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Actin dynamics in host–pathogen interaction

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The actin cytoskeleton and Rho GTPase signaling to actin assembly are prime targets of bacterial and viral pathogens, simply because actin is involved in all motile and membrane remodeling processes, such as phagocytosis, macropinocytosis, endocytosis, exocytosis, vesicular trafficking and membrane fusion events, motility, and last but not least, autophagy. This article aims at providing an overview of the most prominent pathogen-induced or -hijacked actin structures, and an outlook on how future research might uncover additional, equally sophisticated interactions.

Keywords: actin dynamics; bacterial invasion; host–pathogen interaction; viral entry; virulence factors

Cellular actin assemblies

The shape of cells, their movement, phagocytosis, intercellular communication, endo- and exocytosis as well as the distribution of organelles all depend on dynamic reorganizations of the actin cytoskeleton. Actin exists in the cell in two distinct forms: globular actin (G-actin) monomers and filamentous actin (F-actin) polymers. The rearrangement of cellular actin structures is a dynamic, often fast process driven by continuous assembly, disassembly and/or reassembly of actin filaments. This turnover is controlled by multiple factors including major, ubiquitously operating machines, representatives of which are found in all eukaryotes.

Molecular basis of actin polymerization

The first step in making a filament from G-actin monomers is the so-called nucleation, driven by tightly regulated catalytic molecular machines like Arp2/3 complex or members of the formin family of proteins. A schematic overview of the most prominent mechanisms of actin assembly (along with exemplary virulence factors targeting them, see also below) is given in Fig. 1. It is becoming increasingly clear that these and similar machines come as multicomponent complexes, which generate F-actin in response to signals that are transferred onto these machines foremost by Rho-GTPases (see below and Refs [1–3]). In case of Arp2/3 complex, an additional class of proteins or protein complexes, namely the so-called nucleation promoting factors (NPFs) operate as essential intermediates for the activation of actin assembly. Activation of Arp2/3 complex by these NPFs leads to the formation of branched actin networks. Signal-dependent ignition of any of these machines, therefore, results in the spatiotemporally restricted generation of F-actin on cellular membranes.

The WASP family of NPFs in mammals now consists of four subgroups with eight members [4], namely Wasp/N-WASP [5,6], three WAVES [7], and the more recently identified WASH [8] and WHAMM/JMY [9–11] with individual cellular functions [12]. As opposed to the Arp2/3 complex, the formin family, consisting of 15 members in mammals, generates long, unbranched filaments [13]. Although

Abbreviations
EHEC, Enterohemorrhagic *E. coli*; EPEC, Enteropathogenic *E. coli*; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; NPF, nucleation promoting factor; T3SS, type III secretion system.

