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CELL SCIENCE AT A GLANCE

Cellular functions of the ADF/cofilin family at a glance

Georgios Kanellos* and Margaret C. Frame

ABSTRACT

The actin depolymerizing factor (ADF)/cofilin family comprises small actin-binding proteins with crucial roles in development, tissue homeostasis and disease. They are best known for their roles in regulating actin dynamics by promoting actin treadmilling and thereby driving membrane protrusion and cell motility. However, recent discoveries have increased our understanding of the functions of these proteins beyond their well-characterized roles. This Cell Science at a Glance article and the accompanying poster serve as an introduction to the diverse roles of the ADF/cofilin family in cells. The first part of the article summarizes their actions in actin treadmilling and the main mechanisms for their intracellular regulation; the second part aims to provide an outline of the emerging cellular roles attributed to the ADF/cofilin family, besides their actions in actin turnover. The latter part discusses an array of diverse processes, which include regulation of intracellular contractility, maintenance of nuclear integrity, transcriptional regulation, nuclear actin monomer transfer, apoptosis and lipid metabolism. Some of these could, of course, be indirect consequences of actin treadmilling functions, and this is discussed.

KEY WORDS: ADF, CFL1, CFL2, Actin, Actin depolymerizing factor, Cofilin

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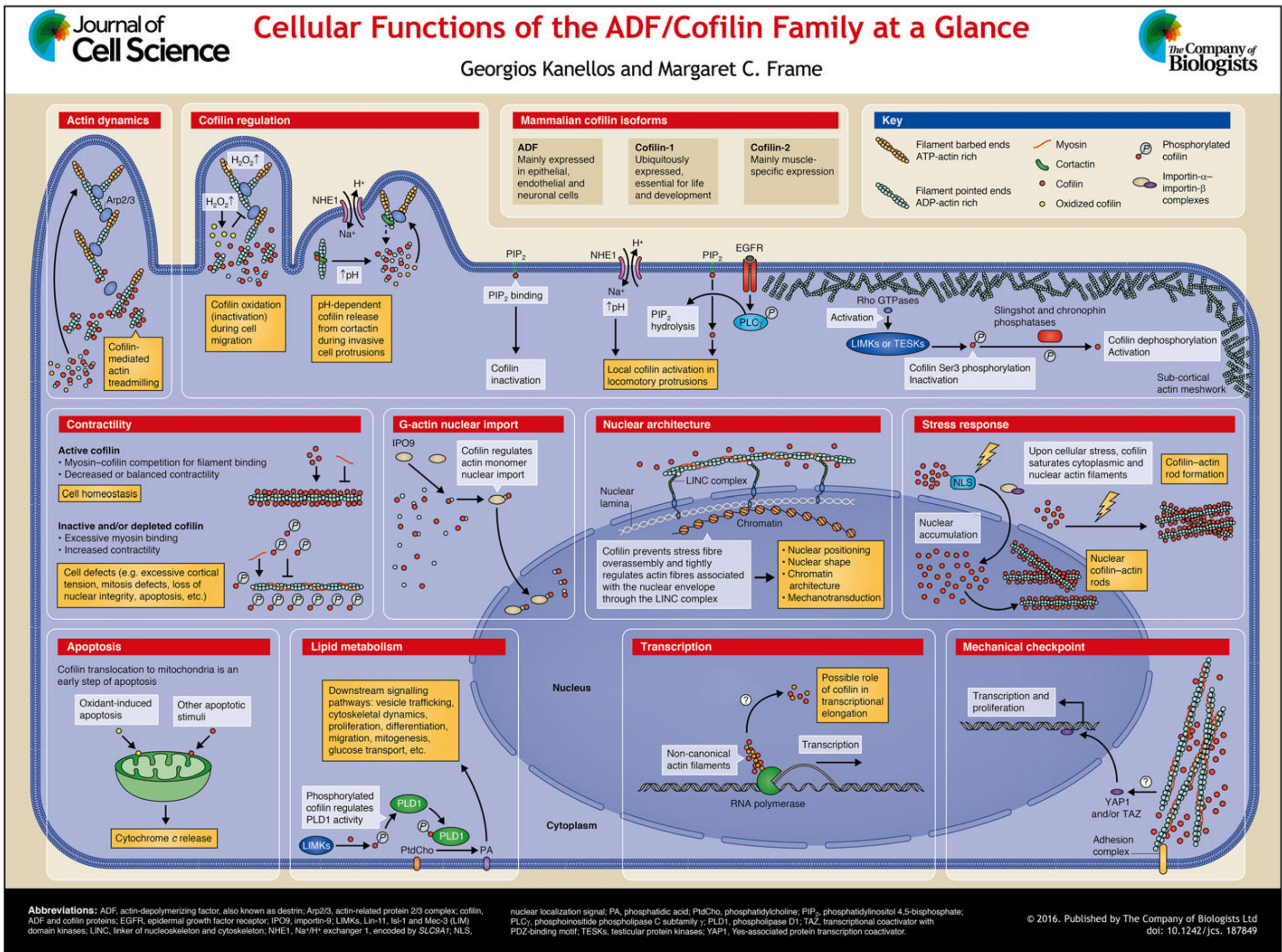
Introduction

