

Review

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Emerging functions as host cell factors – an encyclopedia of annexin-pathogen interactions

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Abstract: Emerging infectious diseases and drug-resistant infectious agents call for the development of innovative antimicrobial strategies. With pathogenicity now considered to arise from the complex and bi-directional interplay between a microbe and the host, host cell factor targeting has emerged as a promising approach that might overcome the limitations of classical antimicrobial drug development and could open up novel and efficient therapeutic strategies. Interaction with and modulation of host cell membranes is a recurrent theme in the host-microbe relationship. In this review, we provide an overview of what is currently known about the role of the Ca²⁺ dependent, membrane-binding annexin protein family in pathogen-host interactions, and discuss their emerging functions as host cell derived auxiliary proteins in microbe-host interactions and host cell targets.

Keywords: host cell targeting; novel therapeutic strategies; pathogen-host interface; virulence factors.

Introduction

Our understanding of pathogenicity (capacity to cause disease in a host) and virulence (level of damage caused in the host), has undergone a profound change. It is no longer thought to be invariably determined by genetic components of the pathogen, but now centers on the microbe-host system. Pathogens rely on their host and the host environmental conditions and are therefore highly adapted to both. Because of their often high mutation rate and the transfer of mobile genetic elements (Stokes and Gillings, 2011; Linero et al., 2012), viruses and bacteria acquire resistance to drugs that target pathogen components. Prominent examples for this are penicillin and amantadine (WHO, 2014). This is expected to be more unlikely for drugs that target host factors (Linero et al., 2012). Given that infectious diseases still account for many deaths worldwide, mainly in poorer societies, suitable therapeutic approaches are needed. In the search for novel targets, host cell factors came recently into focus. By targeting host factors rather than the pathogen itself, broader acting therapeutics could eventually be developed. Many host-directed therapeutic strategies center on drugs that broadly affect inflammation and antimicrobial defense (e.g. cytokines, cytokine-neutralizing antibodies, glucocorticoids, aspirin, ibuprofen), but also include the development of RNA interference-based tools (micro-RNAs, small interfering RNAs) and small molecule inhibitors (Bobbin et al., 2015; Zumla et al., 2016). However, as most approaches are still in preclinical/early clinical phase, their advantages over classical treatments remain largely hypothetical. Development of such precise, target-specific tools demands the comprehensive knowledge of the mechanisms underlying pathogen-host interactions. With this review, we provide an overview of what is currently known about annexin actions in host-microbe relationships. We are covering the latest reports of this rapidly growing field of annexin research to increase their appreciation in these processes.

Annexins are an evolutionary conserved multigene family characterized by their ability to bind to negatively

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charged membrane phospholipids in a reversible and Ca^{2+} -dependent manner (Gerke and Moss, 2002). The salient feature of all annexins is their modular structure - a highly conserved core domain that is responsible for the Ca^{2+} - and phospholipid-binding is preceded by an N-terminal tail, which is variable and unique for each annexin family member, and thought to fine-tune their respective biochemical properties and cellular functions (Gerke and Moss, 2002). Annexins are linked to membrane-related cellular events, mainly Ca^{2+} -regulated membrane association and organization via binding to negatively charged target lipids (especially phosphatidylserine and phosphoinositides) (Gerke and Moss, 2002; Rescher and Gerke, 2004; Gerke et al., 2005). Although annexins are classical cytosolic proteins without signal peptide sequences, at least some of them are also found extracellularly. An ever-increasing number of reports hint at defined functions of this extracellular pool in the transmission of extracellular signals via cell surface receptors, and annexins A1 and A2 have been most extensively studied in this respect (Rescher and Gerke, 2004).

