; van Aelst and D'Souza-Schorey, 1997). Like Ras-family GTP-binding proteins, most Rho proteins act as molecular switches which interact with effectors in the GTP-bound but not the GDP-bound state. Conversion of the GTP- to the GDP-bound state and vice versa, termed GTPase cycling, controls the dynamics of effector protein function and is itself controlled by distinct regulatory proteins. Although the bound GTP is hydrolysed by an intrinsic GTPase activity in most Rho GTP-binding proteins, this reaction has to be accelerated by several orders of magnitude to allow for rapid GTPase cycling. Acceleration of the intrinsic GTPase activity of Rho proteins is achieved by GTPase activating proteins (GAPs) whereby an essential arginine finger is provided by the interacting GAP (Scheffzek et al., 1998; Peck et al., 2002).

Genome wide analysis revealed the existence of >50 Rho GAPs in humans (Bernards, 2003). The activity and substrate range of a Rho GAP in a given cell is thought to depend on many variables such as subcellular localisation, actual expression of substrate GTP-binding proteins and the signalling and developmental state of the cell. Considering these circumstances it becomes clear that Rho GAPs are particularly well suited for fine tuning Rho protein activities (Moon and Zheng, 2003) and this holds particularly true for the bacterial Rho GAP YopE the features and virulence properties of which are summarised in this review (Table 1).

The bacterial Rho GAP YopE from pathogenic *Yersinia* species

The bacterial Rho GAP YopE was initially characterised by the group of Wolf-Watz as a 23 kDa protein encoded by the virulence plasmid pIB1 of *Y. pseudotuberculosis* (Forsberg and Wolf-Watz, 1988). It is also expressed by the close *Y. pseudotuberculosis* relatives *Y. enterocolitica* and *Y. pestis* (Cornelis et al., 1998). YopE was shown to be a crucial virulence determinant of yersiniae in mice infection studies and in parallel it was found to cause a cytotoxic response as reflected by actin filament disruption in HeLa cells and reduced phagocytic capability of mouse macrophages (Rosqvist et al., 1990, 1991). Only a decade later YopE was discovered to function as a Rho GAP (Black and Bliska, 2000; von Pawel-Rammingen et al., 2000). Although the YopE protein displays no sequence homology to eukaryotic GAPs, its crystal structure is highly similar to these and, like eukaryotic GAPs, YopE employs an arginine finger motif (Evdokimov et al., 2002). The catalytic (GAP) domain of YopE ranges

* Corresponding author. Tel.: +49 40 7410 53150; fax: +49 40 7410 54881.
E-mail addresses: m.aepfelbacher@uke.de, m.aepfelbacher@uke.uni-hamburg.de (M. Aepfelbacher).

0171-9335/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2010.12.004

Table 1
Summary of the biochemical and cellular features of YopE.

Features of YopE	Experimental results	References
Effect on host cells	Cytotoxicity, actin filament disruption, activity of type III translocation pore, antiphagocytosis, blockage of reactive oxygen production and IL-1β release	Black and Bliska (2000), Cornelis (2002), Forsberg and Wolf-Watz (1988), Mejia et al. (2008), Rosqvist et al. (1990, 1991) and Schotte et al. (2004)
Rho-protein substrates	RhoA, Rac1, RhoG, CDC42, TC10	Aili et al. (2006), Mohammadi and Isberg (2009) and Roppenser et al. (2009)
Subcellular compartmentalisation	ER- and Golgi localisation through membrane localisation domain (MLD)	Isaksson et al. (2009), Krall et al. (2004) and Roppenser et al. (2009)
Ubiquitination/Proteasomal degradation	Serotype (O8) specific ubiquitination of lysines 62 and −75 lead to proteasomal degradation	Gaus et al. (2011), Hentschke et al. (2007) and Ruckdeschel et al. (2006)

from amino acids 96–219 whereas the N-terminal 15 and 50 amino acids are required for bacterial secretion and translocation into target cells respectively (Cornelis, 2002; Fig. 1A). YopE was reported to exert GAP activity on the Rho family proteins Rho, Rac, Cdc42 and RhoG *in vitro* (Black and Bliska, 2000; von Pawel-Rammingen et al., 2000; Roppenser et al., 2009). Using *Yersinia* infected endothelial cells an initial report suggested that Rac1, but not RhoA or CDC42, is the physiological target of YopE and that the activity of YopE on Rac1 may depend on the upstream pathway by which Rac1 is activated (Andor et al., 2001). Several studies in different cell types concordantly confirmed that YopE induces a drastic reduction in the level of GTP-loaded active Rac1 (Aili et al., 2006; Ruckdeschel et al., 2006; Roppenser et al., 2009). Dependent on the infected cell type and other circumstances of infection, CDC42, TC10 and RhoA may also be targeted by YopE, yet with less efficiency and sometimes after a lag phase (Aili et al., 2006; Roppenser et al., 2009). When trying to identify which Rho proteins are physiologically targeted by a GAP in cells it has to be considered that Rho proteins often are interconnected in a way that one protein activates or inactivates a fellow Rho protein and that these signalling processes often are organised in networks and feedback loops (Jaffe and Hall, 2005; van Aelst and D'Souza-Schorey, 1997). One established way of upstream control of Rac1 is exerted through RhoG and the Elmo/Dock180 module. Binding of active RhoG to a complex of Elmo and Dock180 stimulates the GEF activity of Dock180 leading to GTP-loading of Rac1 (Katoh and Negishi, 2003). Interestingly, besides Rac activity RhoG activity was found to be blocked also by YopE in *Yersinia* infected cells. Using an activity sensor the spatiotemporal dynamics of deactivation of RhoG by YopE at the contact site of living host cells and virulent *Yersinia* could be recorded (Roppenser et al., 2009). Further experiments suggested that YopE can downregulate Rac1 activity by direct interaction and also by interaction with the Rac1 upstream regulator RhoG (Mohammadi and Isberg, 2009; Roppenser et al., 2009).

