The actin cytoskeleton supports a vast number of cellular processes in nonmuscle cells. It is well established that the organization and dynamics of the actin cytoskeleton are controlled by a large array of actin-binding proteins. However, it was only 40 years ago that the first nonmuscle actin-binding protein, filamin, was identified and characterized. Filamin was shown to bind and cross-link actin filaments into higher-order structures and contribute to phagocytosis in macrophages. Subsequently many other nonmuscle actin-binding proteins were identified and characterized. These proteins regulate almost all steps of the actin filament assembly and disassembly cycles, as well as the arrangement of actin filaments into diverse three-dimensional structures. Although the individual biochemical activities of most actin-regulatory proteins are relatively well understood, knowledge of how these proteins function together in a common cytoplasm to control actin dynamics and architecture is only beginning to emerge. Furthermore, understanding how signaling pathways and mechanical cues control the activities of various actin-binding proteins in different cellular, developmental, and pathological processes will keep researchers busy for decades.
central mechanosensory proteins in cells by undergoing conforma-
tional changes and partial unfolding in response to extrinsic or in-
trinsic mechanical forces (Razinia et al., 2012).

The discovery and initial characterization of filamin were soon
followed by identification of a large array of proteins that regulate
different aspects of actin filament turnover in nonmuscle cells. These
include, among others, the actin monomer–binding protein profilin
(Carlsson et al., 1977), actin filament–severing proteins gelsolin and
actin depolymerizing factor (ADF)/cofilin (Yin and Stossel, 1979;
Bamburg et al., 1980), and the heterodimeric capping protein,
which blocks actin filament assembly at their rapidly growing barbed
ends (Isenberg et al., 1980; Cooper et al., 1984). Finally, in the mid-
1990s the first actin filament–nucleating protein, the Arp2/3 com-
plex, was discovered (Machesky et al., 1994; Welch et al., 1997;
Mullins et al., 1998). These and other studies established that actin
can assemble into both protrusive and contractile structures in cells
and that the dynamics of actin filaments is precisely controlled by a
plethora of actin-binding proteins. Even organisms of lower com-
plexity, such as yeasts, express ∼100 proteins that either directly or
indirectly regulate the assembly or organization of the actin cyto-
skeleton (Mishra et al., 2012). In multicellular organisms, including
mammals, this number is significantly higher.

Why do we need such a large and diverse repertoire of actin-
binding proteins? There are a number of reasons. First, actin fila-
ments contribute to a vast number of different cellular processes,
many of which require specific three-dimensional (3D) organization
of actin filaments with distinct dynamic properties (Figure 1). During
adhesion-dependent migration, cells extend thin, actin-rich mem-
brane protrusions—filopodia—at their leading edge to probe the
extracellular environment. Filopodia contain compact, unipolar ac-
tin filament bundles. Actual advancement of the cell edge in migra-
tion is driven by a branched actin filament network called the lamel-

lipodium, which pushes the plasma membrane forward through coordinated
polymerization of actin filaments against the membrane. Many animal cells also contain
bipolar, contractile actomyosin bundles, of-
ten called stress fibers, which contribute to
adhesion, mechanosensing, and tail re-
traction during cell migration (Blanchon et al.,
2014). Moreover, cells harbor several
additional actin filament arrays that gener-
ate force for other processes involving
membrane dynamics, such as endocytosis
and mitochondrial fission (Kaksonen et al.,
2006; Hatch et al., 2014). Finally, it has be-
come evident that actin not only functions in
the cytoplasm, but also executes specific
functions inside the nucleus (Grosse and
Vartiainen, 2013). Because different actin
filament networks are uniquely designed to
execute specific cellular processes, it is not
surprising that partially different sets of pro-
teins control their organization and dynam-
ic processes. Thus actin filaments appear to ex-
hibit a much larger diversity of 3D structures
and contribute to a wider range of cellular
functionalities than microtubules. This might at least partially explain
why cells have a larger number of functionally distinct actin-binding
proteins than microtubule-binding proteins.