Almost all of the microbes in the human body rely on intimate contacts with the membranes of their host cells. Thus, microbes have evolved a wide range of strategies to manipulate the host cellular membranes to their advantage. Initial encounters between a host cell and the pathogen occur at the plasma membrane, and the pathogenic repertoire ranges from the presentation of high affinity ligands to ensure attachment to a subsequent modulation of entry pathways to avoid (extracellular pathogens) or optimize (intracellular pathogens) uptake. Functional changes in endosomes and phagosomes ensure delivery of the microbial genome or establish intracellular persistence. Therefore, it is easy to assume that membrane-organizing proteins are among the host cell proteins involved in pathogenic interaction/rearrangement of intracellular membranes. Moreover, pathogen-induced changes in intracellular Ca^{2+} levels are observed. They arise from perturbed host membrane permeability, tailored activation of cellular Ca^{2+} -responses that support pathogen binding and invasion, and from detection of pathogen-associated molecular patterns (PAMPs) by host cell receptors operating in cell defense (TranVan Nhieu et al., 2004; Zhou et al., 2009). Their biochemical qualities, i.e. their ability to dynamically bind to certain membrane phospholipids in response to elevated intracellular Ca^{2+} levels, make the annexins ideally suited to connect membrane trafficking pathways and signaling. Indeed, annexins seem to operate in the modulation of membrane platforms and in the exocytic and endocytic pathways (Gerke et al., 2005). We suggest that connecting disparate

data on various microbes and intra- and extracellular annexin actions (Table 1) will make it possible to identify common, interrelated themes. Certainly far from the establishment of a unifying concept or unequivocal function, exploring the role of annexins at levels of increasing complexity such as the host-microbe relationship, might uncover their true potential and indeed lead to the development of entirely new types of drugs and treatments.

Annexins as host cell derived virulence factors

Incorporation into virus particles has been observed and reported for several annexins (Figure 1, 1a–c). Proteomic analysis, for example, revealed that purified influenza A virus (IAV) particles contain annexins A1, A2, A4, A5, and A11 (Shaw et al., 2008). Annexin A1 (AnxA1) is one of the most famous family members and there is a wealth of information on its protective anti-inflammatory and pro-resolving effects (Perretti and D'Acquisto, 2009). Because host defense against pathogen attack relies on innate immunity as the first line, pathogens have evolved various strategies to impair and evade the host innate immune defense. Surprisingly, there is only little indication that pathogens utilize AnxA1 to downregulate the host innate immune responses, although this protein is associated with herpesviruses (Kattenhorn et al., 2004; Dry et al., 2008; Loret et al., 2008; Vidick et al., 2013), human immunodeficiency virus type 1 (HIV-1) (Chertova et al., 2006), vesicular stomatitis virus (VSV) (Moerdyk-Schauwecker et al., 2009, 2014), Rift Valley fever virus (Nuss et al., 2014) and IAV (Shaw et al., 2008), and recent evidence points to a function of the AnxA1 receptor FPR2 in IAV replication (Tcherniuk et al., 2016). AnxA2 is a component of herpesviruses (Wright et al., 1994, 1995; Varnum et al., 2004; Zhu et al., 2005; Dry et al., 2008; Loret et al., 2008; Gershon et al., 2012; Vidick et al., 2013), IAV (LeBouder et al., 2008; Shaw et al., 2008; Liu et al., 2012), HIV-1 (Chertova et al., 2006), human papilloma virus (HPV) type 16 (Woodham et al., 2012), hepatitis C virus (HCV) type 1 (Backes et al., 2010), VSV (Moerdyk-Schauwecker et al., 2009, 2014), vaccinia virus (Chung et al., 2006), Rift Valley fever virus (Nuss et al., 2014) and Newcastle disease virus (Ren et al., 2012). AnxA5 is associated with herpes simplex virus (HSV) type 1 (Loret et al., 2008), HIV-1 (Chertova et al., 2006), VSV (Moerdyk-Schauwecker et al., 2009, 2014), Rift Valley fever virus (Nuss et al., 2014), and IAV (Shaw et al., 2008). How and at which stage in the virus infection cycle annexins are acquired remains so far unclear (which

Table 1: Implications of annexins in host-pathogen interactions.

