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**Emerging roles of MICAL family proteins: from actin oxidation to membrane traffic during cytokinesis.**

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## **Abstract**

Cytokinetic abscission is the terminal step of cell division, leading to the physical separation of the two daughter cells. The exact mechanism mediating the final scission of the intercellular bridge connecting the dividing cells is not fully understood, but requires the local constriction of ESCRT-III-dependent helices, as well as remodelling of lipids and the cytoskeleton at the site of abscission. In particular, microtubules and actin filaments must be locally disassembled for successful abscission. However, the mechanism that actively removes actin during abscission is poorly understood. In this Commentary, we will focus on the latest findings regarding the emerging role of the MICAL family of oxidoreductases in F-actin disassembly and describe how Rab GTPases regulate their enzymatic activity. We will also discuss the recently reported role of MICAL1 in controlling F-actin clearance in the ESCRT-III-mediated step of cytokinetic abscission. In addition, we will highlight how two other members of the MICAL family (MICAL3 and MICAL-L1) contribute to cytokinesis by regulating membrane traffic. Taken together, these findings establish the MICAL family as a key regulator of the actin cytoskeleton dynamics and membrane traffic during cell division.

## Introduction

Faithful cell division is crucial for the maintenance of genomic integrity, development, and tissue homeostasis. At the end of cell division, cytokinesis drives the physical separation of the two daughter cells. Cytokinesis begins after chromosome segregation with the formation of a cleavage furrow at the equatorial region. This process involves large-scale deformations of the plasma membrane and is driven by a contractile ring consisting of F-actin and myosin II (Fededa and Gerlich, 2012; Green et al., 2012; Sagona and Stenmark, 2010). After completion of furrowing, the two daughter cells are connected by a microtubule-filled intercellular bridge, which is eventually cut by a mechanism named abscission (Mierzwa and Gerlich, 2014). The machinery that controls cytokinetic abscission assembles on the side of the midbody, a protein-rich structure localized at the centre of the intercellular bridge (Crowell et al., 2014; Elia et al., 2011; Lafaurie-Janvore et al., 2013; Mullins and Biesele, 1977; Schiel et al., 2011). The final scission event is thought to be driven by endosomal sorting complex required for transport (ESCRT)-III-dependent helices that assemble into spirals that pinch the plasma membrane at the abscission site (Guizetti et al., 2011; Mierzwa and Gerlich, 2014; Sherman et al., 2016).

Membrane trafficking within the intercellular bridge plays a crucial role in cytokinetic abscission by locally remodelling both the cytoskeleton and lipids (Cauvin and Echard, 2015; Mierzwa and Gerlich, 2014; Montagnac et al., 2008; Schiel and Prekeris, 2012). To allow constriction of the plasma membrane by the ESCRT machinery, it is indeed important that cytoskeletal elements, such as F-actin and microtubules, are removed from the abscission site (Mierzwa and Gerlich, 2014). Microtubules are cleared by buckling (Schiel et al., 2011) and/or by the microtubule-depolymerizing ATPase Spastin (an enzyme recruited by the ESCRT machinery) (Connell et al., 2009; Yang et al., 2008). However, the mechanisms that remove F-actin in the intercellular bridge are not fully understood. Regarding actin clearance, the small GTPases Rab11A (Schiel et al., 2012) and Rab35 (Dambournet et al., 2011; Klinkert and Echard, 2016) function in parallel to limit polymerisation of F-actin and thus its accumulation within the intercellular bridge. Recently, we revealed an unexpected role for oxidoreduction in triggering local actin depolymerization during cytokinesis (Frémont et al., 2017). We showed that the oxidase MICAL1 is critical for actively depolymerizing F-actin within the intercellular bridge in order to promote the assembly of ESCRT-III filaments and successful abscission.

In this Commentary, we will discuss how the MICAL family members can specifically disassemble actin filaments by oxidation and how their redox-enzymatic activity can be regulated by Rab GTPases. We will also review the physiological roles of this emerging class of enzymes in actin-dependent functions, with a special emphasis on their recently

demonstrated implication in cytokinesis (Frémont et al., 2017; Liu et al., 2016; Reinecke et al., 2015). Other biological aspects of the MICAL family are also mentioned and covered in detail in excellent reviews (Giridharan and Caplan, 2014; Wilson et al., 2016).