Recent evidence indicates that several biochemically and func-
tionally distinct actin filament populations can be generated in cells
from a single actin isoform (Michelot and Drubin, 2011). Those di-
verse actin filament populations can result from the presence of dif-
ferent types of actin filament–nucleating proteins, which generate
filaments that subsequently are selectively decorated by distinct
sets of actin-binding proteins (Michelot et al., 2010; Johnson et al.,
2014). The most extensively studied actin filament–decorating pro-
teins that can alter the biochemical properties of actin are tropo-
myosins. Tropomyosins are a diverse family of long α-helical pro-
teins that bind to the main groove of an actin filament and form
continuous head-to-tail oligomers along filaments. In animals, >30
isoforms can be generated through alternative splicing from four
tropomyosin genes. When bound to filaments, tropomyosin iso-
forms specify the physicochemical properties of actin, and it has
been proposed that different tropomyosin isoforms can recruit spec-
ific downstream proteins to filaments and hence specify different
actin filament populations in cells (Gunning et al., 2015). In addition,
application of external force to actin filaments can affect their struc-
tural properties and thus regulate the binding of proteins to fila-
ments. This phenomenon was recently demonstrated for ADF/cofil-
ins, which selectively bind and sever only flexible actin filaments
while being unable to interact with actin filaments under tension
(Hayakawa et al., 2011). Moreover, studies on experimentally de-
finin actin structures in vitro revealed that the architecture and con-
nnectivity of the actin filament network can affect activities of actin-
associating proteins, including myosins (Reymann et al., 2012;
Ennomani et al., 2016). Thus actin-dependent cellular processes
may require several functionally distinct actin filament populations,
whose interactions with various actin-binding proteins can be further controlled by mechanical force and architecture of the filament network.

Finally, in multicellular organisms, actin filaments often display tissue- or cell-specific functions. Consequently, distinct isoforms of actin-binding proteins with biochemical activities fine-tuned to control filament dynamics or organize specific networks are expressed in a cell-specific manner. For example, unicellular organisms typically express only one actin filament-severing ADF/cofilin protein, but three biochemically distinct ADF/cofilin isoforms are found in mammals, each of which displays specific biochemical properties fine-tuned to control actin dynamics in the specific tissue or cell type where they are highly expressed (Vartiainen et al., 2002; Kremneva et al., 2014).

What lies ahead in actin-binding protein research for the next 40 years? Although during the past few decades we have certainly learned a great deal about the basic principles underlying actin dynamics, there remain several important unanswered questions. The majority of biochemical and cell biological studies on actin-binding proteins have focused on analyzing the functions of individual proteins. However, there is already evidence that, when examined in combinations with each other, actin-binding proteins can display striking new activities relating to the dynamics or organization of actin filaments. For example, binding of ADF/cofilin to actin filaments triggers a change in filament conformation that is critical for their rapid disassembly via the action of another actin-binding protein, Aip1 (McGough et al., 1997; Galkin et al., 2011; Gressin et al., 2015; Jansen et al., 2015). Furthermore, two actin filament barbed-end–binding proteins with opposing activities—heterodimeric capping protein and formins—were shown to interact with each other as a complex that controls actin filament elongation at rapidly growing barbed ends (Bombardier et al., 2015; Shekhar et al., 2015). Finally, it was demonstrated that different actin filament nucleation machineries compete for a limited pool of actin monomers in cells and that the actin monomer–binding protein profilin functions as a “gate keeper” between these nucleators. Profilin delivers actin monomers to power formin- and vasodilator-stimulated phosphoprotein (VASP)–driven filament elongation (Reinhard et al., 1995; Sagot et al., 2002; Evangelista et al., 2002; Romero et al., 2004). Of importance, recent studies revealed that a decrease in the abundance or activity of profilin in cells can lead to an increase in Arp2/3-induced actin filament assembly at the expense of formin- and VASP-polymerized actin filament structures, demonstrating that these different actin assembly machineries indeed compete with each other for actin monomers (Suarez et al., 2015; Rotty et al., 2015).

In the future, it will be important to examine the activities of actin-binding proteins in combination with each other and with other interacting proteins, as well as reconstitute different cellular actin-driven machineries in vitro to reveal the combined effects of various actin regulators in modulating network properties and functions. The latter was already achieved, for example, for the most crucial actin-binding proteins that are required for actin-based motility and for a membrane-associated actomyosin cortex (Loisel et al., 1999; Murrell and Gardel, 2012). However, reconstituting more complex cellular processes remains to be done. Finally, studies focusing on regulation of actin-binding proteins by different biochemical signals and mechanical cues are likely to lead to many surprising findings concerning the principles by which the actin cytoskeleton communicates with cellular signaling pathways and responds to changes in mechanical properties of the extracellular environment of cells.

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