Members of the actin depolymerizing factor (ADF)/cofilin family were first identified in the 1980s because of their ability to bind to actin (Bamburg et al., 1980). They are small proteins (~18 kDa) that



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Box 1. Physiological importance of ADF and cofilins

Cofilins are part of the minimum set of proteins that are essential for actin-based motility *in vitro* (Loisel et al., 1999), as well as for endocytosis, at least in yeast (Okreglak and Drubin, 2007). As a consequence, their importance in embryonic development, health and disease is evident. CFL1 deficiency is embryonic lethal in mice owing to defects in proliferation, polarization and migration of neural crest cells, with the latter leading to subsequent failure of neural tube closure (Bellenchi et al., 2007; Gurniak et al., 2005). ADF is unable to compensate for CFL1 loss *in vivo*, implying that they have non-redundant roles in brain development.

Mutations or deletion of ADF lead to development of cornea disease in mice (ADF is the predominant isoform expressed in cornea), which is marked by aberrant actin cytoskeleton arrangement, epithelial hyperproliferation and eventual blindness (Bellenchi et al., 2007; Ikeda et al., 2003; Verdoni et al., 2008).

Deletion of the genes encoding either ADF or CFL1 has no discernible effect on ureteric bud development in mice, but their co-depletion leads to severely perturbed branching morphogenesis and disrupted cell motility, cell shape and actin structures (Kuure et al., 2010). Moreover, double depletion of ADF and CFL1 (but not single deletion) in the skin epithelium of adult mice also leads to loss of tissue homeostasis, with extensive epidermal thickening, aberrant cell morphology with loss of cell–cell contacts, hyperproliferation and a pronounced accumulation of filamentous actin (Kanellos et al., 2015), indicating that there is some functional redundancy between ADF and CFL1 in some tissue contexts.

CFL2 mutations promote the development of myopathies in humans (Agrawal et al., 2007; Ockeloen et al., 2012). Genetic deletion of CFL2 in mice causes lethality eight days after birth due to severe muscle deficiencies and aberrant actin accumulation within muscle myofibrils, indicating that CFL2 has an important role in muscle development and maintenance (Agrawal et al., 2012). A role of CFL2 in actin monomer exchange in sarcomeres has also been proposed (Gurniak et al., 2014).

Cofilins have also been implicated to be involved at various stages of cancer development and to contribute to tumour progression, invasion and metastasis (Wang et al., 2007, 2004, 2006a). Cofilin could also be involved in the progression of neurodegenerative disorders owing to its role in formation of cofilin–actin rods in response to stimuli that affect normal neuronal function (Bamburg and Bernstein, 2016; Bamburg et al., 2010).

