Actin: Structure, Function, Dynamics, and Interactions with Bacterial Toxins

Sonja Kühn and Hans Georg Mannherz

Abstract Actin is one of the most abundant proteins in any eukaryotic cell and an indispensable component of the cytoskeleton. In mammalian organisms, six highly conserved actin isoforms can be distinguished, which differ by only a few amino acids. In non-muscle cells, actin polymerizes into actin filaments that form actin structures essential for cell shape stabilization, and participates in a number of motile activities like intracellular vesicle transport, cytokinesis, and also cell locomotion. Here, we describe the structure of monomeric and polymeric actin, the polymerization kinetics, and its regulation by actin-binding proteins. Probably due to its conserved nature and abundance, actin and its regulating factors have emerged as prefered targets of bacterial toxins and effectors, which subvert the host actin cytoskeleton to serve bacterial needs.

Contents

S. Kühn
Department of Cell Biology and Infection, Institut Pasteur, Paris, France

H.G. Mannherz (✉)
Department of Anatomy and Molecular Embryology, Ruhr-University, Bochum, Germany
e-mail: hans.g.mannherz@rub.de

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1 Introduction

After host infection, bacteria invade non-phagocytic cells to secure their survival and multiplication. At the same time, they have to evade or block phagocytosis and destruction by professional phagocytes like polymorphonuclear cells (neutrophiles) and macrophages. Both, uptake by non-phagocytic host cells and phagocytosis by macrophages depend on plasma membrane extensions driven by rearrangements of the host actin cytoskeleton. Bacteria have devised a large arsenal of toxins to subvert cellular actin structures for their purposes by attacking actin directly or its manifold regulatory partners. Therefore, a more detailed knowledge of actin and its modes of regulation is essential to fully appreciate the many tricks bacteria have developed to hijack the actin cytoskeleton. Not surprisingly, the study of bacterial toxin actions was in many cases instrumental to deeper understand the fundamental, molecular mechanisms that drive the reorganization of dynamic cellular actin structures (see also Haglund and Welch 2011).

Actin is one of the most ubiquitous proteins in nature. Besides its abundant presence in all types of muscle cells and its participation in muscle contraction, it is present in almost any non-muscle eukaryotic cell (ranging from yeast to mammals) and in most cases in high concentration. It exists also in plant cells, where it fulfils similar functions like in metazoan cells. Furthermore, actin-analog proteins even exist in many bacteria where they may fulfil also cytoskeletal functions (see Gayathri 2016).

The actin protein was first isolated by Bruno Straub in 1943 working in the Laboratory of Albert Szent-Györgyi of the Department of Biochemistry at the University of Szeged (Hungary). Straub was credited for the isolation of actin from skeletal muscle only after the end of the World War II, since his results were initially published in a largely unknown journal edited by the University of Szeged (Straub 1942, 1943; see also Schoenenberger et al. 2011). The existence of two components necessary for muscle contraction had been implicated a century earlier by the work of the physiologists Wilhelm Friedrich Kühne (1837–1900) (University of Heidelberg, Germany), who first isolated a contractile extract from frog muscle—most probably actomyosin (Kühne 1859), and by William Dobson Halliburton (1860–1931) (Kings College, University of London, UK) (Halliburton 1887).

Today, we know much more about actin. In muscle tissues, actin containing thin filaments interdigitate with myosin containing thick filaments. Both types of filaments slide past each other during muscle contraction (Huxley and Niedergerke 1954; Huxley and Hanson 1954). The power for the sliding movement is generated by the head regions of the myosin motor molecule, which possess ATPase activity and the ability to cyclically interact with actin molecules of the thin filaments. The cyclical interaction of myosin motor domains with actin is linked to different steps of ATP binding and ATP hydrolysis. Thus, the chemical energy stored in the β-γ-phosphoanhydride bond of ATP is transformed into mechanical work by the interaction of the motor protein myosin with actin. Similar to muscle contractility, actin in non-muscle cells participates in many motile events like cell locomotion, intracellular transport processes like vesicular movements during exo- or endocytosis, phagocytosis, and cytokinesis, the final stage of mitosis. These motile processes often depend on the interaction of actin with specific myosin variants, but a number of essential motile events are executed also by mere polymerization and depolymerization of actin itself.

In addition, actin-containing filaments are essential for the structural and functional integrity of cells. Maintenance of cell polarity and the formation and stability of surface extensions like lamellipodia, microvilli, or filopodia critically depend on the local architectural stability of networks or bundles of actin filaments.

2 Actin

The actin protein is composed of a single polypeptide chain of 375 amino acid residues (skeletal muscle actin) with a molecular mass of 42 kDa, whose sequence was determined by Elzinga and coworkers (1973). Its amino acid sequence is highly conserved between different organisms, and the actin protein occurs abundantly in eukaryotic cells. Mammals express 6 different actin isoforms encoded by different genes. The actin isoforms are distributed in a tissue-specific manner and classified according to their isoelectric points: the most acidic isoforms being the three α-actins (one specific isoform expressed in skeletal, cardiac, and vascular smooth muscle), the β-actin in contractile structures like the so-called stress fibres (also termed cytoplasmic actin in non-muscle cells), and two γ-actins (one cytoplasmic actin in non-muscle cells and one enteric smooth muscle form) (Rubenstein 1990). Both cytoplasmic actin isoforms are ubiquitously expressed. These different mammalian actin isoforms vary only slightly in their amino acid sequences (Vandekerckhove and Weber 1978). The main differences were observed at their negatively charged N-terminus, whose composition and length vary in an isoform-specific manner.

All mammalian actins exist intracellularly in two main states of organization: the monomeric, globular G-actin or the polymerized, filamentous form (F-actin).

F-actin is physiologically the more relevant form, since it is only F-actin that is able to stimulate the myosin-ATPase activity, which provides the energy necessary for the performance of the cyclical force-producing interactions with myosin motor domains during muscle contraction or other cellular motile events. In addition, non-muscle F-actins often organize into higher-ordered supramolecular structures like stress fibres or bundles present in plasma membrane extension like microvilli or filopodia. Thus, besides its participation in motile events, actin filaments fulfil cytoskeletal functions like stabilizing cell form or specialized membrane extensions.

For these many diverse functions, actin has to be able to specifically interact with a large number of actin-binding proteins. Actin is one of the evolutionarily most conserved proteins, most probably due to its "promiscuous" nature. Actin is designed to interact with a large number (more than 160) of different actin-binding proteins, which regulate its spatial and temporal polymerization to actin filaments and their supramolecular organizations into bundles or networks.

2.1 Actin Structure

The three-dimensional (3D) structure of actin was solved to high-resolution by X-ray crystallography (Kabsch et al. 1985, 1990). Because increasing the ionic strength induces actin polymerization, it was found impossible to obtain crystals of monomeric actin suitable for X-ray analysis. Therefore, binary complexes of actin with an actin-binding protein (ABP) stabilizing it in the monomeric form were employed for crystallization. The 1:1 complex of skeletal muscle α-actin with deoxyribonuclease I (DNase I) was the first complex whose 3D structure was solved (Kabsch et al. 1985, 1990). Subsequently, complexes of skeletal muscle α-actin with gelsolin G1 (McLaughlin et al. 1993) and profilin in complex with cytoplasmic β-actin (Schutt et al. 1993) were determined. Meanwhile, about 80 3D structures of actin in complex with a number of different ABPs or small molecules have been determined, which all confirmed its basic 3D structure (see Dominguez and Holmes 2011).

