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Hans Georg Mannherz *Editor*

The Actin Cytoskeleton and Bacterial Infection

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The Actin Cytoskeleton and Bacterial Infection

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Preface

Three types of cytoplasmic filaments form the cellular cytoskeleton. These filaments often extent from the cell nucleus to the plasma membrane. They stabilise cell shape and provide tensile strength. Different classes of proteins form the different types of filaments: A large number of tissue specific proteins (the intermediate filament proteins) form the intermediate filaments, proteins of the tubulin family build the microtubules, and the actins the microfilaments, which organize the actin cytoskeleton. The cytoskeletal filaments differ in structure and functions. The intermediate filaments have mainly shape stabilising functions. In addition to cell shape stabilising functions the microtubules provide the routes for the intracellular transport of vesicles and form the spindle apparatus during cell division, the actin cytoskeleton participates in a number of motile processes like cell locomotion and more specialized processes as endo- and exocytosis, vesicular transport, and cytokinesis. The supramolecular structure of the cytoskeleton is subjected to constant reorganization according to the cellular activities. In the past the different filament types have been regarded as separate functional entities, recent data, however, strongly indicate structural and functional linkages (as also demonstrated by the contribution by Schwan and Aktories).

Actin is the most abundant protein in many eukaryotic cells. In mammals six tissue-specific isoforms assemble to the actin containing microfilaments, which are often organized into bundles or higher ordered networks. The highly dynamic behaviour of the actin cytoskeleton is regulated by a large number of actin binding proteins (ABP). The ABPs can be grouped into different functional classes, which regulate the assembly or disassembly of actin filaments and are often targets of signalling pathways.

Bacteria have evolved a large arsenal of toxins and effectors, some of which are injected into host cells by type-3 secretion mechanisms to avoid phagocytosis by professional macrophages (see contribution by Aepfelbacher & Wolters). Alternatively, bacterial toxins or virulence factors induce special plasma membrane extensions of non-professional phagocytotic cells, which embrace bacteria leading to their uptake into host cells with the aim to secure their survival by gaining access to nutrients for proliferation, to achieve transport within the cytoplasm or from cell

to cell and finally their release into the environment for further dissemination (see contribution by Stradal & Costa). The actin cytoskeleton is essential for all these processes. Therefore it is not surprising that actin is a preferred target of many bacterial toxins and effector proteins also due to its highly conserved primary and ternary structure and its intracellular abundance.

With the 11 contributions this volume tries to introduce this fast progressing field of research without pretending completeness. Bacteria have evolved a vast number of tricks to secure their survival and proliferation. Therefore only a few bacteria and their toxins and effectors will be introduced with the intention to indicate common modes of action. The structure, properties and dynamic behaviour of actin cytoskeleton itself and the different classes of actin-binding proteins (ABP) will introduced with constant reference to bacterial toxins and effectors, which interfere their functions by modifying their normal behaviour (Kühn & Mannherz).

Bacterial toxins or effectors affect either the actin cytoskeleton by modifying actin directly or subverting certain ABPs or signalling pathways leading to the actin cytoskeleton by modifying small GTP-binding proteins terminating at regulatory ABPs (see contributions by Aepfelbacher & Wolters, Lemichez, and Stradal & Costa). Often a bacterium has developed toxins aiming on both mechanisms to influence the actin cytoskeleton like for instance *Photorhabdus luminescens* (contribution by Lang et al.), *Clostridia* (Schwan & Aktories), *Yersinia* (contribution by Aepfelbacher & Wolters).

A number of bacterial toxins modify actin directly by ADP-ribosylation or cross-linking. These modifications subvert the normal functioning of the actin cytoskeleton and thereby secure bacterial survival by inhibiting phagocytosis. This area will be covered by four contributions, which extend from their cellular effects to structural studies illuminating their selectivity. How phagocytosis is normally able to eliminate bacterial invasion is detailed for the uptake of Borrelia by macrophages in one contribution (Naj & Linder).