Annexin	Pathogen	Role	References
AnxA1	Reovirus/measles virus	Virus-mediated cell-cell fusion	Ciechonska et al., 2014
	High-risk HPVs	HPV-related penile cancer progression	Calmon et al., 2013
	Infectious pancreatic necrosis virus	Antiapoptotic function	Hwang et al., 2007
	Murine cytomegalovirus	Component of virion	Kattenhorn et al., 2004
	Herpes simplex virus type 1	Component of virion	Loret et al., 2008
	Alcelaphine herpesvirus 1	Component of virion	Dry et al., 2008
	Rift Valley fever virus	Component of virion	Nuss et al., 2014
	Murid herpesvirus 4	Component of virion	Vidick et al., 2013
	Human immunodeficiency virus type 1	Component of virion	Chertova et al., 2006
	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2009, 2014
	Hepatitis C virus	negatively affects viral RNA replication	Hiramoto et al., 2015
	Influenza virus	Component of virion	Shaw et al., 2008
		Increased expression during infection, enhances virus propagation through modulation of endosomal trafficking and apoptosis	Arora et al., 2016
AnxA2		AnxA1 in the viral envelope causes FPR2 activation and promotes viral replication through an extracellular-regulated kinase (ERK)-dependent pathway	Tcherniuk et al., 2016
	<i>Mycobacterium tuberculosis</i>	Apoptosis	Gan et al., 2008
	Rous sarcoma virus	Cell transformation	Hayes and Moss 2009
	Human papillomavirus type 16	Virus entry and intracellular trafficking	Woodham et al., 2012; Dziduszko and Ozbun 2013
			Zhu et al., 2005
	Kaposi's sarcoma-associated herpesvirus	Component of virion	
	Alcelaphine herpesvirus 1	Component of virion	Dry et al., 2008
	Rift Valley fever virus	Component of virion	Nuss et al., 2014
	Murid herpesvirus 4	Component of virion	Vidick et al., 2013
	Herpes simplex virus type 1	Component of virion	Loret et al., 2008
	Herpes simplex virus types 1 & 2, and cytomegalovirus	Component of virion	Gershon et al., 2012
		Plasminogen activation	
	Human cytomegalovirus	Component of virion entry	Wright et al., 1994; Wright et al., 1995; Pietropaolo and Compton 1997; Pietropaolo and Compton 1999; Raynor et al., 1999; Varnum et al., 2004; Derry et al., 2007
	Influenza virus	Component of virion	LeBouder et al., 2008, 2010; Shaw et al., 2008; Liu et al., 2012
		Plasminogen activation	
	Human immunodeficiency virus type 1	Component of virion	Chertova et al., 2006; Ryzhova et al., 2006; Harrist et al., 2009; Rai et al., 2010
		Binds Gag protein	
		Gag trafficking	
		Virus assembly	
		Infectivity	
	Hepatitis C virus	Virus assembly	Backes et al., 2010; Saxena et al., 2012
	Enterovirus 71	Binds VP1	Yang et al., 2011
		Entry and infectivity	
	Infectious bronchitis virus	Binds IBV pseudoknot RNA entry	Kwak et al., 2011
	Rabbit vesivirus		González-Reyes et al., 2009
	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2009, 2014
	Respiratory syncytial virus	Virus receptor	Malhotra et al., 2003
	Epstein-Barr virus	Nuclear entry, DNA synthesis, cell proliferation	Luo et al., 2008
	Vaccinia virus	Component of virion	Chung et al., 2006
	Newcastle disease virus	Component of virion	Ren et al., 2012
	Bluetongue virus	Intracellular trafficking release	Beaton et al., 2002; Celma and Roy 2011

Table 1 (continued)

Annexin	Pathogen	Role	References
	<i>Salmonella typhimurium</i>	Invasion	Jolly et al., 2014
	Enteropathogenic <i>Escherichia coli</i>	Invasion	Zobiack et al., 2002
	Enterohemorrhagic <i>Escherichia coli</i>	Invasion	Miyahara et al., 2009
	<i>Pseudomonas aeruginosa</i>	Entry/receptor	Kirschnek et al., 2005
	<i>Mycoplasma hyorhinis</i>	Binding to bacterial protein/infection	Yuan et al., 2016
AnxA3	<i>Mycoplasma pneumoniae</i>	Binding to bacterial protein/infection	Somarajan et al., 2014
	Murid Herpesvirus 4	Component of virion	Vidick et al., 2013
	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2009
AnxA4	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2009, 2014
	Rift Valley fever virus	Component of virion	Nuss et al., 2014
AnxA5	Influenza virus	Component of virion	Shaw et al., 2008
	Herpes simplex virus type 1	Component of virion	Loret et al., 2008
	Rift Valley fever virus	Component of virion	Nuss et al., 2014
	Human immunodeficiency virus type 1	Component of virion	Chertova et al., 2006
	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2009, 2014
	Hepatitis B virus	Entry/putative receptor	Neurath and Strick 1994; De Meyer et al., 1999a,b, 2000; Gong et al., 1999
	Influenza virus	Entry Component of virion	Huang et al., 1996; Shaw et al., 2008
AnxA6		Anti-inflammatory	Berri et al., 2014
	Human immunodeficiency virus type 1	Component of virion	Chertova et al., 2006
	Vesicular stomatitis virus	Component of virion	Moerdyk-Schauwecker et al., 2014
AnxA11	Influenza A virus	Release	Ma et al., 2012; Musiol et al., 2013
	Human immunodeficiency virus type 1	Component of virion	Chertova et al., 2006
	Rift Valley Fever virus	Component of virion	Nuss et al., 2014
	Influenza virus	Component of virion	Shaw et al., 2008