Box 2. Cofilin–actin binding and insights on actin severing

ADF and CFL1 bind to ADP-associated actin subunits with higher affinity compared to ATP- or ADP-P_i subunits, and accelerate P_i release; however, CFL2 has the ability to bind to the latter forms with higher affinity than ADF and CFL1 (Kremneva et al., 2014; Vartiainen et al., 2002; Yeoh et al., 2002). The ADP-actin regions of actin filaments (which represent the 'older' pointed ends, as opposed to the ATP-actin-rich regions that represent the 'newer' elongating barbed ends of the filaments) is where severing preferentially occurs at the boundaries between bare and cofilin-decorated parts of filaments (Suarez et al., 2011). Presumably, this is because cofilin binding results in changes in the properties and structure of the actin filaments, thus making them susceptible to fragmentation (Galkin et al., 2011, 2001; McGough et al., 1997). Hence, the actin-severing function of cofilin is directly related to its concentration relative to actin (the cofilin:actin ratio) at any given time and location. High concentrations of cofilin appear to be able to nucleate actin monomers, as well as to saturate and stabilize actin filaments, whereas lower ratios between cofilin and actin promote filament disassembly because cofilin is only bound sporadically to filaments (Andrianantoandro and Pollard, 2006). Other factors have recently been identified to cooperate with cofilin and to enhance the depolymerization of actin filaments. These factors include actin-interacting protein 1 (Aip1), twinfilin proteins, adenylyl cyclase-associated protein 1 (CAP1) and the coronins (Chen et al., 2015; Gressin et al., 2015; Johnston et al., 2015; Kueh et al., 2008; Mikati et al., 2015; Nadkarni and Brieher, 2014).

In addition to severing, cofilin also de-branches mainly 'older' ADP-bound actin filaments, whereas the Arp2/3 complex, which is the protein complex responsible for nucleating new filaments, introducing 70° branches on F-actin, preferentially binds to newly polymerized ATP-actin segments (Chan et al., 2009; Ichetovkin et al., 2002). However, although cofilin debranching activity has been studied and does occur, another cofilin-like molecule termed glia maturation factor (GMF, of which there are different isoforms) appears to be more specific for the Arp2/3 complex branches and is more efficient than cofilin in mediating debranching (Haynes et al., 2015; Poukkula et al., 2014; Ydenberg et al., 2013).

are widely recognized for their ability to regulate actin dynamics by severing filaments and enhancing their depolymerization. They are found in all eukaryotes, with most mammals expressing three isoforms; in humans and mice these are the actin depolymerizing factor (ADF, also known as destrin), cofilin-1 (CFL1) and cofilin-2 (CFL2) (Maciver and Hussey, 2002). All three are encoded from different genes, and ADF shares 70% amino acid identity with cofilins, whereas the latter share 80% identity at the amino acid level. The functions of individual actin depolymerizing factors are not always clear from the existing literature for several reasons. Many reports addressing the roles of cofilins do not specify which isoform is being studied or what the contributions of individual isoforms are in the context of total cellular actin depolymerizing activities, partly because it is thought that many of their functions overlap. In addition, antibodies often do not discriminate between different isoforms, and sometimes 'rescue experiments' have used cofilin from lower eukaryotes that express only one isoform. For these reasons and for simplicity, we refer to the group of actin depolymerizing factors collectively as cofilin(s), except in cases where the roles for specific isoforms have been defined.

Cofilins are required for normal development, and loss of their tight regulation has severe consequences on tissue homeostasis and organism health (see Box 1). This Cell Science at a Glance article and the accompanying poster aim to introduce non-expert readers to the family of cofilin proteins, and it will also serve as a summary of newer emerging roles and review the latest findings in cofilin research. Hence, we will briefly summarize the well-characterized roles of cofilins in actin treadmilling, the major mechanisms of their regulation and their impact on membrane protrusion and cell motility. Finally, we will discuss emerging aspects of cellular functions of cofilins that expand our knowledge of this crucial family of proteins, besides their direct actions in actin turnover.

