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Review Article

# Unconventional actins and actin-binding proteins in human protozoan parasites

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ABSTRACT

Actin and its regulatory proteins play a key role in several essential cellular processes such as cell movement, intracellular trafficking and cytokinesis in most eukaryotes. While these proteins are highly conserved in higher eukaryotes, a number of unicellular eukaryotic organisms contain divergent forms of these proteins which have highly unusual biochemical and structural properties. Here, we review the biochemical and structural properties of these unconventional actins and their core binding proteins which are present in commonly occurring human protozoan parasites.



## 1. Introduction

Protozoan parasitic infections such as malaria, visceral leishmaniasis, human African sleeping sickness, Chagas disease, giardiasis and amoebiasis are major causes of morbidity and mortality in humans in developing countries. Motility plays a key role in the ability of these organisms to cause infection, by enabling them to penetrate deep into human tissues. Cell motility in eukaryotes is primarily driven by the actin protein and other proteins that regulate it. Thus, it is essential to understand the role of actin and its core binding proteins in host cell invasion and pathogenesis, to be able to devise new methods for the effective management of diseases caused by these pathogens.

## 2. Conventional actins

The actin cytoskeleton, which contains actin as its major protein constituent, plays a key role in several cellular processes such as cell shape regulation, cell motility, intracellular trafficking and cytokinesis (Sheterline and Sparrow, 1994; Pollard and Cooper, 2009). Actin exists in both monomeric or globular (G-actin) and polymeric or filamentous (F-actin) forms. A molecule of G-actin binds to one molecule of ATP and one divalent cation, and shows a weak $Mg^{2+}$-stimulated ATPase activity. Polymerisation of G-actin into F-actin is promoted by the presence of divalent cations under physiological ionic conditions in its ATP-bound state, which is usually followed by hydrolysis of the bound ATP to ADP. The filament end containing ATP-bound actin (barbed end) grows much faster than the ADP-bound actin end (pointed end) by recruiting ATP-bound actin monomers. In a steady state, a dynamic equilibrium is reached where the length of the actin filaments remains constant, with actin monomers continually associating to and dissociating from the ends. Such a state results in establishment of a constant pool of free actin monomers and is called the critical concentration ($C_c$). The $C_c$ is thus defined as the free ATP-G-actin concentration at which the rate of monomer addition to the filament is balanced by the rate of monomer loss, resulting in no net growth of the filament. As the rates of growth of the pointed and barbed ends differ, these two ends have different $C_c$ for actin filament growth. Although the assembly of actin filaments does not depend on its ATPase activity, actin self-assembly is associated with the ATPase cycle, which powers treadmilling (Wegner, 1976).

Analyses of 3D structures of free G-actin (Chik et al., 1996), as well as actin-DNase I and actin-profilin complexes (Kabsch et al., 1990; Schutt et al., 1993), indicate that actin monomers consist of two main domains, originally referred to as large and small, each of which is further composed of two subdomains (Fig. 1). While the small domain comprises subdomain 1 (amino acid residues 1–32, 70–144, 338–375; rabbit α-actin numbering) and subdomain 2 (amino acid residues 33–69), the large domain comprises subdomain 3 (amino acid residues 145–180 and 270–337) and subdomain 4 (amino acid residues 181–269). The main domains

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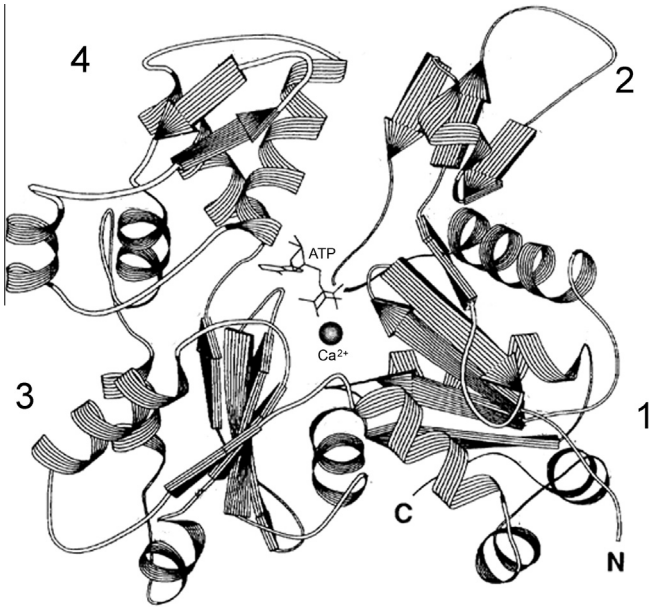


**Fig. 1.** Schematic structure of actin depicting its sub-domains (adapted from Kabsch et al., 1990). This structure originated from the crystal structure of rabbit muscle actin and bovine DNase I complex (PDB code: 1ATN). An ATP molecule and $Ca^{2+}$ are shown in the nucleotide binding cleft; N and C denote amino and carboxy terminii, respectively. The numbers mark sub-domains.

are separated by a cleft containing a tightly bound adenosine nucleotide in complex with a divalent cation. There are 15 amino acid residues that form the nucleotide binding site, of which 13 residues are contributed by subdomains 1 and 3, and the remaining two by subdomain 4. The region that forms the DNase I binding site consists of subdomain 2 (amino acid residues 39–46 and 60–64) and subdomain 4 (amino acid residues 202–204 and 207), of which residues 40–50 from subdomain 2 are highly disordered and form the DNase I binding loop.