generally holds true for virus-embedded host cell proteins), and the association might simply reflect the nature of the specialized membrane domains where viral assembly and budding take place. Viruses that bud from lipid-enriched plasma membrane domains ('rafts') would then incorporate the often raft-associated annexins as part of their host cell membrane-derived viral envelope. Evidence that their presence is of relevance for the viral infection cycle has emerged for annexin A2 (AnxA2), which extracellularly facilitates the conversion of plasminogen to plasmin, and viruses that utilize the host cell plasminogen/plasmin conversion system during infection. For instance, herpesvirus promotes infection through activation of protease-activated receptor 1 (PAR) (Gershon et al., 2012), and AnxA2 on the herpesvirus surface was reported to bind to plasminogen, thus enhancing plasmin production. However, AnxA2 is not essential for viral plasmin generation and its activity might be more modulatory. AnxA2 also contributes to IAV propagation (LeBouder et al., 2008, 2010; Liu et al., 2012), and the functional role of IAV-associated AnxA2 was explored in more detail. For successful infections, the hemagglutinin (HA) in the IAV envelope is first cleaved by host cell proteases. This primes

HA for subsequent and endosomal low-pH-triggered conformational changes, which in turn is required for fusion of the virus envelope with the endosomal membrane and subsequent release of the viral genome into the cytosol. Notably, AnxA2 associated with purified IAV particles mediates plasminogen activation, thereby supporting the proteolytic cleavage of hemagglutinin and enhancing viral replication (LeBouder et al., 2008, 2010). Annexin A5 (AnxA5), which interacts with influenza A and B viruses (Huang et al., 1996), is also incorporated during budding of IAV virions, and counteracts the host innate immune response, particularly the antiviral interferon response, through the inhibition of interferon- γ -mediated activation of signal transducer and activator 1 (STAT1) (Berri et al., 2014). AnxA5 has been shown to specifically bind to a surface antigen of hepatitis B virus (HBV) (Hertogs et al., 1993; Neurath and Strick, 1994), and it was demonstrated that this protein is important for the susceptibility of cells to HBV infection (Gong et al., 1999; De Meyer et al., 1999a,b, 2000). It has to be established whether this also affects host innate immune responses.

Annexins, specifically AnxA2 and A5 within the IAV envelope, might contribute to the pathogenicity and thus