Biochemistry, actin treadmilling and expression of cofilins

Expression of cofilins

Despite their similarity at the amino acid level, cofilins have different degrees of affinity for actin. Initial biochemical characterizations have revealed that although all mammalian isoforms have the ability to bind to filamentous (F)- and globular (G)-actin, ADF and CFL1 are able to bind to and promote steady-state F-actin disassembly to similar extents, whereas CFL2 is less efficient (Vartiainen et al., 2002; Yeoh et al., 2002). This biochemical difference was thought to reflect the fact that ADF and CFL1 are mainly expressed in tissues that benefit from a higher degree of actin turnover. Specifically, ADF is mainly expressed in neuronal, epithelial and endothelial cells, whereas CFL1 appears to be ubiquitously expressed in most adult tissues. CFL2, by contrast, is considered to be restricted to muscle tissue, which is not associated with high actin turnover (Gurniak et al., 2005; Vartiainen et al., 2002). However, a more recent study has implied that ADF, CFL1 and CFL2 all bind to G- and F-actin with similar affinities,

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and, interestingly, ADF and CFL2 are able to sever actin filaments more efficiently compared to CFL1 (Chin et al., 2016). Furthermore, lack of a readily available antibody against CFL2 has delayed deeper understanding of the role of this isoform. We now know that many tissues express all three isoforms, including oligodendrocytes, keratinocytes and cancerous tissue of non-muscle origin (Kanellos et al., 2015; Zuchero et al., 2015), and that CFL2 is a major constituent in human peritoneal mesothelial cells (Herzog et al., 2015). It is possible that tissues that express multiple isoforms might fine tune their expression levels individually in order to tightly control the rates of actin turnover, whereas the types of accessory protein that decorate actin filaments and the expression patterns of cofilin ancillary proteins (see Box 2) modify their efficiency. In turn, this could be linked to their tissue-specific functions. Information on the mechanism of cofilin-mediated actin-filament severing can be found in Box 2.

Localization

By severing, de-branching and enhancing the depolymerization of 'older' filaments, cofilin increases the availability of filament ends, as well as the actin monomer pool, thereby promoting filament and dendritic nucleation and/or elongation (see also Box 2). This makes cofilins pivotal regulators of actin-based membrane protrusion and cell locomotion (reviewed in Bravo-Cordero et al., 2013). However, despite their similarity, it is still under debate whether each isoform fulfils unique roles at the cell periphery; a recent study suggests that they do because ADF or CFL1 depletion leads to characteristic differences in cell migration, focal adhesion turnover and formation of aberrant actin structures for each isoform (Tahtamouni et al., 2013).

In keeping with their role in remodelling the actin cytoskeleton, ADF and CFL1 are enriched in sub-cellular locations that are associated with high actin turnover, specifically in ruffling membranes or actin-based membrane protrusions at the leading edges of motile cells (lamellipodia, invadopodia, etc.), neuronal axons and the contractile rings formed during the final stages of mitosis (Bamburg, 1999; Maciver and Hussey, 2002; Vartiainen et al., 2002). CFL2, by contrast, is predominantly localized between the Z-discs in muscle sarcomeres, where it regulates the length of actin filaments as a result of its ability to bind to and disassemble ATP- or ADP-P_i-bound filaments with greater efficiency when compared with that of ADF or CFL1 (in sarcomeres the barbed ends of the filaments are capped, and actin subunit exchange and length maintenance is primarily restricted to the pointed ends that might contain a mixture of ADP-, as well as ATP- or ADP-P_i-bound subunits) (Kremneva et al., 2014). However, cofilins also contain a nuclear localization sequence (NLS) and are able to translocate to the nucleus, and there is increasing evidence of their involvement in nuclear function (see below).

Regulation of cofilins

Phosphorylation and dephosphorylation

The activity of cofilins is regulated by a plethora of mechanisms, including phosphorylation on residue Ser3 by LIM kinases (LIMK1 and LIMK2) (reviewed in Scott and Olson, 2007) and TES kinases (TESK1 and TESK2), which inhibits their interaction with actin (Toshima et al., 2001a,b) (see poster). The predominant upstream regulators of LIMK activity are the Rho family of small GTPases and the Rho-associated effector kinases. Specifically, phosphorylation (activation) of LIMKs can be downstream of the Rho and Rho-associated protein kinase (ROCK) pathway (Maekawa et al., 1999; Ohashi et al., 2000) or of Rac or Cdc42 that signal through p21-activated kinase (PAK) or myotonic dystrophy kinase-related Cdc42-binding kinase α (MRCKα) activity (Dan et al., 2001; Edwards et al., 1999; Sumi et al., 2001; Yang et al., 1998). LIMKs are also downstream of mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2) activity in endothelial cells (Kobayashi et al., 2006). The regulation of TES kinases is understood to a much lesser extent.