Actin is a rather flat molecule with dimensions of about 5.5 × 5.5 × 3.5 nm (Fig. 1a–c). The molecule is divided into two main lobes of about equal sizes separated by a deep upper cleft whose bottom contains the nucleotide (ATP or ADP) and divalent cation-binding sites (Fig. 1a). A smaller incision is seen at its lower side, which represents the main target area for binding of many ABPs (see later). The two main lobes are connected by a small bridge with the peptide chain crossing twice between the two main lobes. This connection may function as a hinge allowing rotations of the two main lobes relative to each other during G- to F-actin transitions (Oda et al. 2009). Each main lobe is subdivided into two clearly discernible subdomains (SD1–4, see Fig. 1a), which are composed of a central ß-pleated sheet and surrounded by α-helices linked by loops of varying lengths. SD1 and 3 have a similar architecture built from a five-stranded ß-pleated sheet, whereas

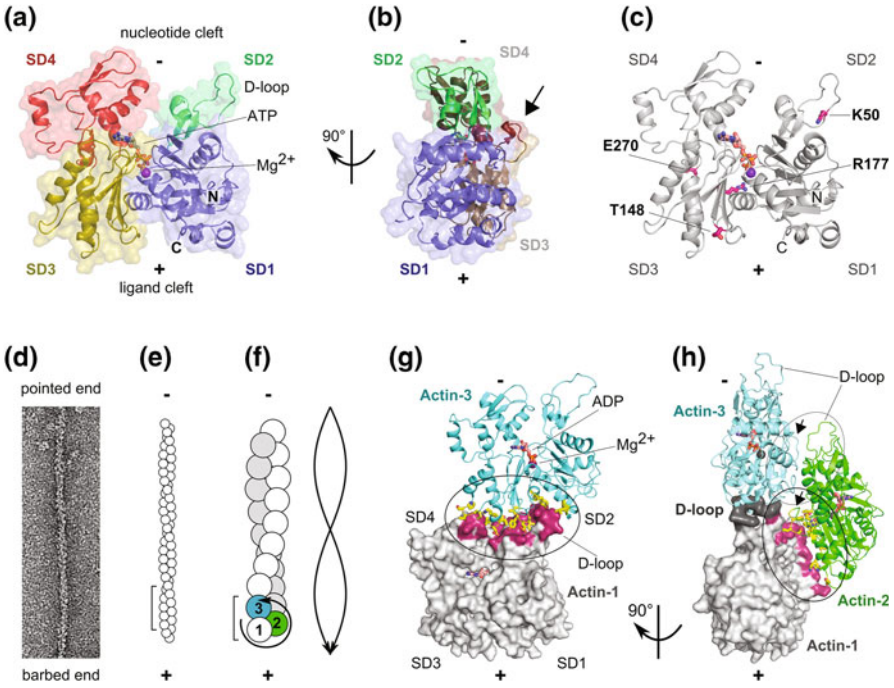

Fig. 1 Structure of G- and F-actin. (**a**–**c**) G-actin structure shown as ribbon with semitransparent surface (**a**–**b**), the ATP nucleotide as stick representation and bound Mg^{2+} cation as sphere. The actin subdomains (SD), pointed (−) and barbed (+) faces are indicated. Front (**a**) and side (**b**) view of G-actin with its different coloured four subdomains (SD1–SD4). Note the two large domains (*blue* SD1 and *red* SD4) and bound ATP at the bottom of the deep nucleotide binding cleft between SD2 and SD4. The hydrophobic ligand-binding cleft that enables interaction of most actin-binding proteins (ABPs) with actin is located on the opposite between SD1 and SD3. The DNase I-binding loop (D-loop) is mainly involved in maintaining important intrastrand F-actin contacts (see **g**–**h**). The *arrow* indicates the hydrophobic plug that forms interstrand contacts (see **h**) (PDB: 1ATN). **c** Sites of direct actin modifications of bacterial toxins. Modified amino acids are indicated in *pink stick* presentation. Arg177 of actin (R177) is ADP-ribosylated by binary toxins like C2 or Iota, while Thr148 (T148) is ADP-ribosylated by the TccC3 toxin. Cross-linking of two actin molecules by bacterial toxins of the MARTX family occurs between Lys50 (K50) and Glu270 (E270). **d**–**h** F-actin subunit organization. **d** shows an electron microscopic image of a single actin filament with depicted pointed (−) and barbed (+) ends, (**e**) the arrangement of the actin subunits within the filament and (**f**) their helical organization (see text). The *bracket* in (**e**) corresponds to the displayed detail in (**f**), while the *bracket* and F-actin protomer numbering at the filament barbed end in (**f**) belongs to the actin dimer and trimer in (**g**–**h**). **g**–**h** Electrostatic and hydrophobic interactions of actin protomers within the actin filament. The contacts are formed between three surfaces: site (I) (actin-1:actin-3), (II) (actin-1:actin-2), and (III) (actin-2:actin-3). Binding sites (II) and (III) are identical. **g** Intrastrand contacts of site I (*circle*) between actin molecules 1 (actin-1, *grey*, as surface) and 3 (actin-3, *blue*, as ribbon) of the long-pitch dimer. Resides of actin-3 (SD1 and SD3) involved in the interaction with actin-1 (SD2 and SD4) are presented as sticks (*yellow*), while the actin-3-binding surface on actin-1 is coloured in *pink*. **h** Interstrand contacts of site II (*black circle*) between actin-1 (*grey*, surface) and actin-2 (*green*, ribbon) of the lateral dimer and of site III (*grey circle*). The hydrophobic plug (see **b**) connects all three actin protomers at the interior of the actin filament and is highlighted (*arrow*). The D-loop of actin-1 (*dark grey*) forms hydrophobic and electrostatic contacts with actin-3 (site I) , while adjacent residues in SD2 of actin-1 are involved in interstrand contacts with actin-2 (site II). Residues of actin-2 involved in the interaction with actin-1 are presented as sticks (yellow), while the actin-2-binding surface on actin-1 is colored in pink

SD2 and SD4 differ in their size and 3D structure. The N- and C-termini are located in SD1. Therefore, SD1 is built from residues 1–32, 70–144, and 338–375, SD2 from residues 33–69, SD3 from residues 145–180 and 270–337, and SD4 from residues 181–269 (Fig. 1a–b).

This basic actin fold was also found in the so-called actin-related proteins (the Arp proteins), which considerably differ in their sequence but are specifically enriched in cell nuclei or present in the cytoplasm of many eukaryotic cells, like within the Arp2/3 complex. In addition, many of the prokaryotic actin-like proteins like MreB and ParM share a high structural homology to actin in spite of high sequence divergences (see Gayathri 2016). Surprisingly, a number of proteins with completely different sequences and functions like hexokinase and the heat-shock protein HSP70 possess also a high structural similarity to actin probably due to a common architecture of their ATP-binding sites (Flaherty et al. 1991).

2.2 *Binding Sites on Actin for Actin-Binding Proteins*

Subdomain 1 appears to be the main binding site for myosin motor heads (see Geeves and Holmes 1999; Behrmann et al. 2012). From SD2 extents a loop that in many solved structures appeared unstructured, but forms the main binding site for DNase I and therefore was named the DNase-binding (or D-) loop (Kabsch et al. 1990). The D-loop is also involved in actin-actin contacts along the long-pitch strand (see below). The small incision at the base between SD1 and SD3 forms an important target area for a large number of actin-binding proteins like gelsolin segment 1 (G1), profilin, cofilin, and thymosin beta 4, which binds to actin with its so-called WH2 domain. WH2 domains are present in a large number of other actin-binding proteins enabling similar interactions with this region of actin (see Dominguez and Holmes 2011).

2.3 *Filamentous (F-) Actin*

The physiologically active form of actin is F-actin. In the test tube, actin can be maintained in monomeric (G-) state only at low-salt conditions of mono- and divalent cations. When raising the ionic strength by addition of cations (KCl to 100 mM and/or to 2 mM $MgCl_2$, in other words to about the intracellular ionic concentrations), actin polymerizes to form filamentous (F-) actin.

Normally, monomeric actin contains firmly bound one molecule of ATP, which is essential for the maintenance of its native configuration. Nucleotide-free actin denaturates rapidly and irreversibly. After incorporation into a growing filament,

the actin-bound ATP is quickly hydrolysed into ADP and inorganic phosphate (Pi). While the ADP nucleotide remains bound to the actin molecule, the Pi is slowly released ($t_{1/2}$ = 6 min).

The actin filament (Fig. 1d–f) has a diameter of about 8.0 to 10.0 nm with the larger domain being in the centre of the filament axis. Cellular actin filaments can be composed of about 1000 actin monomers and attain a length of 1 µm like the thin filaments of skeletal muscles. F-actin can be described as a left-handed two-start long-pitch helix (half pitch rise 380 nm) or as a right-handed generic helix with an inter-subunit raise of 27.5 nm and a 166° rotation angle (Fig. 1f). Since the actins in both strands have the same orientation, the filament ends expose different surfaces of the actin molecule. The contact sites of a single actin subunit with its neighbouring subunits are shown in Fig. 1g–h.

Because F-actin cannot be crystallized, numerous attempts using fibre diffraction procedures or electron microscopy of parallel aligned F-actin (F-actin paracrystals) have been undertaken to elucidate the atomic structure of F-actin at high resolution. Recently, the advancement of cryoelectron microscopy has provided the necessary resolution to define the conformational changes G-actin undergoes when incorporated into F-actin. This structural transition is characterized by a 20° tilt of the two main domains relative to each other resulting in a flatter actin conformation, which appears to be essential for filament incorporation (Oda et al. 2009). Recent data of about 3.7 Å resolution show more clearly the interfilament actin-actin contacts (von der Ecken et al. 2014).