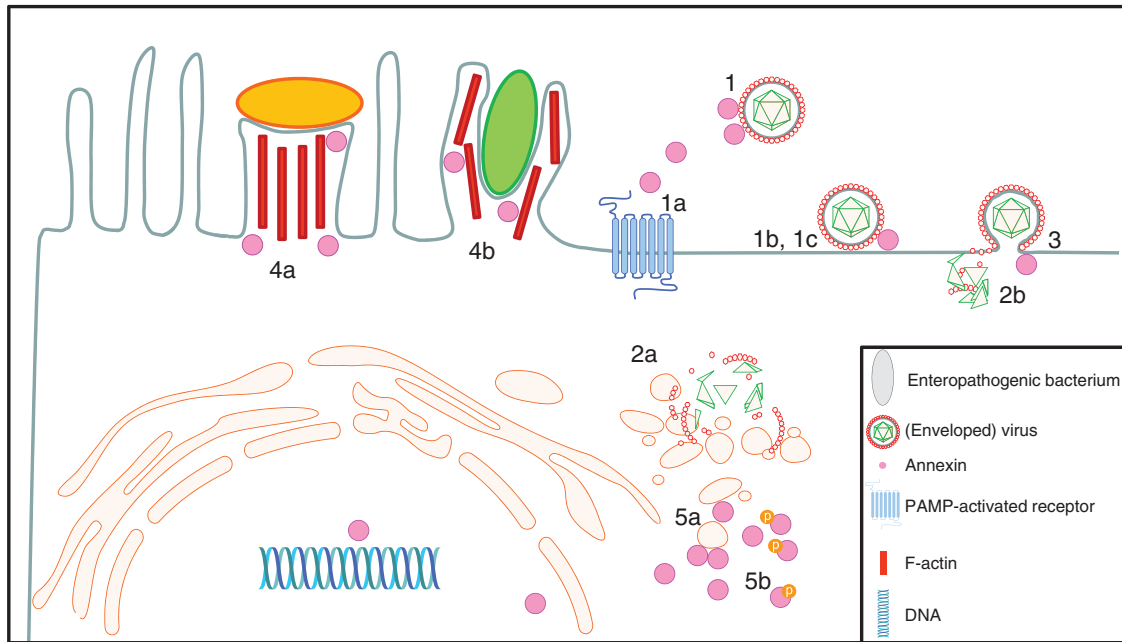


Figure 1: Graphical summary of annexin-microbe interactions.

(1) Virus-associated annexins might (1a) help avoid the host innate immune response by modulating signaling pathways of PAMP-activated receptors (AnxA1/FPR; AnxA5/Stat1), (1b) act as facilitators required for efficient entry, or (1c) serve as ligands/receptors for binding to the cell surface. Intracellularly, annexins might assist in viral replication/assembly at (2a) (specialized) endomembranes or at (2b) the plasma membrane; and/or (3) affect virus budding, leading eventually to incorporation. F-actin binding annexins such as AnxA2 and AnxA8 might help organize membrane platforms for F-actin rearrangements in (4a) EPEC/EHEC and (4b) *Salmonella* infection. Microbe-host cell interaction might (5a) depend on annexin-assisted cellular processes (AnxA1/syncytium formation; AnxA6/endomembrane cholesterol balance and 5b) even affect annexin gene expression levels and post-translational modifications, leading to functional switches (relocalization, recruitment of binding partners) and externalization, as might be required for steps 1b, 1c, 2, 3.

serve as host cell-derived virulence factors. Efficient packaging of the viral genome is a critical step in infections, and viruses with segmented genomes such as IAV must additionally ensure that at least one copy of each segment is present in the progeny virus particles. Hijacking and misusing host cell molecules that are concentrated at the budding zones would provide an efficient and economic means to acquire virulence factors without the need for their genetic encoding and packaging. As a host cell derived virulence factor is tailored to the host after the first successful round of infection, this might even help a host-switching virus to infect a wide range of tissues and/or species.

Certainly, the functional roles of the remaining IAV-associated annexins have not yet been identified. A caveat is that infected host cells produce extracellular vesicles such as exosomes (Schorey et al., 2015). These microvesicles carry a broad variety of host cell proteins. Because they are of the size and density of viruses, microvesicles are a source of contaminating cellular proteins, and careful analysis of the successful separation of the viruses from exosomes is paramount.

Annexins as host cell surface receptors for microbes

Several pathogens, mainly viruses, have been reported to utilize host cell surface associated annexins as receptors for cell binding and entry (Figure 1, 1b–c). The heterotetrameric complex formed by AnxA2 and its interaction partner S100A10 (formerly known as p11, belongs to the S100 protein family of Ca^{2+} binding proteins) was proposed to contribute to the internalization of the most common high-risk human papillomavirus genotype, HPV16, which causes benign and malignant tumors of the mucosal and cutaneous epithelium. HPV16 elicits AnxA2 translocation to the cell surface (Dziduszko and Ozburn, 2013), and the cell surface AnxA2/S100A10 complex is essential for entry of the virus into the host cell, acting presumably as receptor for the HPV minor capsid protein L2 (Woodham et al., 2012; Dziduszko and Ozburn, 2013). Interestingly, small molecule inhibitors of the AnxA2/S100A10 complex substantially reduced HPV16 infection in a cell culture model (Woodham et al., 2015). AnxA2 was reported to interact