Dephosphorylation of Ser3 leads to cofilin activation. The main protein phosphatases known to activate cofilin are slingshot (Niwa et al., 2002) and chronophin (encoded by *PDXP*) (Gohla et al., 2005), although the more generic serine/threonine phosphatases type 1 and type 2A have also been reported to dephosphorylate cofilin (Ambach et al., 2000). Slingshot phosphatases can also interact with and dephosphorylate LIMKs, which inactivates them, thereby adding another level of regulation of cofilin activity (Soosairajah et al., 2005). The phosphorylation-dependent regulation of cofilin is likely to be context-specific and is still incompletely understood.

PIP_2 binding

One of the first cofilin regulatory mechanisms to be identified was phosphatidylinositol 4,5-bisphosphate (PIP_2) binding, which inhibits the interaction of cofilin with actin as they both interact with the same region on the surface of cofilin (Yonezawa et al., 1990; Zhao et al., 2010) (see poster). Cofilin clusters PIP_2 molecules at the membrane through its interaction with multiple PIP_2 headgroups; hence, changes in PIP_2 density can effectively regulate cofilin activity at the membrane (Zhao et al., 2010). Chemoattractants, such as epidermal growth factor (EGF), promote the local activation of cofilin by stimulating its release from PIP_2, following PIP_2 hydrolysis that is triggered by phospholipase C (PLC). In turn, this local cofilin activation induces the formation of membrane protrusions and mediates directional cell motility and chemotaxis (Mouneimne et al., 2006, 2004; van Rheenen et al., 2007). Even the local activation of a chemically modified inactive form of cofilin is sufficient to drive localized protrusions and directional cell migration (Ghosh et al., 2004). Regulation of cofilin through PIP_2 binding appears to be confined to subcortical actin networks and is independent of LIMKs (Song et al., 2006). In fact, the general inhibition of cofilin through LIMK-mediated phosphorylation acts in concert with its local activation through release from phospholipids to permit cellular directional sensing. This is one possible explanation of why LIMK inhibition, which generally leads to higher levels of active (unphosphorylated) cofilin, results in loss of cell directionality and impaired invasion in three-dimensional matrices (Mouneimne et al., 2006; Scott et al., 2010).

Regulation through pH

Cofilin activity is also affected by the intracellular pH, and the Na^+-H^+ exchanger NHE1 (encoded by *SLC9A1*) plays an important role in this regulation. Firstly, the cofilin-induced clustering of PIP_2 (see above) is sensitive to pH, with higher pH inhibiting clustering; this can decrease PIP_2 density at the membrane, leading, in turn, to increased local cofilin release and activation, and pH-dependent membrane protrusions and motility in response to growth factor stimulation (Frantz et al., 2008; Zhao et al., 2010). Secondly, cofilin activities, with respect to actin severing and filament depolymerization, are regulated by pH, with cofilin being more potent at increased pH (pH 8) (Yeoh et al., 2002). Thirdly, local pH variations influence the binding of cofilin to cortactin, another cofilin-interacting protein, evident in invasive protrusions of breast cancer cells. An increase in pH, mediated by NHE1, results in the

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release of cofilin from cortactin; this activates cofilin and promotes cell invasion (Magalhaes et al., 2011).

Other regulatory mechanisms

Other types of regulation of cofilins that have been reported include phosphorylation of CFL1 by viral (v)-Src at Tyr68 (a residue that is not present in ADF), which 'marks' the protein for ubiquitylation and subsequent degradation through the ubiquitin–proteasome pathway (Yoo et al., 2010). It is unclear whether this represents a physiological mechanism that is normally carried out by cellular (c)-Src. Furthermore, oxidation of cofilin cysteine residues in motile cells regulates its binding to actin and affects cell motility (Cameron et al., 2015). The same modification has been reported to influence cofilin activities during apoptosis (see Apoptosis section below). Lastly, mechanosensitive regulation of cofilin actin binding and severing activity has been reported (Hayakawa et al., 2011; Tojkander et al., 2015). Cofilin appears to preferentially bind to less-tensile actin filaments and to mediate their degradation, whereas filaments under tension are protected from cofilin-mediated fragmentation. This mechano-regulation has been shown to be important for the maturation of contractile stress fibres in cells (Tojkander et al., 2015).