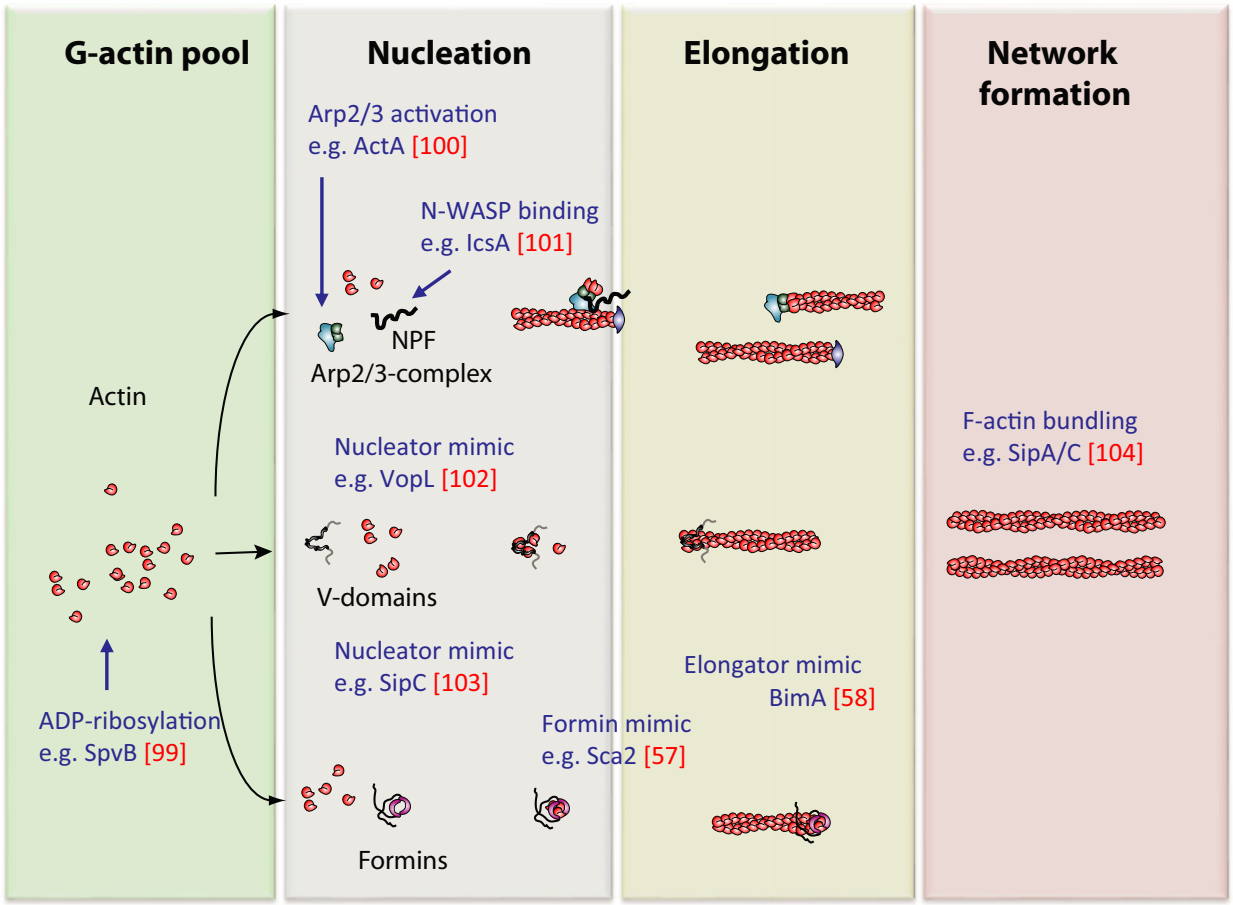

Fig. 1. Molecular mechanisms of actin filament assembly and their targeting by virulence factors. Actin filament turnover is tightly regulated by catalytic nanomachines and their cofactors (for details, see text or Ref. [12]). Assembly of F-actin is manipulated at virtually every level by bacterial virulence factors. The columns mark the phases of F-actin production and the virulence actors (blue) are placed about where they affect filament turnover. The factors depicted are only few examples and the list is far from being complete. Nonetheless, for the future, we expect many more virulence factors and/or mechanisms to be identified that affect these and other steps of dynamic actin turnover such as severing or capping. Note that the molecular mechanisms evolved by bacteria to nucleate/elongate actin are not identical, but at best similar to those of the host cell as drafted in the chart.

certain formins are implicated in the formation of filopodia, which are finger-shaped cell protrusions [14] or of myosin-decorated stress fibers [13], sheet-like protrusions termed lamellipodia embody the most prominent Arp2/3 complex-mediated actin structure. Last but not least, consecutive copies of G-actin-binding domains, such as WH2 (WASP homology 2, also termed V domains for Verprolin homology domain) domains, are capable of generating filaments and represent an additional but in comparison still understudied class of actin nucleators [15]. This class comprises members as different as Spire [16], Cobl (Cordon-bleu, [17]), leiomodin in muscle [18,19], or the bacterial factors VopL and VopF from *Vibrio* sp. [20,21]. Finally, stability and turnover of actin filaments are controlled by a multitude of modulatory activities such as severing, capping or bundling, which determines, for example, texture, durability, or longevity of the given structure built. Together, we are still facing huge gaps in our understanding of how actin structures in living cells are formed through the concerted biochemical activities that we already know—aside from the unknown. A schematic overview of some actin-nucleating gears and their preferred location of action—if known—are provided in Fig. 2.