### **The MICAL-family proteins: structure, domain organization and cellular functions**

The first members of the MICAL family (molecule interacting with CasL), human MICAL1 and *Drosophila* Mical, were discovered independently in 2002. Human MICAL1 was found in a screen for CasL-interacting protein in a thymus cDNA library (Suzuki et al., 2002), and *Drosophila* Mical in a screen for proteins binding to the cytoplasmic domain of the Semaphorin receptor Plexin A (Terman et al., 2002). While in *Drosophila*, there is only one *Mical* gene, two more *MICAL* genes (*MICAL2* and *MICAL3*) were identified in vertebrates (Fischer et al., 2005; Pasterkamp et al., 2006; Suzuki et al., 2002; Terman et al., 2002; Weide et al., 2003). In addition, MICAL-like encoding genes have been described: one in *Drosophila* (*Mical-L*), and two in mice and humans (*MICAL-L1* and *MICAL-L2*) (Nakatsuji et al., 2008; Sharma et al., 2010; Terai et al., 2006; Terman et al., 2002) (Fig. 1). Members of the MICAL family are multidomain proteins that contain an N-terminal flavoprotein monooxygenase (MO) domain essential for the actin-depolymerizing activity (Alqassim et al., 2016; Nadella et al., 2005; Siebold et al., 2005) (see below); a calponin homology (CH) domain typical of actin binding proteins; a LIM domain (Lin-11, Isl-1 and Mec-3); and a Rab binding domain (RBD) with motifs initially proposed to form a coiled-coil domain (Giridharan and Caplan, 2014; Wilson et al., 2016) (Fig. 1).

Pioneer work demonstrated that *Drosophila* Mical is involved in Plexin-mediated axonal repulsion *in vivo*, downstream of the Semaphorin 1a (Sema-1a)-Plex A pathway (Terman et al., 2002). Remarkably, MICAL uses its enzymatic domain to directly bind and oxidize F-actin in order to disassemble the actin cytoskeleton during repulsive axon guidance (Hung et al., 2011; Hung et al., 2010; Terman et al., 2002). MICAL family proteins have now been implicated in many key cellular functions that depend on dynamic actin cytoskeleton remodelling (Fig. 2). For example, MICAL proteins are required for several aspects of neuronal biology (Beuchle et al., 2007; Bron et al., 2007; Hung et al., 2013; Hung et al., 2010; Kirilly et al., 2009; Lundquist et al., 2014; Luo et al., 2011; Morinaka et al., 2011; Pasterkamp et al., 2006; Schmidt et al., 2008; Terman et al., 2002; Van Battum et al., 2014), cell viability (Ashida et al., 2006; Loria et al., 2015; Zhou et al., 2011), cancer (Deng et al., 2016; Loria et al., 2015; Mariotti et al., 2016), immunity (Lee et al., 2013), skeletal muscle morphology and function (Beuchle et al., 2007; Hung et al., 2013), cardiovascular integrity

(Yang et al., 2015), bristle development (Hung et al., 2011; Hung et al., 2013), cell shape (Aggarwal et al., 2015; Giridharan et al., 2012b), membrane trafficking (Bachmann-Gagescu et al., 2015; Grigoriev et al., 2011), and regulation of nuclear actin (Lundquist et al., 2014). The newly described roles of MICAL family members in cytokinesis will be discussed in detail below.

MICAL-like proteins have been implicated so far in the endocytic pathway (Abou-Zeid et al., 2011; Bahl et al., 2016; Cai et al., 2014; Giridharan and Caplan, 2014; Rahajeng et al., 2010; Rahajeng et al., 2012; Reinecke et al., 2015; Sharma et al., 2010; Sharma et al., 2009; Terai et al., 2006), exocytosis (Sun et al., 2016), phagosomal maturation (Dumas et al., 2015), synaptic development (Nahm et al., 2016), neurite outgrowth (Kobayashi et al., 2014a; Kobayashi et al., 2014b; Kobayashi and Fukuda, 2013), cell division (Reinecke et al., 2015), cell shape (Kanda et al., 2008; Sakane et al., 2012; Sakane et al., 2013), cell junctions formation (Nakatsuji et al., 2008; Sakane et al., 2012; Yamamura et al., 2008), cell migration (Sakane et al., 2016) and cancer development (Ioannou et al., 2015; Zhu et al., 2015). As MICAL-like proteins lack the N-terminal monooxygenase domain involved in F-actin oxidation and disassembly, we will essentially focus this Commentary on MICAL proteins. More details about MICAL-like functions can be found in previous reviews (Giridharan and Caplan, 2014; Rahajeng et al., 2010).