with HCMV glycoprotein B (Bold et al., 1996; Pietropaolo and Compton, 1997), and, together with S100A10, to facilitate CMV infection (Derry et al., 2007) probably through enhanced fusion of the virus with the host cell (Raynor et al., 1999). However, this was not confirmed in another study (Pietropaolo and Compton, 1999). AnxA2 on the host cell surface was also implicated in the entry of enterovirus type 71 (EV71), the causative agent of the hand, foot, and mouth disease, to the host cell surface, possibly by binding the viral capsid protein VP1 on the cell surface (Yang et al., 2011). Rabbit vesivirus (RaV) virions might also depend on interaction with AnxA2 for their attachment and internalization (González-Reyes et al., 2009). Moreover, AnxA2 was isolated as a potential respiratory syncytial virus (RSV) receptor on epithelial cells (Malhotra et al., 2003). AnxA2 was reported as a receptor for *Pseudomonas aeruginosa* (Kirschnek et al., 2005), but has been recently implicated in *Mycoplasma* infection as well. Two independent studies demonstrated for *Mycoplasma hyorhinis* and *Mycoplasma pneumoniae* that AnxA2 binds to certain bacterial proteins or serves as receptor (Somarajan et al., 2014; Yuan et al., 2016), and targeting this interaction has been demonstrated to be of therapeutic potential (see below).

Intracellular interactions with microbes

Many viruses and bacteria exploit the host cell membrane systems to survive and replicate intracellularly, and they might need additional help from annexins (Figure 1, 2–5). AnxA1 was identified to Ca^{2+} -dependently interact with fusogenic reptilian reovirus p14 and measles virus F and H proteins. Virus-induced syncytiogenesis is caused by viral proteins that mediate plasma membrane fusion of neighboring cells to generate large multi-nucleate cells. While the initial membrane fusion and the formation of a small pore was not affected, intracellular AnxA1 was shown to promote the subsequent pore expansion required for efficient syncytium formation (Ciechonska et al., 2014). AnxA2 is an interaction partner for HIV-1 Gag in productively infected macrophages, with the interaction most likely taking place at the limiting membrane of endolysosomes. Depletion of AnxA2 led to incomplete Gag processing and reduced infectivity of released virions, suggesting a role for AnxA2 in HIV-1 assembly (Ryzhova et al., 2006). Supporting this, AnxA2/S100A10 complex was present in highly purified virions (Chertova et al., 2006). A direct interaction of AnxA2 with HIV-Gag

has also been demonstrated at raft microdomains in the plasma membrane of 293T cells, although the supporting effect on HIV particle production (Harrist et al., 2009) could not be confirmed in another study (Rai et al., 2010), suggesting that AnxA2 is not universally essential for either HIV-1 assembly or Gag processing, but is required for the production of infectious HIV-1 particles specifically in macrophages. Nonenveloped bluetongue virus protein NS3 was shown to bind the AnxA2 interaction partner S100A10. Interestingly, the S100A10 binding site on NS3 mimics the S100A10 binding site on AnxA2 and efficiently competes for S100A10 binding (Beaton et al., 2002; Celma and Roy, 2011). AnxA2 is also linked to the production of HCV particles, presumably through interaction with HCV proteins. In infected cells, AnxA2 localizes to and supports formation of HCV-induced unique subcellular membrane structures, the endoplasmic reticulum (ER)-derived sites of HCV replication termed ‘membranous web’ (Lai et al., 2008; Backes et al., 2010; Saxena et al., 2012). Furthermore, AnxA2 was identified as a binding protein for the pseudoknot RNA of infectious bronchitis virus, thereby regulating frameshifting efficiency and possibly acting as an antiviral factor (Kwak et al., 2011). Annexin A6 (AnxA6) is another annexin implicated in IAV infection and two studies revealed a negative regulatory impact on IAV replication and propagation. AnxA6 is crucial for maintaining a balanced cholesterol content in cellular membranes. Elevated AnxA6 levels interfere with cholesterol trafficking out of the endosomal system, thereby causing a shift in cholesterol contents from the plasma membrane to the late endosomes (Cubells et al., 2007). AnxA6 was shown to indirectly regulate IAV replication by lowering the availability of cholesterol at the plasma membrane. In cells overexpressing AnxA6, IAV particles budding from the host cell plasma membrane are equipped with an envelope that is strongly reduced in cholesterol and exhibit severely impaired infectivity (Musiol et al., 2013). A second study also observed defective budding in AnxA6-overexpressing cells and identified AnxA6 as host cell factor that interacts with a proton-selective ion channel incorporated in the viral envelope, the M2 protein (Ma et al., 2012). The lowered late endosomal pH causes the M2 proton channel to conduct protons, leading to acidification of the viral interior and subsequent release of viral ribonucleoprotein from the virus envelope (Pielak and Chou, 2011). In infected cells, newly synthesized lipid envelope proteins are trafficked from the ER through the Golgi and the trans-Golgi network (TGN) to viral assembly sites at the plasma membrane. At this step in the viral infection cycle, M2 activity counteracts the acidic luminal Golgi pH to protect the nascent HA protein

from protonation-induced conformational change (Pielak and Chou, 2011). While it is quite clear that AnxA6 negatively affects IAV infection, it remains to be determined whether and how AnxA6-controlled host cell cholesterol balance and AnxA6-M2 interaction are linked.