Rho GTPases signaling to actin assembly

Signaling pathways regulated by proteins of the Rho GTPase family are involved in many cellular functions, ranging from cell polarization, migration, cell division,

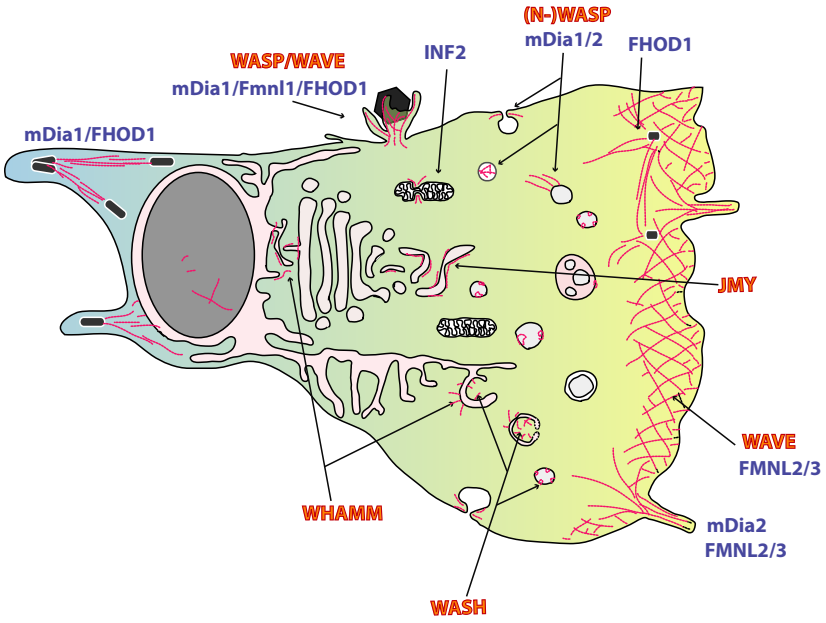

Fig. 2. Cellular actin assemblies. Gross structure of cell membranes with actin assemblies and the respective Arp2/3-complex activators (in red) or formins (in blue) that were described to contribute to their formation. The listing cannot be complete and requires continuous revision, as our knowledge on the cellular roles of these actin-generating nanomachines is continuously growing. Original references for the mentioned actions of NPFs and formins are numerous and can be found in recent competitive reviews [2–4,11–13,15]. Note that pathogens were found capable to usurp many if not most of these actin assemblies and that the currently unseen ones are expected to be found in the future.

and vesicle trafficking to transcription and inflammatory reactions, just to name a few [22].

Rho GTPases cycle between an inactive, GDP-bound state and an active, GTP-bound state. They undergo conformational changes during cycling between states, which in turn is controlled by other classes of GTPase-binding proteins [23]. So-called guanine nucleotide exchange factors (GEFs, [24]) regulate their activation by facilitating the exchange of GDP for GTP, whereas GTPase-activating proteins (GAPs) enhance their intrinsic hydrolase activity leading to inactivation [25]. In the GTP-bound state, the GTPase binds to a given downstream effector, igniting a signaling cascade. Finally, guanine nucleotide dissociation inhibitors (GDIs) function to maintain Rho GTPases in an inactive GDP-bound state [26] and/or protect them from degradation [27]. The small GTPase activation cycle is schematically depicted in Fig. 3. The Rho GTPase family comprises 20 members in humans [28], with the best characterized members being RhoA, Rac1, and Cdc42.

RhoA has been shown to be involved in the formation of stress fibers, while Rac is responsible for the formation of actin-rich protrusions termed lamellipodia. Cdc42 can instead contribute to the formation of various protrusions and to endomembrane trafficking, although it is still mostly associated with the formation of finger-like filopodia. Owing to their conserved and crucial roles in controlling actin cytoskeleton turnover, cell survival, and proliferation, Rho GTPases are a prime target for virulence mechanisms of bacterial pathogens [29–31]. It is worth mentioning here that bacterial virulence factors have evolved sophisticated examples of molecular mimicry, that is, harboring analogs of GTPase-regulatory factors such as GEFs, GAPs, and GDIs (highlighted and referenced in Fig. 3).