Within the filament, each actin subunit contacts four neighbouring actins: two longitudinally related actins along the long-pitch strand and two lateral subunits of the neighbouring strand. The contacts between actin subunits along the long-pitch strands are more numerous and apparently stronger than the interstrand contacts. The longitudinal contacts are formed between subdomains 2 and 4 of each subunit with subdomain 3 of the respective upper subunit (Fig. 1g, h). This contact area contains a number of electrostatic and hydrophobic interactions (highlighted in Fig. 1g, h). The residues involved are 166–169 (SD 3) to 41–45 (SD2), residues 286–289 (SD3) to 202–204 (SD4), and residues to 322–325 (SD3) to 243–245 (SD4) of the upper to the lower subunit (see Fig. 1g, h; for review see Mannherz 1992). A major hydrophobic contribution is provided by the D-loop (SD2) with the lower surface of the ß-pleated sheet of SD3 of the actin subunit above, where it contacts residues around Tyr169 (von der Ecken et al. 2014).

The main interstrand contact is formed by a loop (the so-called hydrophobic plug, Holmes et al. 1990; see also Dominguez and Holmes 2011) that extends into the middle of the filament from SD3 to SD4 of each actin subunit and contacts a pocket formed by the interface of two adjacent actin subunits of the opposing strand (arrows in Fig. 1b, h). This loop is formed by residues extending from Pro264 to Ser271, but contrary to its original denomination forms salt bridges between the three actins (Fujii et al. 2010). It contacts residues 40–45 and 63–65 (SD2 of the lower opposing subunit) and residues from 166 to 171 and 285 to 289 (SD3 of the opposing upper subunit) (reviewed in Mannherz 1992; von der Ecken et al. 2014).

2.4 Actin Dynamics: Polymerization Behaviour

Actin proteins in living cells are in a tightly regulated, dynamic equilibrium between the G- and F-actin states. The spontaneous assembly of monomeric to filamentous actin is a two-step polarized condensation process, which depends on an initial nucleation phase followed by a rapid elongation process until the steady state is reached. During the nucleation phase, an actin oligomer consisting of three or four G-actin subunits has to be assembled for elongation to occur (Fig. 2). The formation of actin nuclei represents the rate-limiting step. It is kinetically highly unfavoured, since the net negative charge at physiological pH hinders the formation of actin dimers ($k_+ = 10\ \mu M^{-1}\ s^{-1}$, $k_- = 10^6\ s^{-1}$ giving a K_d of 100 mM). A more stable nucleus is formed only after binding of a third actin protomer ($k_+ = 10\ \mu M^{-1}\ s^{-1}$, $k_- = 10^3\ s^{-1}$ giving a K_d of 0.1 mM), or requires even the stabilizing effect of binding to a fourth one. Further actin monomers assemble at both sides of this actin nucleus during early filament elongation (Wegner and Engel 1975; Gilbert and Frieden 1983; Tobacman and Korn 1983; Sept and McCammon 2001).

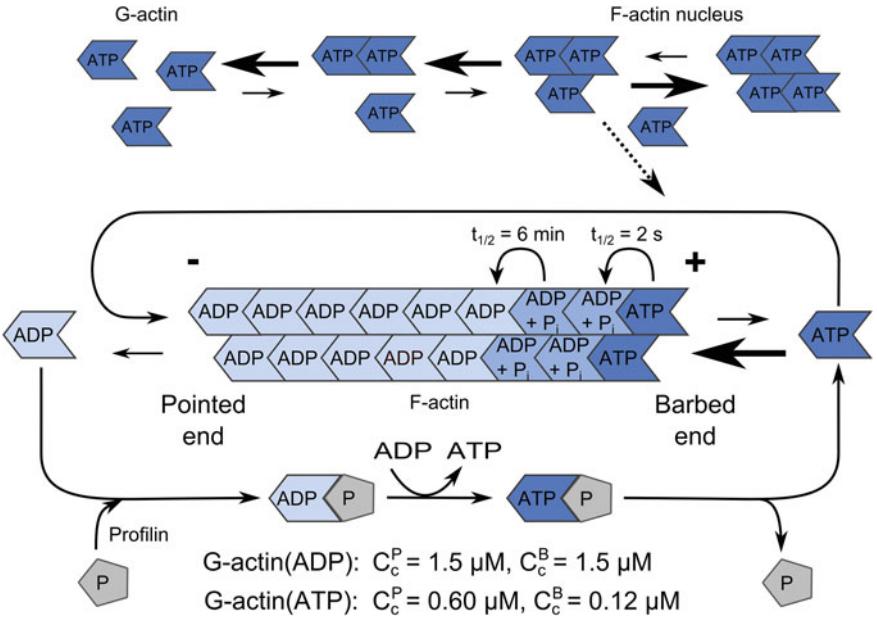

Fig. 2 The nucleation and polymerization (treadmilling) process of the G- to F-actin transition. The *upper row* illustrates the formation of nuclei (dimer to tetramer) from ATP-containing actin molecules and the cyclic scheme the treadmilling process of F-actin with barbed end (+) ATP-containing actin subunits and pointed end (−) ADP-containing subunits. The critical concentrations for ATP- or ADP-actin addition to the pointed (P) or barbed (B) end are shown (for detail, see text). $t_{1/2}$ describes the half time for ATP hydrolysis or for P_i dissociation from the actin protomers after ATP hydrolysis

Due to the structural polarity of F-actin, two filament ends with different exposed surfaces and with distinguishable elongation rates are generated: the fast-growing plus or barbed end (+) and the slow-growing minus or pointed end (−) (Fig. 2). With the different rates of actin monomer addition and dissociation, the barbed end of F-actin elongates up to ten times faster than the pointed end (rate constants (+)-end: $k_+ = 12\ \mu M^{-1}\ s^{-1}$, $k_- = 1.4\ s^{-1}$; (−)-end: $k_+ = 1.3\ \mu M^{-1}\ s^{-1}$, $k_- = 0.3\ s^{-1}$) (Pollard et al. 2000). During F-actin growth, the concentration of soluble actin monomers decreases, until at steady state G-actin molecules exchange with the filament ends without increasing the total F-actin amount. The equilibrium concentration of the monomeric G-actin pool at steady state is termed the critical concentration C_c. Monomeric actin polymerizes at G-actin concentrations above C_c, while lower values lead to F-actin depolymerization. The C_c values for ATP-G-actin differ at both filament ends, and the C_c is much lower at the barbed end ($C_c^+ = 0.12\ \mu M$ vs. $C_c^- = 0.60\ \mu M$). Monomeric actin polymerizes at the barbed end at G-actin concentrations above C_c^+ and below C_c^-, while the pointed end depolymerizes. This process is termed treadmilling and describes the presence of equal net rates of F-actin barbed end assembly and pointed end disassembly after reaching the steady-state G-actin concentration of 0.12 μM. During the treadmilling cycle, the overall length of the actin filament remains constant (Kirschner 1980; Wegner 1976, 1982; Bonder et al. 1983; Bugyi and Carlier 2010).

In filamentous actin, the actin-bound ATP nucleotide is irreversibly hydrolysed to ADP and inorganic phosphate ($0.3\ s^{-1}$) followed by the slow release of the phosphate ($0.002\ s^{-1}$) (Pollard and Weeds 1984; Carlier et al. 1984; Korn et al. 1987; Pollard et al. 2000). The ATP hydrolysis rate of actin is thereby slower compared to the assembly kinetics at the barbed end (see Fig. 2). This leads to an F-actin barbed end with terminal actin protomers enriched in ATP or ADP + Pi, while the remaining filament is in the ADP-bound state. Thus, actin polymerization is an energy-consuming process that results in two ends that differ with regard to their structure and kinetics, but also with their energy charge.

Notably, the intrinsic treadmilling rate of actin and thereby the net flux of actin protomers from the filament's barbed end to the pointed end is very slow. The fast, polarized assembly of actin filaments found in living cells is solely achieved by the interaction of actin with actin-binding proteins (ABPs) which tightly regulate the treadmilling process (see Sect. 3).