New and emerging roles of cofilins

Contractility

Co-depletion of ADF and CFL1 in HeLa cells causes the accumulation of abnormal F-actin structures, which is mediated by excessive myosin-II activity (Wiggan et al., 2012). It has long been known that cofilin and myosin and/or tropomyosin compete for binding to actin filaments (Nishida et al., 1984), and this could be important for distinguishing actin filaments on the basis of distinct properties and molecular composition (Bryce et al., 2003). However, a physiological relevance of this competition has only recently been reported, where it was shown to regulate intracellular contractility, which if left unchecked in the absence of ADF and CFL1, leads to excessive force generation and multiple cellular defects, such as unrestrained membrane blebbing, as well as impaired chromosome segregation and cell division (Wiggan et al., 2012). The effects are less prominent when ADF is depleted alone and are most severe after co-depletion of both ADF and CFL1, implying functional redundancy in this cellular context (Hotulainen et al., 2005; Wiggan et al., 2012). Increased contractility following ADF and CFL1 co-depletion has also recently been reported, where it was shown that a similar mechanism involving tight control of contractile actin fibres by cofilins regulates nuclear architecture and ultimately cell viability (see below) (Kanellos et al., 2015). Interestingly, another consequence of dysregulation of the actin cytoskeleton occurs during neuritogenesis. In differentiating neurons, the ADF- and CFL1-mediated remodelling of the cortical actin cytoskeleton facilitates normal neurite differentiation. ADF and CFL1 loss results in an aberrant actin cytoskeleton with a denser, presumably more tensile, actin cortex that obstructs appropriate microtubule positioning and protrusion, blocking neuritogenesis (Flynn et al., 2012).

Apoptosis

During induction of apoptosis with staurosporine, active (lacking phosphorylation of Ser3) cofilin can translocate to mitochondria before cytochrome *c* release, and this has been shown to be crucial for the initiation of cell death (Chua et al., 2003). TGF-β-induced apoptosis in human prostate cancer cells also requires mitochondrial translocation of cofilin (Zhu et al., 2006). Although cofilin action during induction of apoptosis (albeit not completely understood) might suffice for the initiation of the process, other actin-binding proteins could be involved, for example CAP1 (Wang et al., 2008). Furthermore, oxidation of cysteine residues in active cofilin is essential for its translocation to mitochondria during oxidant-induced apoptosis. Following its translocation, cofilin promotes cytochrome *c* release by affecting the permeability of the mitochondrial permeability transition pore (Klamt et al., 2009). However, cofilin oxidation is dispensable when apoptosis is induced by stimuli other than reactive oxygen species (Klamt et al., 2009) (see poster). Translocation of cofilin to mitochondria also mediates amyloid-β-induced neurotoxicity in a process that involves Ran-binding protein 9 (Woo et al., 2015). In addition to these roles at early stages of apoptosis, cofilin might also be involved in the regulation of apoptosis-associated morphologies during the later stages, such as in apoptosis-associated bleb formation (Mannherz et al., 2005).

Nuclear actin transport

Actin regulates fundamental nuclear processes, such as transcription, reprogramming and gene activation (reviewed in Miyamoto and Gurdon, 2013). Hence, cells appear to have evolved very sophisticated mechanisms for tight regulation of the balance between the amount and state of actin in both the cytoplasm and nucleus. One of the mechanisms that contribute to the maintenance of nuclear actin levels is mediated by an interaction between cofilin and importin-9 (Dopie et al., 2012) (see poster). Actin does not have a classic NLS, whereas cofilins have a bipartite NLS that allows for their efficient shuttling into the nucleus (Munsie et al., 2012). Importin-9 interacts with actin in a cofilin-dependent manner (likely to be mediated through the cofilin NLS), and this interaction is crucial for the nuclear transport of actin, which in turn influences the level of transcription (see also below) (Dopie et al., 2012).