Actin structures induced or hijacked by bacteria

A subgroup of pathogenic bacteria invades their host cells such as nonphagocytic gut epithelium cells by stimulating uptake processes reminiscent of phagocytosis, macropinocytosis, or endocytosis. All these entry pathways converge on actin polymerization, although the phenotypic appearance is rather diverse. Historically, these invasion pathways were classified into so-called 'trigger' and 'zipper' mechanisms [32,33], either accompanied by excessive membrane ruffling mediated by large, lamellipodia-like membrane folds, or alternatively, accompanied by much smaller, local actin rearrangements, respectively. Today, however, we know that this classification is not always as sharp between entry strategies of pathogens, and that bacteria can quite flexibly employ various entry pathways in different experimental systems that are not necessarily observed in their native target cells *in vivo*, which are usually much less accessible to experimental manipulation than established tissue culture models. Much work remains to be done in this area. Notwithstanding this, the virulence factors utilized and their molecular mechanisms of functions established in simplified, *in vitro* systems remain correct, although their output effects may be quantitatively and qualitatively different in cells of differentiated tissue.

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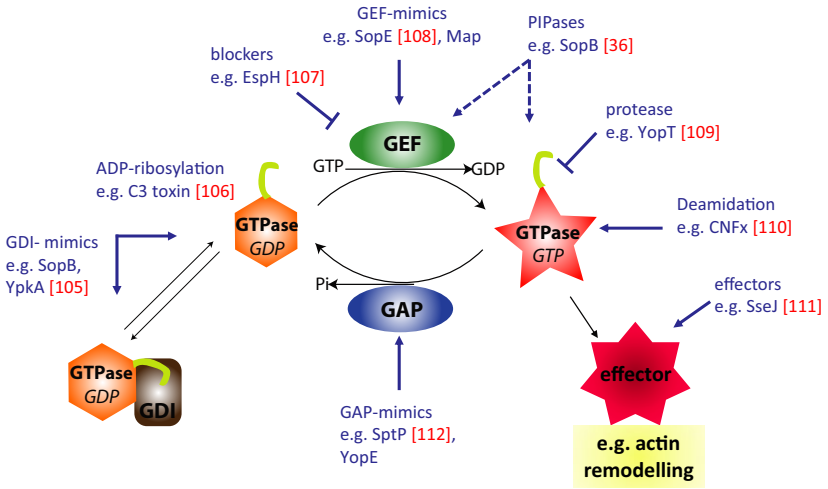

Fig. 3. The Rho-GTPase activation cycle and its manipulation by virulence factors. Rho GTPases cycle between an inactive GDP-bound state and an active GTP-bound state. So-called GEFs regulate their activation, whereas GAPs enhance their intrinsic hydrolase leading to inactivation. In the GTP-bound state, the GTPase binds to its downstream effectors. Finally, GDIs keep Rho GTPases in an inactive state and protect them from degradation. The small GTPase activation cycle is targeted by bacterial virulence factors at virtually every step. Virulence factors (in blue) are certainly not complete but just exemplary for entire families of factors and the identification of more virulence determinants and mechanisms is expected from future research. Targeting of these processes with small molecules might pave the way to novel pathoblockers or anticancer drugs.

For the trigger type of entry utilized for instance by *Shigella flexneri* or *Salmonella enterica* serovar Typhimurium, the pathogen transfers effector proteins into the host's cytoplasm (see T3SS below), inducing fierce, local actin polymerization, causing the plasma membrane to lift up and around the bacterium in order to envelop it. This is similar in appearance to the formation of phagocytic cups or large structures mediating macropinocytosis and engages virtually the same signaling and actin assemblies [34,35]. More recent research has uncovered, however, that pathogens can elicit many more and much more diverse responses in cells to induce their entry, engaging additional GTPases and actin-dependent mechanisms unrelated to those initially identified, such as Rho-mediated contractility [36] or SPIRE- and formin-induced actin polymerization [37,38].