3 Interactions with Actin-Binding Proteins (ABPs)

A large number of about 160 different actin-binding proteins (ABPs) have been identified so far. Almost all actin-binding proteins either control cellular actin assembly dynamics or maintain the supramolecular F-actin organization and its connection to other cellular components (see Fig. 3). There are only few examples known where G-actin influences the enzymatic activity of another protein: first, the inhibition of DNase I activity (Mannherz et al. 1980). DNase I is an extracellular or serum protein, and its chromatin-degrading activity will harm any cell when gaining

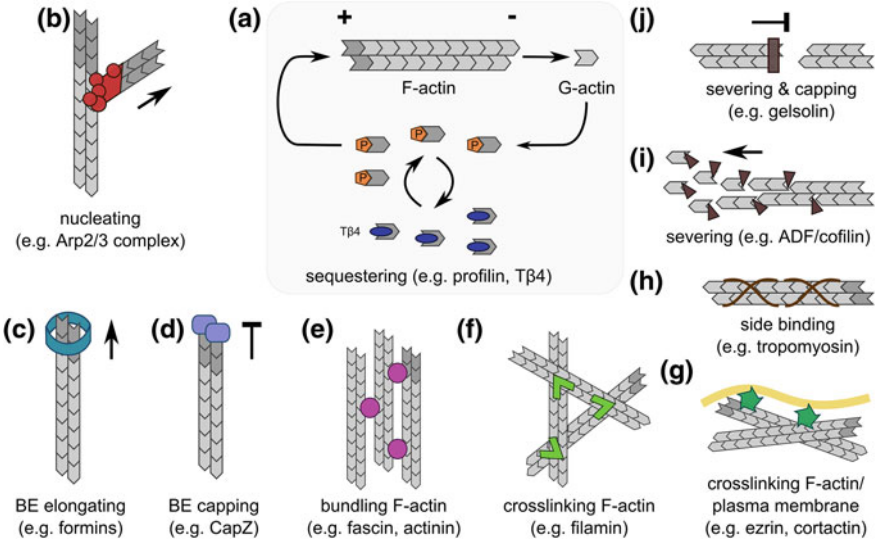

Fig. 3 The effect of the diverse classes of actin-binding proteins (ABPs) on actin dynamics. **a** F-actin in equilibrium with G-actin that can be sequestered by binding to profilin (P) or thymosin beta 4 (Tß4). **b–j** The effects of different ABP classes on F-actin are illustrated (for details see text)

access to the nucleus. DNase I inhibition by G-actin appears to be a safeguard mechanism to prevent chromatin degradation when DNase I inadvertently diffuses into cells with damaged plasma membranes (Eulitz and Mannherz 2007). Secondly, the kinase activity of a bacterial toxin (Yersinia YopO protein; see later) is stimulated by G-actin binding (Trasak et al. 2007; Lee et al. 2015; see also Aepfelbacher and Wolters 2016). Furthermore, binding of G-actin within the cytoplasm to MAL (or MRTF: myocardin-related transcription factor) negatively regulates the transcriptional activity of the serum response factor (SRF) by preventing the translocation of MAL into the nucleus. Only dissociation of the G-actin-MAL complex liberates MAL to diffuse into the cell nucleus and to stimulate SRF transcriptional activity leading to the expression of SRF-dependent genes (Olson and Nordheim 2010).

Apart from these examples, most ABPs that influence cellular actin dynamics either induce the formation of F-actin nuclei to overcome the energetic barrier of F-actin nuclei formation, or regulate the polymerization behaviour of F-actin. The latter is achieved either by increasing the rate-limiting step of the treadmilling cycle (e.g. severing and pointed end depolymerization), by manipulating the F-actin assembly dynamics by increasing the barbed end elongation rate or preventing the assembly of additional G-actin protomers, or by destabilizing F-actin by side binding and severing. In cells, the supramolecular organization of actin is maintained by cross-linking and bundling actin filaments and by connecting actin filaments with other cytoskeletal filaments or cellular membranes (Fig. 3). Most actin regulators share redundant functions with others and can be grouped into different classes according to their general activity (Fig. 3). The main groups are briefly

introduced below. Of note, many of these proteins are hijacked by bacterial pathogens to induce the pathogen-required temporal and spatial regulation of the host actin regulatory machinery.

3.1 G-actin-Sequestering Proteins

The maintenance of a cellular, monomeric actin pool is of importance, because the elongation rate of barbed ends depends on the availability of ATP-G-actin. This cellular G-actin pool is bound in 1:1 complexes to sequestering proteins like the abundant G-actin-binding proteins profilin and β-thymosins (main component: thymosin beta 4 or Tβ4) that prevent spontaneous F-actin nucleation (Rosenblatt et al. 1995; Fig. 3a). This sequestered G-actin pool is in rapid equilibrium with monomeric, unsequestered actin but not with the filamentous one. β-Thymosins form 1:1 complexes with preferably ATP-G-actin, which are by themselves polymerization resistant and thereby responsible for the maintenance of the high intracellular pool of unpolymerized actin. Since the affinity of β-thymosins to actin is only in the micromolar range (K_d = 1–5 μM), the actin in this complex is in rapid equilibrium with free and assembly-competent G-actin. Only the often high intracellular concentration of β-thymosins (up to 500 μM in non-muscle cells) can establish an appreciable amount of sequestered actin (see Fechheimer and Zigmond 1993; Mannherz and Hannappel 2009).

Profilin enhances the ADP exchange rate of G-actin leading to sequestered profilin:ATP-G-actin complexes. Like many other actin-binding proteins, profilin interacts with the ligand-binding cleft of actin at the edge of subdomains I and III (for review, see Dominguez and Holmes 2011). As a consequence, profilin does not participate in pointed end assembly and enhances thereby the processivity of treadmilling. Additionally, profilin effectively competes with β-thymosins for ATP-G-actin binding due to its higher binding affinity for actin. In contrast to Tβ4, profilin-actin is able to associate specifically with the barbed end of F-actin to participate in its elongation. Profilin-bound actin therefore represents the main cellular source of actin for polymerization and serves as a kind of carrier between the non-available Tβ4-actin pool and free F-actin barbed ends. Interestingly, some bacterial actin modifications specifically interfere with actin binding to Tβ4, but not to profilin (see Sect. 4.1).

3.2 F-actin-Nucleating Proteins and Their Nucleation-Promoting Factors (NPFs)

Proteins that initiate the polymerization of actin filaments by stabilizing actin nuclei involve the Arp2/3 complex and its nucleation-promoting factors (NPFs), multiple WH2 domain-containing proteins, and some formin family members (Fig. 3b, c).

The main cellular F-actin-nucleating machinery represents the Arp2/3 complex that initiates branched actin filaments at the sides of pre-existing mother filaments (Fig. 3c) and that is involved in the formation of the branched actin meshwork in membrane ruffles and lamellipodia (Mullins et al. 1998a, b; Amann and Pollard 2001; Lai et al. 2008). Nevertheless, the activation of the Arp2/3 complex by additional nucleation-promoting factors (NPFs) is necessary for efficient F-actin nucleation activity. NPFs are WH2 domain-containing proteins and provide an actin monomer for the formation of an actin trimer with the two actin-related proteins of the Arp2/3 complex (Higgs and Pollard 1999; und 2001; Robinson et al. 2001; Goley and Welch 2006). The NPFs WASP, N-WASP, and the WAVE complex are mainly involved in actin remodelling at the plasma membrane and often manipulated by invading pathogens (Pollard and Borisy 2003; Veltman and Insall 2010). Some formins have been described to possess F-actin nucleation activities. This class of autoinhibited nucleators is activated by GTP-binding proteins of the Rho family, which thereby links the organization of the intracellular actin cytoskeleton to extracellular signals.

3.3 F-actin-Elongating Proteins

This group of ABPs comprises Ena/VASP proteins and formins that both processively elongate F-actin barbed ends and promote the dissociation of barbed end assembly antagonists (Fig. 3c). Besides their nucleation activity, dimeric formins are prominent barbed end trackers that support the fast formation of long straight filaments and compete with capping proteins for barbed end binding (Pruyne et al. 2002; Paul and Pollard 2009; Shekhar et al. 2015; Bombardier et al. 2015). Formins bind profilin-G-actin with their FH1 domain and F-actin barbed ends with their ring-shaped FH2 domain that processively incorporates new actin monomers into the filament (Goode and Eck 2007; Kühn and Geyer 2014). Tetrameric Ena/VASP proteins contain WH2 domains and interact with G- and F-actin to add profilin-bound actin monomers to the bound filament barbed end (Bachmann et al. 1999; Applewhite et al. 2007; Breitsprecher et al. 2008; Ferron et al. 2007; Bear and Gertler 2009).

3.4 F-actin-Capping Proteins

The large and diverse group of capping proteins bind with high affinity to the ends of actin filaments and prevent further subunit association or dissociation (Fig. 3d). They vary in their abundance, domain composition, and F-actin-binding affinity. Most capping proteins interact with F-actin barbed ends to block filament growth, like gelsolin or capping protein (CP) (Cooper and Sept 2008; Silacci et al. 2004). There are only a few pointed end capping proteins, like tropomodulin in muscle tissue.