Transcription

As mentioned above, nuclear actin and cofilin influence transcriptional activity in cells. This is supported by the fact that CFL1 is part of the RNA polymerase II transcriptional machinery (Obrdlik and Percipalle, 2011). Specifically, CFL1 has been found to have a role in transcriptional elongation of gene-coding sequences and apparently does not associate with untranslated regions or promoters. Silencing of CFL1 leads to significantly reduced transcriptional activity, and the association of both RNA polymerase II and actin with gene coding regions is also affected, highlighting the importance of CFL1 as part of the transcriptional apparatus (Obrdlik and Percipalle, 2011). Interestingly, restoring nuclear actin levels independently of cofilin does not restore normal transcription, suggesting that cofilin is not simply controlling transcription through regulation of monomeric actin availability (Dopie et al., 2012).

Nuclear architecture

The depletion of both ADF and CFL1 leads to severe perturbation of cellular and adult tissue homeostasis, which is marked by the uncontrolled assembly of contractile actin filaments and consequent increase in tension that promotes nuclear deformation and loss of cell viability (Kanellos et al., 2015). A specialized subset of contractile actin fibres (which are tethered into focal adhesions) pass over the nucleus and regulate nuclear shape – these are collectively termed the 'actin cap' (Khatau et al., 2009; Kim et al., 2012). The linker of nucleoskeleton and cytoskeleton (LINC) complex, which localizes to the nuclear envelope, physically connects these actin

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cap fibres with the nuclear lamina (reviewed in Starr and Fridolfsson, 2010). Disruption of this LINC-complex-mediated connection relieves nuclei from the increased forces exerted on them by the aberrant tensile filaments that arise after ADF and CFL1 depletion, and restores nuclear integrity and normal shape (Kanellos et al., 2015). This implies that the actin cap fibres are regulated by cofilins in cells and that this regulation is crucial for nuclear positioning and migration, as well as probably all the cellular features and responses that are controlled by the link between the actin cytoskeleton and the nuclear envelope (Starr and Fridolfsson, 2010) (see poster). Interestingly, increased tension in actin filaments that are linked to the nuclear lamina promotes the formation of indentation sites in the nuclei of normal endothelial cells, and this affects chromatin condensation (Versaevel et al., 2014). This emphasizes the importance of tight regulation between actin filaments and acto-myosin contractility, mediated by cofilins, for nuclear morphology and function, including chromosome regulation.

Lipid metabolism

Cofilin that is phosphorylated at Ser3 was long considered to be an inactive form of cofilin without any biological function; however, this might not be the case as it has been shown to interact directly with phospholipase D1 (PLD1) (Han et al., 2007). Phospholipase D proteins comprise a family of enzymes that catalyse the conversion of phosphatidylcholine (PtdCho), a phospholipid found in biological membranes, to phosphatidic acid (PA), thereby triggering downstream signalling (reviewed in Exton, 2002; Wang et al., 2006b). PLD1 activity is regulated by Rho signalling (Schmidt et al., 1999), which also controls the activity of LIMKs and subsequent cofilin phosphorylation. The interaction of cofilin phosphorylated at residue Ser3 with PLD1 is required for the translocation of PLD1 to the plasma membrane and the stimulation of its activity (Han et al., 2007). The activity of PLD1 can be inhibited by constitutively active cofilin (Ser3 to Ala mutation), silencing of cofilin expression or overexpression of slingshot phosphatase, whereas it is enhanced by overexpression of cofilin or a mimetic of cofilin that is phosphorylated at Ser3 (Ser3 to Asp mutation). This confirms that even in its phosphorylated, presumed inactive form, cofilin is likely to fulfil important biological roles (Han et al., 2007).