The zipper mechanisms which are utilized, for example, by pathogenic *Yersinia and Listeria* species are initiated by bacterial surface proteins that serve as 'fake' ligands of host cell surface receptors. The receptor becomes activated and signals across the plasma membrane, which leads to highly localized actin polymerization events, reminiscent perhaps to those accompanying clathrin-mediated endocytosis of the receptor. In the case of *Listeria*, two such mechanisms operate in parallel: one receptor-ligand mimicry involves binding of bacterial Internalin A (InlA) to host E-cadherin [39]; the second mechanism concerns the c-MET receptor tyrosine kinase binding to InlB [40], triggering of which during invasion of HeLa cells is accompanied by clathrin recruitment, supporting the idea of pathogen-induced receptor endocytosis [41]. In contrast, *Yersinia* utilizes the cell adhesion machinery through binding to the transmembrane protein β1-integrin through the bacterial surface protein invasin [42].

Bacterial virulence factors and Rho GTPases

A common virulence feature of gram-negative gastrointestinal bacterial pathogens is the delivery of proteins directly into the host cell cytoplasm. The bacteria inject virulence factors, also known as effectors, via a syringe-like nanomachine named Type III secretion system (T3SS), evolutionary related to the flagellum. While T3SSs are conserved in composition and function among different species, each bacterium secretes an individual set of effectors [43] thought to serve establishment of the individual niche. For instance, *Salmonella* and *Shigella* species are intracellular pathogens that trigger their uptake into nonphagocytic gut epithelial cells [44]. Invasion into host cells of these bacteria depends on the activation of Rho GTPases by the concerted action of sets of T3 effectors that mediate prominent actin rearrangements resulting in engulfment of the bacteria [33]. Quite distinct from those, members of the Enteropathogenic *E. coli* (EPEC)/ Enterohemorrhagic *E. coli* (EHEC) group (also known