3.5 F-actin-Bundling and Cross-linking Proteins

Existing actin filaments can form long straight bundles with the help of bundling proteins, whose length determines the distance between individual filaments inside an actin bundle (Fig. 3e). Villin and fimbrin fulfil this function in bundles within microvilli in epithelial cells, while fascin is involved in F-actin bundling in filopodia (Bretscher and Weber 1979; Edwards and Bryan 1995; Khurana and George 2008). Besides bundling, F-actin can be organized into large, cross-linked, and netlike actin webs by proteins of the filamin family (Fig. 3f; Razinia et al. 2012). Cross-linking does also occur to mediate the interaction of F-actin with cellular components like the plasma membrane as it has been described for members of the ezrin/radixin/moesin (ERM family; Niggli and Rossy 2008) and spectrin protein families (Broderick and Winder 2005) (Fig. 3g). The ERM proteins attach F-actin network to the cytoplasmic face of the plasma membrane by binding with their N-terminal FERM domain to integral membrane proteins. By their preferred binding to F-actin, these proteins stabilize also the filamentous form of actin. Spectrin molecules form with short actin filaments a network within the so-called terminal web of microvilli bearing epithelial cells or the membrane cytoskeleton of red blood cells.

3.6 F-actin-Stabilizing Proteins

The large group of tropomyosins encompasses elongated proteins built from an α-helical coiled-coil, which stabilize existing actin filaments by side binding along both long-pitch strands of F-actin (Fig. 3h; von der Ecken et al. 2014; Pittenger et al. 1994; Wang and Coluccio 2010).

3.7 F-actin-Severing Proteins

The diverse group of severing proteins consists of two main families, ADF/cofilin and gelsolin-like proteins, that are responsible for the sudden, rapid breakdown of straight or branched actin filaments (Fig. 3i; Andrianantoandro and Pollard 2006; Nag et al. 2013). They bind at least transiently to G-actin forming either 1:1 or 1:2 complexes and fragment F-actin in a Ca^{2+}-dependent (gelsolin) or Ca^{2+}-independent (ADF/cofilin) manner.

After severing actin filaments, proteins of the gelsolin family remain associated with the newly formed barbed end and prevent further monomer addition. Gelsolin proteins thereby act as capping proteins, while the uncapped pointed end rapidly disassembles (Fig. 3j; Silacci et al. 2004). Severing proteins also promote filament polymerization depending on the physiological status of the cell. Members of the

ADF (actin-depolymerizing factor)/cofilin protein family bind specifically to ADP-bound G- and F-actin and facilitate indirectly barbed end assembly. They act in synergy with barbed end capping proteins to cause an increase in the critical concentration of the pointed end (C_c^-) by enhancing its disassembly rate, which increases the monomeric ATP-actin concentration and stimulates spontaneous nucleation and faster barbed end polymerization of new actin filaments (Carlier et al. 1999; Tania et al. 2013). Thus, severing contributes to the turnover of actin filaments and may result in a net increase in the F-actin amount.

3.8 Regulation of the Activity and Localization of ABPs

Many ABPs are directly regulated downstream of key extra- and intracellular signalling cascades that control actin dynamics during cell migration, cytokinesis, exocytosis, and endocytosis (Pollard and Cooper 2009). The activity and spatial localization of these actin regulators depend on Ca^{2+} ion concentrations, phosphatidylinositol phosphate interactions, active Rho GTPases, phosphorylation by kinases, and their recruitment by membrane-bound scaffold proteins. As an example, the nucleation-promoting factors N-WASP and WAVE complex of the F-actin-nucleating Arp2/3 complex are effectors of the Rho GTPases Cdc42 and Rac1, respectively. In addition, they become activated and recruited by kinase phosphorylation (e.g. Abl and ERK2), phospholipid binding at the plasma membrane ($PI(4,5)P_2$ and PIP_3, respectively), and interaction with membrane-bound adapter proteins like IRSp53 and Nck. Rho GTPases themselves are activated by extracellular stimuli and intracellular signal cascades that involve tyrosine phosphorylation, changes in the lipid composition of membranes, and scaffold proteins. Of note, Rho proteins themselves represent targets of bacterial toxins (see Lemichez 2016). Severing proteins of the gelsolin family are regulated by Ca^{2+} ion levels and phosphorylation, while their activity is inhibited by the phospholipid $PI(4,5)P_2$.

4 Examples of Bacterial Proteins that Subvert the Host Actin Cytoskeleton

In contrast to the above described eukaryotic actin-binding proteins, bacteria have developed actin cytoskeleton regulators, which either directly mimic and functionally override some of the ABP functions, or covalently modify actin in order to functionally disturb the actin cytoskeleton. The following paragraphs try to give a brief introduction into the considerable diversity of the strategies that bacterial pathogens have developed to attack or to survive in host eukaryotic cells (as summarized also in Tables 1, 2 and 3).

Table 1 G-actin modifying bacterial toxins

Bacterium	Toxin	Actin modification	Effect on F-actin cytoskeleton	Reference
Clostridium botulinum	C2	R177 ADP-ribosylation	Depolymerization	Aktories et al. (1986)
Clostridium perfringens	Iota	R177 ADP-ribosylation	Depolymerization	Stiles and Wilkins (1986), Simpson et al. (1987)
Clostridium difficile	CDT	R177 ADP-ribosylation	Depolymerization	Perelle et al. (1997)
Clostridium spiroforme	CST	R177 ADP-ribosylation	Depolymerization	Popoff and Boquet (1988), Simpson et al. (1989)
Bacillus cereus	VIP	R177 ADP-ribosylation	Depolymerization	Han et al. (1999)
Salmonella enterica	SpvB	R177 ADP-ribosylation	Depolymerization	Otto et al. (2000), Tezcan-Merdol et al. (2001), Hochmann et al. (2006)
Aeromonas salmonicida	Aext	R177 ADP-ribosylation, Rho GAP activity	Depolymerization	Braun et al. (2002), Fehr et al. (2007), Litvak and Selinger (2007)
Photorhabdus luminescens	Photox	R177 ADP-ribosylation	Depolymerization	Visschedyk et al. (2010)
Aeromonas hydrophila	VahC	R177 ADP-ribosylation	Depolymerization	Shniffer et al. (2012)
Streptococcus pyogenes	SpyA	ADP-ribosylation of actin and vimentin	Depolymerization	Coye and Collins (2004), Icenogle et al. (2012)
Photorhabdus luminesens	Tc toxins (e.g. TccC3)	T148 ADP-ribosylation	Actin clustering	Lang et al. (2010)
Vibrio cholerae	RtxA (MARTX family)	K50-E270 cross-linking of G-actin	Depolymerization	Fullner and Mekalanos (2000), Satchell (2011), Sheahan et al. (2004)
Vibrio cholerae	VgrG1	K50-E270 cross-linking of G-actin, ADP-ribosylation	Depolymerization	Durand et al. (2012), Suarez et al. (2010)

Table 2 Direct manipulation of actin dynamics by bacterial effectors

Protein	Bacterium	Strategy (details and involved host proteins)	Effect on actin cytoskeleton	References
TARP	*Chlamydia trachomatis, C. pneumoniae*, *C. muridarum*, *C. caviae*	Mimicking host nucleators, indirect Arp2/3 complex regulation, recruitment of membrane-actin crosslinking proteins (actin binding domains: WH2, poly-Pro, FAB1/2; PI3K, FAK, vinculin recruitment; Rac1 GEF activation)	F-actin nucleation at bacterial entry site via Arp2/3 complex-dependent and independent mechanisms F-actin bundling	Jewett et al. (2006). Lane et al. (2008), Jiwani et al. (2013)
BimA	*Burkholderia pseudomallei, B. mallei*	Mimicking host Ena/VASP F-actin-tracking proteins (Actin-binding domain: WH2)	F-actin nucleation, barbed end elongation, bundling, uncapping	Sitthidet et al. (2010), Benanti et al. (2015)
VopL	*Vibrio parahaemolyticus*	Mimicking host Ena/VASP F-actin-tracking proteins (Actin-binding domain: WH2, FH1-like)	F-actin nucleation Stress fibres formation	Yu et al. (2011), Liverman et al. (2007)
VopF	*Vibrio cholerae*	Mimicking host Ena/VASP F-actin-tracking proteins (Actin-binding domain: WH2, FH1-like)	F-actin nucleation, elongation, uncapping, actin sequestering, filopodia formation, tight junction alterations	Dziejman et al. (2005), Pernier et al. (2013)
Sca2	*Rickettsia conorii*	Mimicking host formin F-actin assembly proteins (Actin-binding domain: WH2, FH1-like, FH2-like)	F-actin nucleation, profilin-dependent processive elongation, uncapping	Haglund et al. (2010), Madasu et al. (2013)
YopO	*Yersinia enterocolitica*	Actin-induced kinase activity for ABPs (inactivation of gelsolin, cofilin, VASP, EVL, Dia1, INF2, WASP), Rho GDI	F-actin disruption, impaired phagocytosis by misregulation of host ABPs	Trasak et al. (2007), Lee et al. (2015)