In the filamentous form, subdomains 2 and 4 of one actin monomer interact with subdomains 1 and 3 of another actin monomer. The amino acid residues that are contributed by subdomain 2 include sequences that form part of the DNase I binding site. The actin filament is then stabilised through a loop of 11 amino acids (residues 262–272) that include a four-residue (266–269) hydrophobic plug. This loop inserts into a hydrophobic pocket formed by subdomains 2 and 3 of adjacent monomers on the opposing strands. The first structural model of actin filament was proposed by Holmes et al. (1990) based on the x-ray diffraction pattern of oriented F-actin gels. This model has been further refined by Oda et al. (2009). The actin monomers are arranged in a two-start filament with a half pitch of 37 nm and a rise of 2.75 nm per monomer. The width of the filament is within a range of 7–10 nm.

Many proteins (>100) interact with and influence the functions of actin by modulating its dynamics in the cellular environment (Pollard et al., 2000; dos Remedios et al., 2003). These proteins are largely structurally and functionally conserved in various eukaryotes. While some of these proteins affect the availability of the polymerisable pool of free actin monomers by controlling the sequestration or nucleotide exchange, others control filament formation and stability through their nucleating, capping, crosslinking, bundling, depolymerising and severing activities. In addition, cellular signalling molecules and small GTPases control actin remodelling by regulating the activities of actin-binding proteins and other effectors (Mackay and Hall, 1998).

Thus, the most distinguishing characteristics of all canonical actins are the formation of long and stable filaments in the presence of ATP and divalent cations, especially $Mg^{2+}$, at or above their $C_c$ (about 1.5 μM); characteristic two-start filament structure, the width of which ranges between 7 and 10 nm (Oda et al., 2009); ability to bind to DNase I and inhibit its activity; stabilisation of filaments by phallotoxins and destabilisation by cytochalasin D or latrunculin B (Reisler, 1993; Wakatsuki et al., 2001); and regulation by a set of about 20 canonical actin-binding proteins which include actin depolymerising factor (ADF)/cofilins, twinfilin, profilin, gelsolin, CAP/Srv2, formin, Arp2/3 complex, β-thymosin, troponin, filamin, fimbrin, villin, actinin, plastin, spectrin and CapZ. However, actins present in various human protozoan parasites such as *Plasmodium*, *Toxoplasma*, *Entamoeba*, *Giardia*, *Trypanosoma* and *Leishmania* spp. display highly unusual characteristics that differ from those of the canonical actins. In this review, we focus on the biochemical, structural and functional aspects of these unconventional actins and actin-binding proteins.

## 3. Unconventional actins

Phylogenetically, the Amoebae, the Apicomplexa and the Trypanosomatida actins form three distinct clades. While amoebae actins, together with human and rabbit actins, form clade 1, apicomplexan and trypanosomatid actins form clades 2 and 3, respectively (Fig. 2). Interestingly, *Giardia* actin forms a separate group and lies between clades 1/2 and clade 3 on the phylogenic tree (Fig. 2), and is the most divergent form of eukaryotic actins (Fig. 3, Tables 1 and 2). Although the overall domain structure is similar in all of the actins, there are subtle differences between the lengths and exact locations of the secondary structure elements in their amino acid sequences (Fig. 3), which may give rise to differences between the properties of actins from the same or different clades of parasites. In the following sub-sections, we summarise these properties of apicomplexan, amoebae, trypanosomatid and *Giardia* actins.

### 3.1. Apicomplexan actins

The apicomplexan group of parasites contain a large number of unicellular eukaryotic organisms including *Plasmodium* and *Toxoplasma*, which parasitise vertebrate (including human) and invertebrate hosts. While *Plasmodium* spp. such as *Plasmodium falciparum* and *Plasmodium vivax* are the main causative organisms of human malaria, *Toxoplasma gondii* causes parasitic pneumonia of the eyes, brain, liver and heart of humans. These parasites have a unique mode of movement called gliding motility, which is essential for invasion of the host cells (Morrissette and Sibley, 2002). As actin polymerisation is required in gliding motility and host cell invasion by both *Plasmodium* spp. and *T. gondii* (Miller et al., 1979; Dobrowolski and Sibley, 1996; Wetzel et al., 2003), the structure and polymerisation properties of *Plasmodium* and *Toxoplasma* actins have been more thoroughly investigated than other protozoan parasite actins.

*Plasmodium* spp. express two isoforms of actin, of which the major isoform (actin I) is abundant and is expressed throughout the life cycle of the parasites, whereas the minor isoform (actin II) is present only in the gametocytes and mosquito stages including sporozoites, the parasite form that is transmitted to the vertebrate host by mosquitoes (Wesseling et al., 1988; Deligianni et al., 2011; Lindner et al., 2013). Actin I is an essential component of the parasite motor machinery responsible for the unique gliding motility and host cell invasion by the parasites (Miller et al., 1979; Wetzel et al., 2003), while actin II is only required in male gametogenesis (Vahokoski et al., 2014). These two isoforms in *Plasmodium* differ not only from canonical actins but also from each other. Both of these isoforms hydrolyse ATP more efficiently than α-actin and, unlike canonical actins, form short oligomers