Mechanical checkpoint

Another emerging role for cofilins is the regulation of cell proliferation in response to mechanical cues, which promote cofilin-mediated actin cytoskeleton remodelling, in turn influencing the activity of the transcriptional co-activators YAP1 and TAZ (encoded by *WWTR1*) (Aragona et al., 2013). Activity of the YAP and TAZ pathway is crucial for cell proliferation, during development, and has also been implicated in various diseases such as cancer (reviewed in Piccolo et al., 2014). Increased mechanical tension upon cofilin depletion, and subsequent stabilization of actin stress fibres, promotes the nuclear localization of YAP and TAZ, and enhances transcription and proliferation (Aragona et al., 2013). Conversely, cytoskeleton remodelling, or release of tension through chemical inhibition of contractility, inhibits proliferation, and these responses are independent of the classic Hippo pathway that is mediated by the effector kinases MST1 and MST2 (also known as STK4 and STK3, respectively), and LATS1 and LATS2 (Aragona et al., 2013). This crosstalk between the actin cytoskeleton and the YAP and TAZ pathway has been shown to be the main driver of a subset of uveal and skin melanomas. Cells from these uveal melanomas have reduced levels of cofilin activity, which promote actin cytoskeleton stability and activation of YAP. YAP activation is sensitive to inhibition of either contractility or actin polymerization in this context as well (Feng et al., 2014).

Taken together, these findings demonstrate that cells can sense the architecture of the microenvironment and respond to mechanical cues by cofilin-dependent remodelling of the actin cytoskeleton, eliciting responses that are vital for cell fate and tissue homeostasis.

Stress response

As well as an NLS, cofilin also has a nuclear export signal (NES), which mediates its transport out of the nucleus (Munsie et al., 2012). Under stress conditions, such as heat shock, osmotic stress or ATP depletion, cofilin accumulates in the nucleus where it excessively binds to, and saturates, actin filaments, resulting in the formation of cofilin–actin ‘rods’. Although this has been shown to occur mainly inside the nucleus, cytoplasmic rod formation has also been observed (Bamburg, 1999; Munsie et al., 2012). Rod formation is involved in the development of neurodegenerative disorders, such as Huntington’s and Alzheimer’s, as the cofilin–actin rods are part of the protein inclusions that contribute to the disruption of neurite function (Bamburg and Bernstein, 2016; Minamide et al., 2010, 2000). Although not completely understood, it has been proposed that rod formation under conditions of stress could serve as a means for the cell to prevent excessive ATP hydrolysis associated with actin filament turnover, which would make ATP unavailable for other cellular stress responses (Bernstein et al., 2006). Because cofilin–actin rod formation is a rapid response when cells are stressed *in vitro*, it has been suggested that rod formation could be a potential candidate for the targeted treatment and prevention of neuronal degenerative disorders (Bamburg et al., 2010).

Concluding remarks

Cofilins have long been implicated in a diverse and multifaceted array of cellular functions and, as discussed above, many new and unexpected roles have emerged. We think it likely that many more will emerge in the coming years. However, it remains to be established whether the ‘newer’ biological roles of cofilins are a direct result of their actin-severing activities or whether these proteins also have as yet unappreciated activities as adaptor proteins and so contribute to the scaffolding of specific molecular complexes in space and time. Despite a wealth of information on their cytoplasmic functions, the nuclear roles of cofilins (and indeed of actin itself) are only now becoming clearer. Roles of nuclear actin appear to include its association with all three RNA polymerases, as well as in controlling the movement of entire chromosomes (Chuang et al., 2006; Miyamoto and Gurdon, 2013), yet the regulation of these activities and the consequences of their misregulation for health and disease are not well understood. We postulate that cofilins are likely to contribute to the activities of nuclear actin, and future work will surely address this.

There is evidence that deformability of the nuclear envelope is a property of cancer cells that facilitates successful cell migration and invasion through complex ‘crowded’ environments, and actin mediates this deformability (Davidson et al., 2014; Thiam et al., 2016). This is in keeping with our own data that the activities of ADF and CFL1 in cancer cells are required for maintenance of cell shape, nuclear integrity and cell viability, and loss of ADF and CFL1 causes irreversible nuclear deformation (Kanellos et al., 2015). Taken together, the current evidence points to a growing list of diverse biological roles for cofilins, as well as to the crucial nature

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of their actions and their multi-layered regulation that are frequently perturbed in disease.

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