Together these cell biological findings indicate that the RhoG-Rac1 signalling axis is the preferred but likely not exclusive target for downregulation by YopE. This is comprehensible because Rac proteins (Rac1 and its relative Rac2) control a myriad of cell functions and many of these are crucial in the immune system (Vicente-Manzanares and Sanchez-Madrid, 2004; Burridge and Wennerberg, 2004). In fact, Rac1 and RhoG have been implicated in many immune functions known to be subdued by *Yersinia* in a YopE-dependent manner. Such cell functions for instance include phagocytosis, transendothelial migration, IL-1β-production and superoxide anion production (Cornelis, 2002; Schotte et al., 2004; Condliffe et al., 2006; Nakaya et al., 2006; van Buul et al., 2007).

YopE acts back on Yop-translocation

YopE has an additional function as a negative regulator of the *Yersinia* type III secretion system which delivers the Yop-effectors inside the cells. The current concept is that signals initiated by *Yersinia* cell infection activate RhoA, B or C and stimulate actin polymerisation which then somehow supports Yop delivery. Once the Yops are translocated, the activities of YopE and to a lesser extent also of YopT prevent pore formation as part of a negative feedback loop (Mejia et al., 2008). Thus, YopE can shut down its own delivery and the delivery of all other Yops (Mejia et al., 2008; Aili et al., 2008).

Intracellular membrane localisation determines YopE specificity

Despite earlier reports that indicated a cytosolic localisation, YopE has convincingly been demonstrated to bind to cellular membranes and colocalise with the endoplasmic reticulum and Golgi (Krall et al., 2004; Roppenser et al., 2009; Fig. 1B). A region comprising amino acid residues 54–75 (termed membrane localisation domain; MLD; Fig. 1A) was found to mediate the membrane binding (Krall et al., 2004). YopE constructs lacking the MLD displayed a cytoplasm-like fluorescence (Fig. 1B) and colocalisation with the target GTP-binding proteins Rac1 and RhoG at membranes was abrogated. Yet, the GAP activity of MLD-deleted YopE towards cellular Rac and RhoG was unchanged and the activity towards TC10 and RhoA even increased considerably as reported in one study (Roppenser et al., 2009). Another study could not detect differences in intracellular activity of YopE towards RhoA upon deletion of the MLD (Isaksson et al., 2009). These investigations suggest that the intracellular localisation of YopE crucially determines its specificity towards target Rho GTP binding proteins. Upon removing its membrane binding domain the substrate specificity of YopE decreases which however does not negatively affect some of its cellular effects such as cytoskeleton disruption and regulation of Yop-effector translocation (Roppenser et al., 2009; Isaksson et al., 2009). Notably, however, the restricted and tightly controlled substrate specificity brought about by intracellular compartmentalisation appears to be important for the function of YopE *in vivo*, a conclusion which is based on the finding that a *Y. pseudotuberculosis yopEΔMLD* mutant presented a strongly attenuated virulence in a mouse infection model (Isaksson et al., 2009).

Ubiquitination and degradation of YopE modulates bacterial virulence

Microbial proteins like YopE that gain access to the interior of a host cell and interfere with cell regulation will be sensed as foreign and putatively dangerous. In eukaryotic cells the proteasome is the most important non-lysosomal proteolytic system that removes potentially detrimental proteins by a mechanism involving ubiquitination (Shabek and Ciechanover, 2010). Consequently, several bacterial virulence factors are subjected to ubiquitination and proteasomal degradation which can on one hand limit bacterial disease. On the other hand bacteria have found ingenious ways of exploiting the proteasomal system for their own purposes (Angot et al., 2007; Hicks and Galan, 2010; Rytkonen and Holden, 2007). YopE appears to be the only Yop of *Yersinia* that is

Download English Version:

https://daneshyari.com/en/article/2178617

Download Persian Version:

https://daneshyari.com/article/2178617

Daneshyari.com