Table 3 Examples for indirect strategies of actin cytoskeleton manipulation by bacterial effectors

Protein	Bacterium	Strategy (details and involved host proteins)	Effect on actin cytoskeleton	References
ActA	*Listeria monocytogenes*	Direct Arp2/3 complex activation (mimics NPF N-WASP; actin binding domains: VCA, poly-Pro)	F-actin assembly into tail-like structures for bacterial motility	Domann et al. (1992), Kocks et al. (1992), Gouin et al. (2005)
RickA	*Rickettsia conorii*	Direct Arp2/3 complex activation (mimics NPFs for recruitment and activation)	F-actin nucleation	Gouin et al. (2004, 2005)
BimA	*Burkholderia thailandensis*	Direct Arp2/3 complex activation (mimics NPFs for binding and activation; actin binding domains: VCA, poly-Pro motif)	F-actin nucleation	Sitthidet et al. (2010), Welch and Way (2013), Benanti et al. (2015)
IcsA (VirG)	*Shigella flexneri*	Indirect Arp2/3 complex activation (recruits and activates N-WASP; actin binding domains: VCA, WH2, poly-Pro motif)	F-actin nucleation	Goldberg et al. (1993), Suzuki et al. (2002), Cossart (2000)
TccP (EspFu)	*EHEC/EPEC*	Indirect Arp2/3 complex activation (activates NPFs N-WASP and WASP by release from autoinhibition)	F-actin nucleation	Campellone et al. (2004), Cheng et al. (2008)
EspF	*EHEC/EPEC*	Indirect Arp2/3 complex activation (activates NPF N-WASP; actin, profilin, Abcf2, SNX9 binding)	F-actin nucleation, tight junction disruption, antiphagocytosis	Alto et al. (2007), Peralta-Ramírez et al. (2008)
Tir	*EHEC/EPEC*	Recruitment of membrane-bound scaffold proteins and ABPs (phosphorylation by host kinases leads to recruitment of Nck, IQGAP1, IRSp53, IRTKS)	F-actin nucleation and polymerization; actin pedestal formation	Brown et al. (2008), Campellone et al. (2002), Weiss et al. (2009), Campellone (2010), Gruenheid et al. (2007), de Groot et al. (2011)
IpaA	*Shigella flexneri*	Recruitment of membrane-actin crosslinking proteins (Vinculin)	Depolymerization of F-actin, reduction of adhesion	Bourdet-Sicard et al. (1999), Ramarao et al. (2007), Tran Van Nhieu (1997)
SptP	*Salmonella typhimurium*	Regulation of F-actin bundling and crosslinking proteins (phosphatase for villin, vimentin), regulation of Rho GTPases (GAP for Rac1, Cdc42)	Downregulation of actin cytoskeleton remodelling after bacterial entry	Fu and Galán (1999); Murli et al. (2001); Lhocine et al. (2008)

(continued)

Table 3 (continued)

Protein	Bacterium	Strategy (details and involved host proteins)	Effect on actin cytoskeleton	References
EspL	*EHEC/EPEC*	Regulation of membrane-actin crosslinking proteins (annexin 2)	F-actin accumulation, formation of pseudopod-like structures	Miyahara et al. (2009)
CagA	*Heliobacter pylori*	Phosphorylated by host kinases (effects on cortactin, ezrin, vinculin)	Actin cytoskeleton rearrangements that promote cell motility, cell scattering, and elongation	Tegtmeyer and Backert (2011), Rieder et al. (2005), Stein et al. (2002)
SipA	*Salmonella typhimurium*	Antagonizes with severing proteins (displaces ADF/cofilin, villin and gelsolin from F-actin, barbed end protection from severing and depolymerization)	Decreases critical concentration, F-actin stabilization, membrane ruffling for bacterial internalization	Zhou et al. (1999a, b), McGhie et al. (2001), Lhocine et al. (2008)
Ceg14	*Legionella pneumophilia*	Competition with sequestering proteins (profilin)?	Direct F-actin binding, inhibition of actin polymerization	Guo et al. (2014)
C3 toxins	e.g. *Clostridium botulinu,* C. limosum, *Staphylococcus aureus*	Direct modification of Rho GTPases (ADP-ribosylation prevents Rho GEF activation)	Inhibition of Rho interaction with its effectors, stress fibre depolymerization	Genth et al. (2003), Chardin et al. (1989)
SopE/E2	*Salmonella typhimurium*	Regulation of Rho GTPases (GEF for Cdc42, Rac1)	F-actin polymerization, membrane ruffling	Friebel et al. (2001), Hardt et al. (1998)
CadF (FlaA, FlaB)	*Campylobacter jejuni*	Modulation of Rho GTPase signaling (fibronectin binding, Cdc42 and Rac1 activation via integrin β1-FAK-Src-PI3K-Vav2 and FAK-DOCK180/Tiam-1)	Actin cytoskeleton rearrangements, membrane ruffling	Krause-Gruszczynska et al. (2011), Boehm et al. (2011), Eucker and Konkel (2012)

(continued)

Table 3 (continued)

Protein	Bacterium	Strategy (details and involved host proteins)	Effect on actin cytoskeleton	References
SteC	*Salmonella typhimurium*	Activation of kinase signaling pathways	F-actin remodelling around SCV	Poh et al. (2008), Odendall et al. (2012)
IpaC	*Shigella flexneri*	Recruitment of kinases (c-Src activates signal cascades for Rho GTPase activation, regulation of ABPs)	Actin reorganization, formation of filopodia and lamellipodia	Tran Van Nhieu et al. (1999), Mounier et al. (2009)
IpgD	*Shigella flexneri*	Modification of membrane lipid composition (dephosphorylates PIP_2 to PI(5)P)	Dissociation of actin cytoskeleton from plasma membrane, F-actin remodelling	Niebuhr et al. (2002), Mellouk et al. (2014)
SopB (SigD)	*Salmonella typhimurium*	Modification of membrane lipid composition (dephosphorylates PIP_3 to PI(3)P)	Actin cytoskeleton rearrangements, membrane ruffling	Zhou et al. (2001), Terebiznik et al. (2002), Mallo et al. (2008)

Bacterial pathogens produce a huge variety of virulence factors that are able to target and to modify host proteins to interfere with or to inhibit cellular functions. Pathogens actively hijack the actin cytoskeleton to invade host cells, to move inside the host cytosol, to facilitate cell-to-cell spread, and to secure their survival by blocking phagocytosis. All these purposes require the controlled rearrangement of cellular actin structures. First, bacteria need to disrupt the membrane-associated, cortical actin cytoskeleton prior to cell invasion to access the host cytosol. For intracellular motility and cell-to-cell spread, pathogens secondly initiate actin filament nucleation and polymerization. Newly formed, free actin barbed ends (e.g. via the Arp2/3 complex) finally need to be elongated and bundled to generate the required pushing forces for the engulfment of pathogens and for intracellular pathogen motility. In order to secure their survival, bacteria often impede engulfment and destruction by professional phagocytotic host cells. Many bacteria have developed mechanism to block their engulfment by inhibiting actin reorganizations necessary for phagocytosis. Interestingly, most bacterial effectors do not directly interact with monomeric or filamentous actin but modulate host actin ABPs that regulate actin dynamics. A number of effects of bacterial virulence factors will be discussed in to more detail in later chapters.

4.1 Direct Interactions of Bacterial Effectors with Actin

4.1.1 Direct Modifications of G-actin

The intracellular abundance of actin and its essential role for many cellular functions have made it a preferred target for infectious agents, a fact that appears to be supported by its highly conserved sequence and structure. A number of bacteria have developed toxins that directly target actin (for review, see Aktories et al. 2011; see also Table 1 and Lang et al. 2016). Among these, two groups of toxins can be differentiated, which covalently modify actin. ADP-ribosyltransferases ADP-ribosylate actin at Arg177 (e.g. C2 toxin of *Clostridium botulinum*; Aktories et al. 1986) or Thr148 (e.g. TccC3 toxin of *Photorhabdus luminescens*; Lang et al. 2010) to induce the inhibition or promotion of actin polymerization, respectively, with the aim to secure their survival by inhibiting phagocytosis (see Lang et al. 2016).