Apart from viruses, several pathogenic bacteria were postulated to interact with AnxA2 at certain stages of their life cycle (Figure 1, 4a,b). Due to its ability to bind actin and membranes, AnxA2 is one of the key players linking actin dynamics and membrane platforms in mammalian cells. AnxA2 is involved in a wide variety of actin-driven membrane processes including cell-cell adhesion, epithelial cell polarity, membrane ruffling and endocytosis (Rescher and Gerke, 2004; Rescher et al., 2004, 2008; Hayes et al., 2006; Hayes and Moss, 2009; Grieve et al., 2012), cellular mechanisms that are often exploited by pathogenic organisms to invade the host. Furthermore, AnxA2 is localized at the apical plasma membrane and the microvillar brush border of intestinal epithelial cells (Danielsen et al., 2003). These surfaces are often targeted by enteric bacterial pathogens that use contact-dependent type 3 secretion systems (T3SS), especially to undermine the actin cytoskeleton at their site of contact and invasion (Patel and Galán, 2005). *Salmonella* is one example of pathogenic bacteria employing such a T3SS to trigger its uptake into nonphagocytic epithelial cells. Due to this secretion system, *Salmonella* effector proteins are directly delivered across the plasma membrane into the host cell, where they can induce dynamic actin-driven ruffling of the membrane and, as a result, internalization of the pathogen into a modified phagosome. In particular, the ability of AnxA2 to function at dynamic actin remodeling platforms in cellular membranes seems to be of particular relevance. The facultative intracellular pathogen *Salmonella typhimurium* recruits AnxA2 through the effector protein SopB, – which is delivered into the host by the T3SS to *Salmonella* invasion sites (Jolly et al., 2014). Prior to its recruitment to the plasma membrane, AnxA2 most probably interacts with S100A10. This in turn is a prerequisite for engagement with the exceptionally large protein AHNK (Benaud et al., 2004). Most likely, AHNK and the AnxA2/S100A10 complex then participate in the reorganization of the actin cytoskeleton needed for efficient invasion. Enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) both induce the host cell to form filamentous actin-rich protrusions (pedestals) beneath the sides of bacterial attachment. Interestingly, both pathogens specifically recruit AnxA2 to these pedestals (Zobiack et al., 2002; Miyahara et al., 2009). In EHEC infections, the EHEC effector protein EspL2 binds directly to the F-actin-aggregating AnxA2, increasing its activity

and, hence, promoting invasion (Miyahara et al., 2009). In both cases, relocation of AnxA2 depends on the bacterial effector Translocated intimin receptor (Tir), which is delivered into the host actively translocated into the host cell via the T3SS. Tir was postulated to bind to the C-terminal region of AnxA2 directly, thereby recruiting it to the site of bacterial adherence (Munera et al., 2012). Ca^{2+} is most likely needed for membrane recruitment (Zobiack et al., 2002), however, the impact of Ca^{2+} on direct protein-protein interaction with bacterial effectors was not investigated. AnxA2 is a phosphatidylinositol (4,5) bisphosphate-interacting protein (Hayes et al., 2004; Rescher et al., 2004), a lipid that serves as a hub for the docking of actin-organizing proteins (Kwik et al., 2003) and is also found concentrated beneath the bacterial attachment sites (Zobiack et al., 2002). Interestingly, AnxA8, which also binds F-actin and phosphatidylinositol (4,5) bisphosphate (Goebeler et al., 2006), is also present at EPEC adhesion sites (Goebeler et al., 2006). Whether the direct interaction with bacterial proteins is an AnxA2-specific feature or shared by at least AnxA8 remains to be established. Collectively, the observation that *Salmonella*, EPEC as well as EHEC have independently developed separate mechanisms to take advantage of the function of AnxA2 (and maybe other annexins) in actin-remodeling indicates that this protein might be of critical importance for enteric pathogens in general.