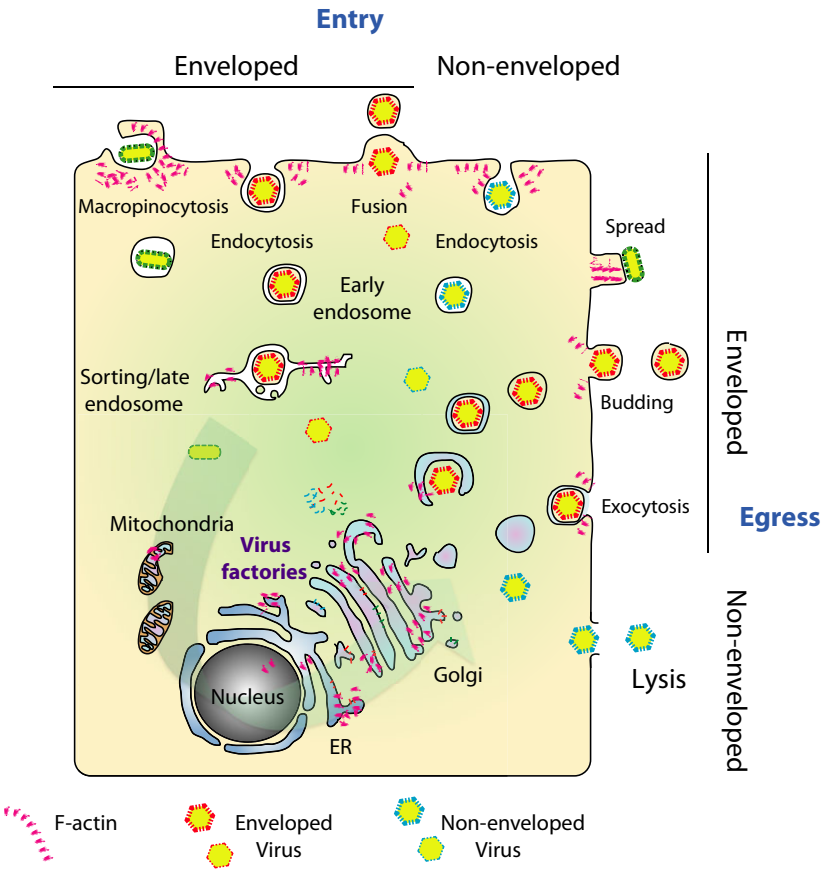

Fig. 4. Virus infections harness actin assembly at membranes at all stages. Schematic representation of cellular locations where virus infection and propagation engages membranes and actin dynamics. The figure focuses on entry (upper side) and egress (right side) and only hints at the multiple possibilities of where virus assembly can take place such as ER and Golgi compartments. Virtually, every type of membrane and actin assembly is utilized by one or the other virus. Hence, it is not surprising that even mitochondria [98] or inhospitable places like peroxisomes can be exploited for virus propagation. Therefore, the figure must remain superficial and just repeats common themes. For instance, the term 'endocytosis' stands for all types of endocytosis not only clathrin-mediated mechanisms.

as A/E lesion pathogens) are primarily extracellular, adhering to the surface of gut epithelial cells. Doing so, they induce loss of microvilli and induce formation of so-called actin-rich pedestals underneath their attachment points. These bacteria also deliver T3 effectors to manipulate the actin cytoskeleton [45].

In the last decade, work by Alto and colleagues was instrumental for the identification of a novel T3SS effector family, the WxxxE family of bacterial GEF mimics. Subsequent crystal structures revealed that WXXXE proteins in fact share the fold with Salmonella T3 effectors SopE/SopE2, also harboring GEF activity, and uncovered the elegant GEF mimicry mechanism [46–48].

In addition to these bacterial GEFs, also GAP and GDI mimics, or enzymes that modify GTPases for constitutive activation or inactivation exist, enabling manipulation of the host GTPase-signaling landscape at various levels. All these factors have been described in comprehensive reviews [30,49,50] and some representative examples are given in Fig. 2.

Activation of specific individual Rho GTPases and corresponding actin-generating machines engaged by these model pathogens were studied in detail over the past 20 years, but this has posed more questions than were answered. For instance, it is still in the dark how Rho is activated by the *Salmonella* phosphatidyl-phosphate phosphatase SopB [36], or why *Shigella* harbors bacterial GEFs for the functionally antagonistic host GTPases Rac1 and RhoA [48,51,52] or how it recognizes tricellulin upon host contact [53], just to name a few. While quite some biochemical details on individual, bacterial virulence factors are now established, their intricate interplay—as they come as a cocktail—and a more holistic understanding of their profound effects in the host is galaxies away.

Bacterial virulence factors and actin

The simplest mechanism of attacking the actin cytoskeleton is targeting it directly by modifying toxins, causing cross-linking of actin or ADP-ribosylation. These modifications either result in stimulation of actin polymerization or block it [reviewed in Ref. 54]. Bacterial virulence factors may also have modulatory functions such as actin bundling, as it was described for *Salmonella* SipA [55]. Molecular mimicry of actin regulatory factors can occur at all levels (also compare