A number of *clostridial bacteria* inject ADP-ribosyltransferases into host cells, which at least transiently form direct complexes with actin. The structure of the complex of G-actin with the ADP-ribosyltransferases from *Clostridium perfringens* (iota toxin) is shown in Fig. 4a (Tsuge et al. 2008; for further detail, see Tsuge et al. 2016). Actin Arg177-ADP-ribosylation introduces a bulgy side group in close proximity to the interstrand actin-actin interface and thereby interferes with the addition of a further actin subunit to the plus end (for further detail, see Schwan and

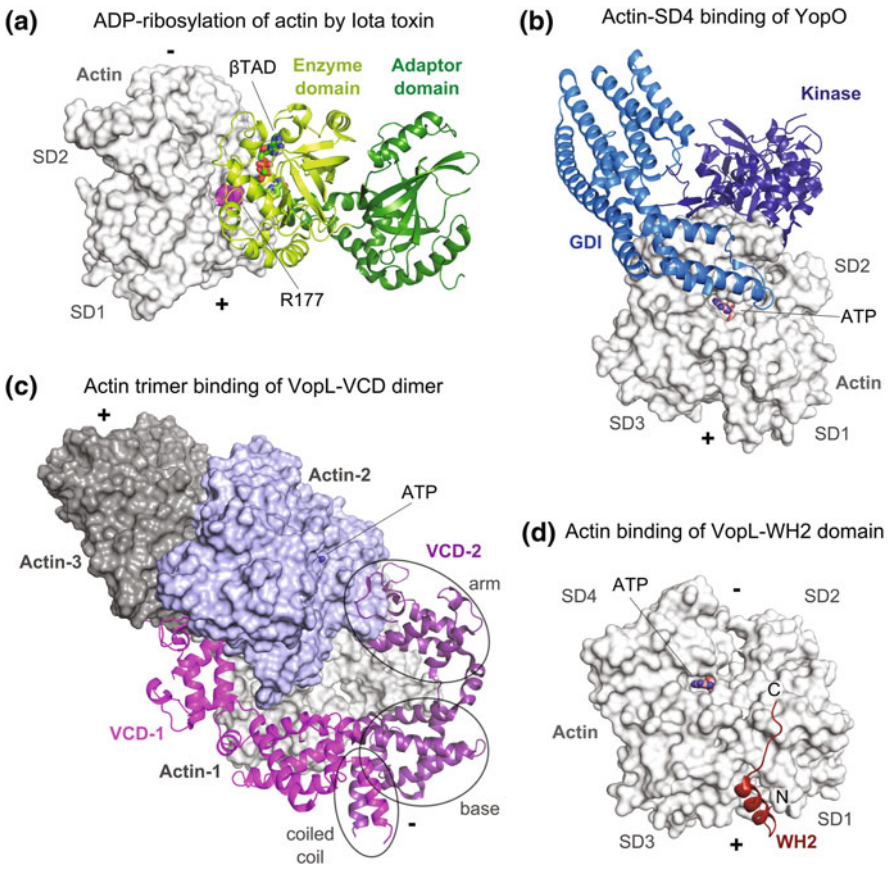

Fig. 4 Complex structures of actin with directly actin-binding bacterial proteins. Actin molecules are depicted as surface representation and bacterial proteins as ribbons, the actin subdomains (SD), pointed (−) and barbed (+) ends are indicated. **a** The ADP-ribosylating iota toxin consists of an N-terminal adaptor domain (*dark green*) with a C-terminal enzymatic domain (*light green*). The Arg177 residue of actin (R177) is highlighted in pink and in close proximity to βTAD (non-hydrolysable NAD analog) bound to the enzymatic iota domain (PDB: 3BUZ; Tsuge et al. 2008). The adaptor domain interacts with the binding domain (not shown) of the binary iota toxin. **b** Structure of the ABP-phosphorylating YopO effector in complex with G-actin. The front view on actin is shown with the YopO GDI domain (*GDI* guanine-nucleotide dissociation inhibitor) in *light blue* and the kinase domain in *dark blue* (PDB: 4CI6; Lee et al. 2015). Both, the GDI and the kinase domain interact with the pointed face of actin-encircling subdomain 4 (SD4). **c–d** The bacterial effector VopL comprises two different actin-binding domains: the VCD (VopL C-terminal domain; **c**), and three WH2 domains (WASP homology 2; **d**). VopL dimerizes with the coiled coil in the VCD (VCD-1: chain A, *pink*, VCD-2: chain B, *purple*). In addition, each VCD consists of arm and base regions, which interact with actin. The VCD-actin complex has been crystallized with three actin protomers in F-actin-similar conformation [strand 1: actin-1 and actin-3, strand 2: actin-2 (*light blue*)]. Note that the WH2 domain-binding sites at the barbed faces of actin protomers 2 and 3 are not occupied by the VCD dimer and would allow WH2 domain binding (PDB: 4M63; Yu et al. 2011; Zahm et al. 2013). **d** The cross-linked VopL-WH2-actin complex was crystallized with the first (residues 130–160) of the three WH2 domains of VopL (PDB: 3M1F; Rebowski et al. 2010). The WH2 domain of VopL (*red*) adopts the typical fold of WH2 domains as found, for instance, in WASP and thymosin ß4 and binds identically with its amphipathic helix to the hydrophobic ligand-binding cleft between SD1 and SD3. N- and C-termini are indicated

Aktories 2016; Lang et al. 2016). In contrast, Thr148 ADP-ribosylation interferes with the binding of ABPs, which sever F-actin or stabilize its monomeric form. Thr148 is located between subdomains 1 and 3 representing a major target zone for a number of actin-binding proteins (see Lang et al. 2016).

A different covalent modification of actin leading to interference with its normal cycling is achieved by bacterial toxins, which cross-link actin molecules (e.g. RtxA of *Vibrio cholerae* (Fullner and Mekalanos 2000). These toxins catalyse the cross-linking of actin to dimers and larger oligomers, which are unable to polymerize and thereby interfere with the dynamic behaviour of the actin cytoskeleton (see Kudryashova et al. 2016).

4.1.2 F-actin Dynamics Modifying Bacterial Proteins

During the last years, an increasing number of bacterial effectors has been identified that modify actin filament dynamics (Table 2). These pathogenic factors mimic host nucleation or elongation factors and hold similar structural and functional motifs or follow akin strategies. Some bacteria mimic WH2 domain-containing nucleation factors like the F-actin nucleator TARP (*Chlamydia spp.*), which binds directly with its WH2-like domain to G-actin to initiate new actin filaments upon oligomerization (Jewett et al. 2006). In addition, TARP is able to indirectly activate F-actin nucleation by the Rac1-WAVE-Arp2/3 pathway (Lane et al. 2008) and to bundle actin filaments (Jiwani et al. 2013).

Another group of bacterial effectors mimic host Ena/VASP F-actin-tracking proteins like BimA of *Burkholderia* pathogens (*Burkholderia pseudomallei, B. mallei*). BimA utilizes WH2 motifs and poly-proline-rich regions to nucleate, elongate, and bundle actin filaments (Sitthidet et al. 2011; Benanti et al. 2015). A further example for Ena/VASP mimics are the closely related, dimeric pathogen effectors VopF and VopL (*Vibrio cholera* and *Vibrio parahaemolyticus*). Both contain multiple WH2 domains with actin filament nucleation activity and poly-proline-rich sequences for profilin-actin binding (see Fig. 4c, d for more details; Dziejman et al. 2005; Liverman et al. 2007; Yu et al. 2011). VopF tracks F-actin barbed ends for processive filament elongation and competes with capping proteins to uncap pre-existing filaments (Pernier et al. 2013) like Ena/VASP or formin proteins (Shekhar et al. 2015). SAXS studies of actin-sequestering, dimeric VopF constructs seem to support the role of VopF as barbed end tracker (Avvaru et al. 2015). By contrast, a pointed end nucleation model has been proposed for VopL based on the complex structure of the dimeric VopL-VCD domain with an actin trimer (Fig. 4c; Zahm et al. 2013).

The closest bacterial mimic of a formin described so far is the Sca2 effector of *Rickettsia conorii*. Sca2 possesses strong formin-like F-actin assembly properties and imitates with its N- and C-terminal domains the filament barbed end elongating, dimeric FH2 domains of formins. It possesses, in addition, a profilin-binding FH1 domain for the recruitment of profilin-actin and an actin-binding WH2 domain (Haglund et al. 2010; Madasu et al. 2013).