### **Mechanisms of MICAL-induced actin-depolymerization**

Knowing the precise mechanism of how MICAL proteins act on the actin cytoskeleton and how their activities are fine-tuned in space and time are essential for understanding the physiological functions of MICALs in normal cells, as well as in the context of disease (Wilson et al., 2016).

The N-terminal monooxygenase domain of MICAL proteins is structurally related to the nicotinamide adenine dinucleotide phosphate (NADH)-dependent monooxygenase domain of the flavin adenin dinucleotide (FAD)-containing enzyme *p*-hydroxybenzoate hydroxylase (PHBH) (Cole et al., 2005; Nadella et al., 2005; Siebold et al., 2005). MICAL enzymes bind FAD and use NADPH and O<sub>2</sub> in redox reactions that lead to F-actin disassembly (Hung et al., 2011; Hung et al., 2010; Lundquist et al., 2014; Schmidt et al., 2008; Vitali et al., 2016). Several mechanisms have been proposed to explain how MICALs induce F-actin disassembly. As a monooxygenase enzyme, MICALs produce reactive oxygen species (ROS) such as H<sub>2</sub>O<sub>2</sub> *in vitro* and *in vivo* (Giridharan and Caplan, 2014; Morinaka et al., 2011; Nadella et al., 2005; Zhou et al., 2011). *In vitro* experiments show that ROS molecules can modify cysteine, methionine and tryptophan residues of actin, which

affects actin polymerization and depolymerisation (Fedorova et al., 2010; Milzani et al., 2000; Nadella et al., 2005). Thus, MICAL1 could promote F-actin depolymerization through the production of locally high levels of diffusible  $H_2O_2$ , which has been shown to increase when F-actin binds to MICAL (Vitali et al., 2016). In contrast to the  $H_2O_2$  production hypothesis, MICALs have been found to use F-actin as a direct substrate to oxidize two actin methionines (M44 and M47) into methionine sulfoxide (Hung et al., 2011; Hung et al., 2010). In addition, a direct contact between MICALs and F-actin appears to be compulsory for depolymerization, whereas incubation with high concentrations  $H_2O_2$  (40 mM) alone are insufficient to depolymerize actin filaments (Frémont et al., 2017; Hung et al., 2011). Interestingly, MICAL-mediated actin oxidation is selectively reversed by methionine sulfoxide reductases (SelR in *Drosophila*, MsrBs in mammals), which prevent F-actin disassembly induced by MICAL *in vitro* (Hung et al., 2013; Lee et al., 2013). Consistently, SelR counteracts Mical in multiple actin-dependent cellular processes in *Drosophila*, including axon guidance, synaptogenesis, muscle organization and mechanosensory development (Hung et al., 2013). In mammals, MsrB1 has a regulatory role as a MICAL1 antagonist in orchestrating actin dynamics and macrophage function (Lee et al., 2013). Whether SelR and MsrBs also counteract MICAL1 function during cytokinesis is an open question that should be addressed in future studies.