Fig. 1): The *Listeria* surface protein ActA for instance mimics an NPF and recruits and activates Arp2/3 complex for actin tail formation. On the contrary, *Shigella* IcsA mimics an NPF-activating signal and releases autoinhibition of the host cell NPF N-WASP, which then recruits and activates Arp2/3 complex. These mechanisms lead to actin assembly at the bacterial surface in the cytoplasm followed host cell invasion. A further upstream type of mimicry is represented by Vaccinia Virus A36R or EPEC Tir, both of which mimic receptor tyrosine kinase (RTK) signaling through the plasma membrane [56]. This leads to the recruitment of the RTK-Adapters such as Nck, in turn igniting the N-WASP-Arp2/3 cascade and mediating actin tail formation at the plasma membrane abutting the pathogen upon clustering of the pathogenic receptor mimic. Alternative types of actin tail formation are exerted through bacterial actin nucleators like the Rickettsial protein Sca2 or the Burkholderial BimA, mimicking nucleation factors that generate long unbranched filaments with activities reminiscent of formins or Spire [57] or of the Ena/VASP family of actin polymerases [58]. Remarkably, in case of BimA, different *Burkholderia* species have evolved this protein to either operate as Ena/VASP mimic (*B. pseudomallei* and *mallei*) or Arp2/3 complex activator (*B. thailandensis*), which confirmed the versatility and flexibility of virulence factor evolution to serve the specific pathogen's need [59]. These and similar bacteria, residing and spreading inside host cells in an actin polymerization-dependent fashion, have to exit the phagosome in order to unfold these features. Others like *Salmonella* remain in the membrane cover, and instead mature and remodel it to establish it as their specific niche. It is intuitive that this type of membrane remodeling will again involve Rho GTPases and actin dynamics, but the exact contributions of specific host cell factors are still in the dark.

Actin and the viral life cycle

Viruses depend as obligatory intracellular parasites on multiple functions of their host cell. Thus, viral infections unsurprisingly alter the regular functions of a cell to support replication and production of new virions. A prime aspect of this conversion is profound reorganization of the actin cytoskeleton, accompanying most if not all stages of the viral life cycle, from entry through replication and assembly to egress (Fig. 4) [60]. One characteristic hallmark of viruses is their cellular and host tropism [61]. In the absence of virus-compatible host cells, they do not replicate at all. Two distinct subtypes of cellular viral tropism were described, namely receptor-dependent and -independent tropisms. This means that restriction of viral replication occurs either on the cell surface (receptor-dependent entry) or intracellularly (post-entry steps) through molecular incompatibilities. The state of differentiation of a given cell dictates its gene expression pattern, which in turn enables (or prohibits) viral infection and propagation. Interestingly, several viruses can transform cells, which can be seen as an active step to design their new homes for persistence. This process also profoundly changes host cell proliferation and motility, often leading to tumor formation and metastasis. However, these processes will not be discussed here because it mostly is not an immediate form of host–pathogen interaction [62,63]. Nevertheless, it is worth to consider that these viruses apparently prefer to reside in motile and proliferating cells.

Virus entry

In the first step of viral infection, virions engage the cell surface, subsequently penetrating the cell membrane and entering the cytoplasm.

Prior to internalization, many viruses show a cell-surface-surfing behavior, which is proposed to carry them from initial contact sites, for instance filopodial protrusions [64], to areas amendable for penetration into the cytoplasm, for example, sites with high-endocytic activity. This process was shown to depend on actin and myosin II motor activity and likely be driven by myosin II-dependent actin retrograde flow in these structures [65].

For subsequent internalization, the cortical actin meshwork is thought to embody a physical barrier that has to be overcome, which can be achieved by actin cytoskeleton remodeling [66]. Virions can ignite signaling and induce internalization of their hijacked receptor, taking a ride on, for example, clathrin- or caveolin-mediated endocytosis. Some virions utilize macropinocytosis or other clathrin-independent paths into the cell, all involving actin in one of the other way [reviewed in Refs 67,68]. Apparently, viruses have learned to hijack the full spectrum of endocytic mechanisms to gain access to the cells.

Moreover, enveloped viruses such as HIV, HRSV, or HSV [69–71] may also gain entry by directly fusing with the cell's plasma membrane, which involves action of Rho GTPases and actin in a way that is not fully understood. Future research may identify correlates of this process in nonpathogenic cell fusion processes of the host, as found, for example, in muscle cell precursors or inflammatory macrophages forming giant cells.

Finally, attachment of virions to host cells may promote uptake of additional virions by stimulating signals rendering the host more susceptible. Herpes simplex virus (HSV), as an example, induces the extension of cell surface protrusions spiked with more attachment sites for more virions [72,73].