Conversely, *Salmonella spp.* inject into host cells among many other effectors SipA (invasion-promoting toxin), which promotes actin polymerization and stabilizes existing actin filaments at the cytoplasmic face of the plasma membrane underneath the adhesion site of the bacterium. SipA was shown to clamp two actin subunits from opposing strands leading to localized F-actin stabilization that induces membrane ruffles to internalize the adherent bacterium (Lilac et al. 2003).

A completely different mode of direct actin interaction undergoes the bacterial effector YopO (or YpkA) after injection into host cells by *Yersinia enterocolitica.* Binding of YopO to G-actin had been shown to stimulate the YopO protein kinase activity, which is essential for inhibiting phagocytosis (Trasak et al. 2007). The 3D structure of the 1:1 YopO-actin complex has been solved (Lee et al. 2015). YopO consists of three main domains: a membrane binding, a protein kinase, and a Rho GTPase-binding domain that inhibits guanine-nucleotide dissociation. The kinase and Rho GTPase-binding domains wrap around SD4 of preferentially cytoplasmic G-actin, preventing its polymerization (Fig. 4b). Activated YopO kinase phosphorylates ABPs that interact with the available binding area between SD1 and SD3 (Lee et al. 2015; see also Aepfelbacher and Wolters 2016). Among these ABPs are polymerization-promoting proteins like formins and VASP, and NPFs like WASP, but also F-actin-fragmenting factors such as gelsolin and cofilin. Thus, YopO uses the bound G-actin as bait for proteins involved in phagocytosis, which are subsequently inactivated by YopO (Lee et al. 2015).

4.2 Manipulation of Actin-Binding Proteins by Bacterial Effectors

4.2.1 Recruitment and Regulation of the Host F-actin Nucleation Machinery

Besides mimicking F-actin nucleation function, many pathogens either directly or indirectly target the Arp2/3 complex, the major host actin nucleator (see Table 3). *Listeria* directly recruits the Arp2/3 complex with ActA, an N-WASP-mimicking bacterial effector (Domann et al. 1992; Kocks et al. 1992; Gouin et al. 2005; see also Pillich et al. 2016), to induce F-actin nucleation for actin-based motility in the host cytosol. *Shigella* on the other hand injects the bacterial adaptor IcsA for indirect Arp2/3 complex activation via N-WASP recruitment (Goldberg et al. 1993; Cossart 2000; Suzuki et al. 2002). Other pathogens like *Rickettsia conorii* and *B. thailandensis* mimic NPFs (RickA, BimA) (Gouin et al. 2004, 2005; Welch and Way 2013; Benanti et al. 2015; Sitthidet et al. 2010), while EHEC and EPEC activate N-WASP (TccP, EspF) (Alto et al. 2007; Peralta-Ramírez et al. 2008; Campellone et al. 2004; Cheng et al. 2008) (see Stradal and Costa 2016).

A further, more indirect strategy is the recruitment of membrane-associated scaffold proteins that provide a localization and activation platform for the host F-actin nucleation machinery. A prominent example is the EPEC effector Tir, which induces the recruitment and activation of N-WASP-binding and activating proteins like Nck, IQGAP1, and IRSp53 (Weiss et al. 2009; Campellone 2010; Campellone et al. 2002; Gruenheid et al. 2007; Brown et al. 2008; de Groot et al. 2011; see also Stradal and Costa 2016). Another method to position the Arp2/3 complex is obtained by the recruitment of vinculin, which directly interacts with F-actin and Arp2/3. Virulence factors of several bacteria were found to utilize vinculin for actin cytoskeleton rearrangements including *Rickettsia* (Sca4), *Chlamydia* (TARP), EHEC/EPEC (Tir-talin), and *Shigella flexneri* (IpaA).

4.2.2 Interactions of Bacterial Effectors with Actin-Binding Proteins (ABPs)

Bacterial pathogens have developed effectors that not only manipulate actin filament nucleation and elongation, but also differently affect cellular actin dynamics by hijacking host F-actin capping, bundling, cross-linking, severing, or sequestering proteins. It was recently shown that the alteration of the activity of the F-actin barbed end capping, bundling, and filament severing protein villin by SptP is required for the invasion of *Salmonella* (Lhocine et al. 2015). Annexin 2, a cross-linking protein that connects membrane-bound protein complexes with the actin cytoskeleton, is stimulated by the EHEC/EPEC factor EspL to form pseudopod-like structures (Miyahara et al. 2009). *Heliobacter*'s CagA on the other hand hijacks host signalling pathways to regulate F-actin/plasma membrane cross-linkers like vinculin, ezrin, and cortactin (Tegtmeyer and Backert 2011; Rieder et al. 2005; Stein et al. 2002). Host severing proteins (ADF/cofilin, gelsolin) disrupt actin filaments and release G-actin required for the polymerization of new filaments. The *Salmonella* effector SipA has been found to antagonize with severing proteins for direct F-actin binding and to protect filament ends from severing and depolymerization (Zhou et al. 1999a, b; McGhie et al. 2001; Lhocine et al. 2015). The recently identified Ceg14 effector (*Legionella pneumophila*) might compete with the G-actin-sequestering profilin and directly bind F-actin, which could lead to the inhibition of actin polymerization (Guo et al. 2014).

4.2.3 Manipulation of Host ABP Regulation: Rho GTPases, Kinases, and Phospholipids as Bacterial Targets

In addition to direct interaction with host ABPs, pathogens have developed numerous strategies to upstream manipulate their activity and spatial localization.

Many actin-binding proteins are downstream effectors of Rho GTPases. To modulate the host actin cytoskeleton, bacterial pathogens effectively target Rho GTPase signalling at various stages leading to the activation or inhibition of

GTPase function (Aktories 2001). Altered Rho GTPase activity or availability interferes with downstream Rho GTPase effector proteins and the corresponding signalling pathways responsible for actin cytoskeleton rearrangements. Bacterial toxins usually effectuate Rho GTPase targeting via three different strategies. First, they directly catalyse enzymatic post-translational modifications to block GTPases in their active or inactive states (e.g. ADP-ribosylation of RhoA by the C3 toxin of *C. botulinum*; Aktories 2001; Genth et al. 2003; Chardin et al. 1989). Other bacterial factors manipulate or mimic host regulators of Rho GTPase activity, like the RhoGEF-mimicking virulence factors SopE/E2 (*Salmonella typhimurium*) (Hardt et al. 1998; Friebel et al. 2001). The third strategy is to modulate host Rho GTPase signalling pathways by the stimulation of cell surface receptors (e.g. CadF of *Campylobacter jejuni*; Krause-Gruszczynska et al. 2011; Boehm et al. 2011; Eucker and Konkel 2012).

Several bacterial pathogens interfere with host kinases to indirectly manipulate signalling cascades of the actin cytoskeleton. The *Salmonella* kinase SteC, for example, promotes the formation of an F-actin meshwork by the activation of a MEK/ERK/Myosin IIB signalling pathway (Poh et al. 2008; Odendall et al. 2012), while the *Shigella* virulence factor IpaC localizes the Src kinase at bacterial entry sites, which regulates the activity of several ABPs (Tran Van Nhieu et al. 1999; Mounier et al. 2009).

Another strategy to induce F-actin clearance beneath the plasma membrane is to modify the lipid composition of host membranes by hijacking phosphatidylinositol phosphate metabolism pathways. For example, the *Shigella* phosphatase IpgD facilitates bacterial escape into the cytosol (Niebuhr et al. 2002; Mellouk et al. 2014), while the inositol polyphosphatase SopB of *Salmonella* enables bacterial uptake into host cells (Zhou et al. 2001; Terebiznik et al. 2002; Mallo et al. 2008).

Finally, some bacteria induce actin rearrangements by the recruitment of membrane-associated host scaffold proteins that provide a localization and activation platform for ABPs (as described in Sect. 4.2.2).

5 Conclusions

Due to its high abundance in eukaryotic cells and its conserved structure, actin has become a preferred target of bacterial toxins, which either directly modify actin or mimic or modulate actin-binding proteins, which regulate its supramolecular organization. In addition, a number of bacterial toxins modify signalling cascades that regulate actin cytoskeleton dynamics. Thus, bacteria hijack and divert actin functions for host cell entry, their survival, their transport within the host cell cytoplasm, and intercellular spread. Here, we presented the actin structure, its organization into higher organizational structures, and its basic dynamic behaviour, which might all become modified by the many different bacterial toxins and effectors.

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