ADP-ribosyltransferases also modify small GTP-binding proteins of the Rho-family resulting in dramatic effects in their signalling capacity to the actin cytoskeleton (one special contribution and part of a number of other contributions). Alternatively, extracellular gastrointestinal pathogens manipulate the signalling pathways leading to the actin cytoskeleton by secreting virulence factors that modify the GTPase cycle of host Rho-GTPases thus securing their survival (This topic will be covered with two contributions (by Lemichez and Stradal & Costa).

Some intracellular bacteria hijack the actin cytoskeleton to their own needs by presenting on one pole bacterial proteins that lead to the assembly of a comet-like F-actin tail able by rapid actin subunit treadmilling to propel the pathogen within the cytoplasm of the host cell and for further dissemination from cell to cell (see contribution by Pillich et al.).

In many instances the analysis of the action of bacterial toxins and virulence factors provided important clues to the function of endogenous ABPs or even helped to identify ABPs, which were binding partners of certain bacterial effector proteins like the Arp2/3 complex as partner of ActA of Listeria. Future studies on

bacterial toxins will certainly uncover new insights into their functions and also unravel new aspects of the inherent regulatory mechanism of the actin cytoskeleton, which might also lead to the development of new antibiotic drugs. As a further outlook in this perspective this volume includes a description of the bacterial actins, which are different in primary but similar ternary structure to their eukaryotic counterparts, and their interactors (contribution by Gayathri).

Bochum, Germany Hans Georg Mannherz

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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.

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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

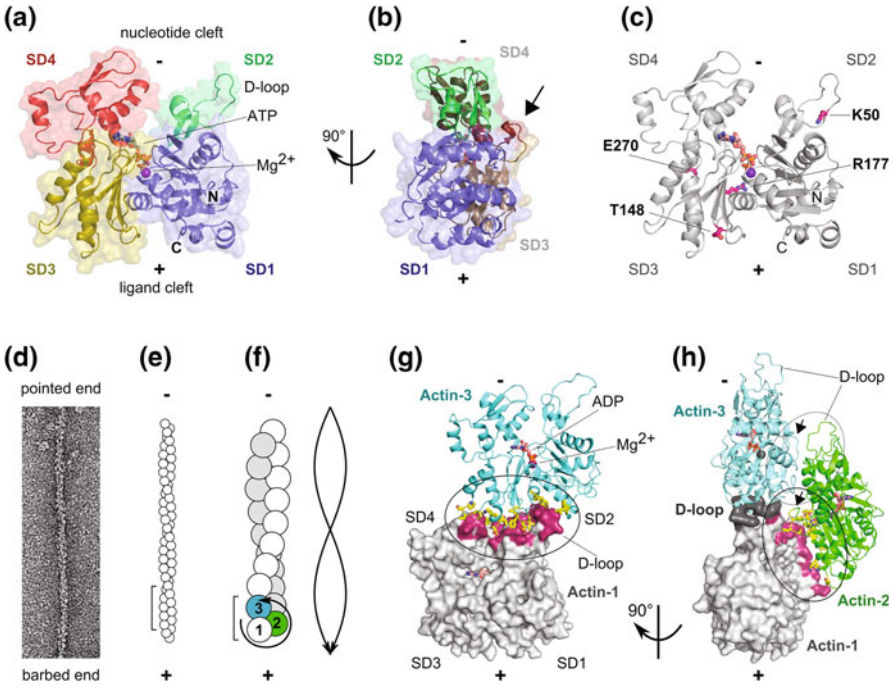

Fig. 1 Structure of G- and F-actin. (**a**–**c**) G-actin structure shown as ribbon with semitransparent surface (**a**–**b**), the ATP nucleotide as stick representation and bound Mg^{2+} cation as sphere. The actin subdomains (SD), pointed (−) and barbed (+) faces are indicated. Front (**a**) and side (**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