Annexins and pathogen-induced reprogramming of target cells

Pathogens often manipulate the host cell gene expression profile or induce post-transcriptional modifications of host cell proteins. Those changes might be beneficial for the pathogen (such as enhanced or depressed host cell proliferation, or impaired immune response) or reflect the global cell response to the pathogenic attack. The following section concentrates on changes of annexin expression patterns and/or posttranscriptionally modified annexins in infected cells that correlate directly with pathogen success (Figure 1, 5). However, whether the different annexin expression levels reflect the pathogen's specific attempt to deregulate certain cellular processes or the host cell's efforts to fight the pathogenic attack is mostly unclear. AnxA1 was reported to negatively affect HCV RNA replication (Hiramoto et al., 2015), whereas increased expression of AnxA1 in IAV-infected cells was reported to positively affect virus propagation (Arora et al., 2016). AnxA1 overexpression was also seen in

high-risk HPV-positive penile squamous cell carcinoma and might depend on HPV E6 oncoprotein (Calmon et al., 2013). Higher levels of AnxA2 expression were observed in Kaposi's sarcoma-associated herpesvirus (KSHV)-infected cells and might support KSHV latency (Paudel et al., 2012). Latent membrane protein 1 (LMBP1) encoded by Epstein-Barr virus was shown to activate protein kinase C, resulting in AnxA2 phosphorylation and nuclear translocation, with the nuclear pool of AnxA2 supporting DNA synthesis and cell proliferation (Luo et al., 2008). HPV16 was reported to cause epidermal growth factor receptor (EGFR)-dependent activation of src kinase, resulting in phosphorylation and extracellular translocation of AnxA2 to function in HPV16 internalization (Dziduszko and Ozburn, 2013). Virulent strains of *Mycobacterium tuberculosis* induce proteolytic cleavage of AnxA1 on the surface of infected cells, thereby interfering with formation of the apoptotic envelope (Gan et al., 2008). Instead, infected macrophages undergo necrotic cell death, leading to release of viable bacilli and enhanced infection.

Future perspectives – annexin as drugs or drug targets

Growing concern regarding the increasing number of drug-resistant pathogen strains has called for novel therapeutic approaches. An emerging strategy is to target events involved in the early processes of pathogen-host cell recognition, namely the binding of pathogen components to receptors on the host cell surface. While blocking host cell receptors through high-affinity antagonists seems a logical and straightforward approach, this will also inhibit their normal cellular function and thus lead to undesirable side effects. For example, many HIV-1 strains use the C-C chemokine receptor 5 (CCR5) as a co-receptor for entry and infection of immune cells (Murphy, 2001), and CCR5 inhibitors are a novel class of antiretroviral drugs used in HIV therapy. However, CCR5 together with RANTES (CCL5), one of the several CCR5 agonists, appear to be involved in an antiviral response to other chronic infections (Schuh et al., 2002; Sanchooli et al., 2014). Therefore, refined antagonists that selectively target HIV-CCR5 binding without disrupting CCR5 activation by endogenous agonists might be safer therapeutic tools (Fätkenheuer et al., 2005). As listed above, extracellular annexins, and prominently AnxA2, were implicated in a vast amount of pathogen interactions with their target host cells. Synthetic annexin analogs that efficiently compete binding of the pathogen ligands to the native annexin on the host cell surface might be better

suited to efficiently impair microbe attachment without disturbing cellular functions. Indeed, the potential of such approaches has already been successfully explored: a synthetic AnxA2 N-terminal polypeptide was shown to remove *Mycoplasma hyorhinis* cell culture contaminations more efficiently and less toxic compared to commercial antibiotics (Yuan et al., 2016). Often, host cell damage is caused by pathogen toxins that recognize host cell membrane receptors. *Mycoplasma pneumonia* synthesizes the exotoxin community acquired respiratory distress syndrome (CARDS) toxin, which has been shown to recognize AnxA2 as a functional receptor. Indeed, targeting AnxA2 with an anti-AnxA2 monoclonal antibody was reported to reduce toxin binding and internalization (Somarajan et al., 2014). AnxA5 was reported to bind and affect lipopolysaccharide (LPS), a major endotoxin of Gram-negative bacteria, and might be of therapeutic use in Gram-negative sepsis (Rand et al., 2012). Overall, annexins might evolve as promising targets in pathogen-host cell interactions, yet data are still sparse, leaving ample room for promising and exciting research opportunities.

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