Intracellular trafficking, replication, assembly, and egress of virions

Dynamic actin turnover was shown to have strong effects on some viruses during their propagation in the host [74–77]. However, we are just beginning to distinguish the relative contributions of actin dynamics to these steps, using for instance super-resolution video microscopy. On one hand, it is reasonable to assume that complex structures such as some virus factories in the cell center will strongly rely on intact actin dynamics to support rearrangements of ER and Golgi in response to virion production. However, there is much more to be explored in this phase of the viral life cycle: actin impacts on eukaryotic gene expression directly [78,79] and indirectly [80,81] and, in addition, contributes to chromatin organization through nuclear F-actin assemblies, but how precisely remains to be established [82]. Although these aspects of actin dynamics are incompletely understood and notoriously difficult to visualize, even less is known about usurpation of them by virions. Nevertheless, several indications for the participation of these cellular processes in virion production/assembly have emerged [83–88].

Lately, we have witnessed an explosion of knowledge on autophagy. Autophagy comes in various flavors in the cell, but is accompanied by distinct membrane remodeling events that all involve actin dynamics [89] mostly downstream of Arp2/3 complex-dependent and the corresponding NPFs WASH, WHAMM, and JMY [90–93]. Not surprisingly, therefore, this cellular process is also connected to the life cycle of various viruses. Although some have evolved to evade autophagy in the cell, others appear to have modified autophagy for their own benefit. However, the connection between actin dynamics, autophagy, and viral infection is still comparably vague and I would like to refer to two excellent recent reviews summarizing this emerging field [94,95]. Future research will have to define whether virions directly target actin dynamics during manipulation of the autophagic flux, or if this connection is indirect.

Next, budding and egress steps of viral pathogens again involve passing through the plasma membrane, which necessarily requires actin rearrangements. It is known, for instance, that some viruses including HIV induce actin-based protrusions/microvilli [96] and that actin depolymerization diminishes viral yield. Moreover, cell to cell spread of this virus involving the viral Env and GAG proteins is actin-dependent, and indeed, HIV-GAG directly interacts with F-actin [97].

Finally, virus spread may also be promoted by direct induction of actin structures. As a prominent example, vaccinia virus and other members of the poxvirus family are well known for inducing prominent actin structures below the plasma membrane following budding, again generating actin comet tails now considered important for efficient viral dissemination. Comparable structures are induced at the cell surface through signaling across the plasma membrane by pathogenic *Escherichia coli*, for instance of the EPEC or EHEC type (see above and Ref. [31]). Although certainly more static than Vaccinia virus tails (see above) and thus specifically called actin pedestals, these structures are believed to mediate translocation of the bacteria along the plasma membrane and perhaps onto neighboring cells. This emphasizes how the same pathways and machineries can lead to distinct output responses, which must depend on the overall molecular inventory of host cell proteins regulating these pathogen-induced actin structures.

Together, due to the intimate contact and obligate dependence of the virus on the host cell equipment, coevolution has shaped a multitude of strategies that all either directly utilize manipulation of actin (dis-)assembly or at least take into account that the targeted membrane is under control of actin dynamics. Future work needs to dissect the differential contribution of signaling and actin assembly factors to the steps of individual viral life cycles (Fig. 4).

Concluding remarks

All intracellular and even some extracellular pathogens subvert the host cell cytoskeleton to promote their own survival, replication, and dissemination. A study of these microbes has led to important discoveries concerning not only the specific infection mechanism at play but also regarding the specific function of cytoskeletal regulatory pathways and cellular mechanisms. Importantly, the cellular pathways involved may harbor attractive therapeutic targets to fight such infections. However, to reach this goal, much work is required to tease apart 'bystanders', recruitment of which just accompanies these processes, from 'drivers', directly utilized by the pathogen, which might embody promising targets. Aim of such approaches is not necessarily to kill the microbe, which would pose a selection pressure to developing further resistances, but to tune

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down the dynamics of a given infection allowing the host to eradicate the intruder by itself. Novel systematic analyses, including systems biology level comprehension of these processes and molecular biology down to atomic resolution, are required to enlighten the delicate interaction processes between pathogen and host.

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Author contributions

TS drafted the manuscript and drew the figures. TS and MS wrote the manuscript.

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