Mechanistically, it has been proposed that the selective oxidation of methionines 44 and 47 in F-actin results in filament disassembly as it affects key residues at the interface between two successive actin subunits in actin filaments (Hung et al., 2011). Interestingly, *in vitro* total internal reflection fluorescence (TIRF) microscopy experiments using single fluorescently-labelled actin filaments attached on a coverslip by immobilized myosins showed that *Drosophila* Mical induces severing of actin filaments, defining Mical as an F-actin severing enzyme (Hung et al., 2011). We recently reinvestigated the mechanism of actin disassembly by human MICAL1 and *Drosophila* Mical. Surprisingly, we found that MICAL1 induces rapid depolymerization from both ends of the filaments with no sign of severing (Frémont et al., 2017). This was observed both when filaments were immobilized as previously described (Hung et al., 2011), or when filaments were attached to microfluidic chambers by one of their ends (Frémont et al., 2017). We also found that, once oxidized by MICAL1, actin filaments depolymerize at a high rate even when the enzyme is removed from the solution (Frémont et al., 2017). Altogether, these observations are consistent with a mechanism of MICAL oxidation weakening the longitudinal interactions between actin subunits within filaments, which makes the filaments more fragile and leads to enhanced F-actin depolymerization rates from both ends (Fig. 3). In agreement with our observations, recent *in vitro* experiments using *Drosophila* Mical also show little severing activity, and instead report an enhanced susceptibility of oxidized actin filaments to fragmentation-

inducing conditions, such as mechanical stress (pipetting) or severing by cofilin (Grintsevich et al., 2016). In light of these newer data, the originally observed severing activity might have resulted from experimental stresses, such as interactions of actin filament with the surface they were attached to, a high fluorescent labelling and/or illumination conditions.

### **Oxidation-dependent depolymerization of F-actin by MICAL1 during cytokinetic abscission**

Until recently and despite the importance of MICAL-mediated actin-oxidation in F-actin dynamics, nothing was known with regard to its potential role during cell division. As already mentioned, local F-actin depolymerization is critical for cytokinetic abscission. In our recent study, we have now revealed that successful abscission critically relies on F-actin clearance by MICAL1 in human cells and by Mical in *Drosophila* cells (Frémont et al., 2017). First, using genome-edited cell lines, we found that MICAL1 localizes first to the midbody then in a zone closely apposed to the abscission site just before the final cut. Functionally, MICAL1-depletion induces a local accumulation of F-actin at the bridge and induces a dramatic bulging of the plasma membrane and cortex around the midbody as assessed by correlative light-microscopy and scanning electron microscopy EM (for practical details, see Crowell et al., 2014; Fremont and Echard, 2017). This accumulation of F-actin in bridges owing to MICAL1 depletion delays and sometimes completely inhibits abscission. In agreement with this, reducing F-actin levels in intercellular bridges by using non-toxic, low doses of Latrunculin-A restores normal abscission kinetics in MICAL1-depleted cells. Importantly, the abscission defects observed upon MICAL1 depletion can be rescued by the expression of GFP-tagged, siRNA-resistant MICAL1, but not by a redox-dead version of MICAL1 that is unable to oxidize F-actin. Taken together, these results demonstrate that oxidation-dependent F-actin depolymerization in the bridge by MICAL1 is required for successful abscission (Fig. 4). It should be pointed out that approximately half of the MICAL1-depleted cells undergo abscission with normal timing, suggesting that additional mechanisms yet unknown must exist in order to clear F-actin from intercellular bridges in the absence of MICAL1 (Frémont et al., 2017). This could be achieved by other, partially redundant pathways that either promote actin depolymerization or inhibit actin polymerization.

It has been proposed that the accumulation of F-actin in bridges inhibits the assembly of ESCRT-III-filaments or their constriction at the abscission site, which could explain the observed abscission defects (Dambournet et al., 2011; Mierzwa and Gerlich, 2014; Schiel et al., 2012). Using the ESCRT-III component CHMP4B as a reporter, we found that F-actin

accumulation in MICAL1-depleted cells blocks the recruitment of ESCRT-III to the abscission site, but not its initial recruitment to the midbody (Frémont et al., 2017). These findings reveal that F-actin depolymerization is critical for the correct localization of the ESCRT-III pool that is actively involved in abscission. Interestingly, chemical stabilization of F-actin in bridges with Jasplakinolide treatment also reduces ESCRT-III recruitment to the abscission site. Mechanistically, local F-actin accumulation at/or close to the abscission site might constitute a physical barrier that prevents the recruitment of ESCRT-III proteins. Alternatively, local accumulation of actin could also perturb membrane tension within the bridge, which has been proposed to drive the translocation of ESCRT-III components from the midbody to the abscission site (Elia et al., 2012). In conclusion, oxidation of F-actin by the redox enzyme MICAL1 is critical for local F-actin clearance, which is critical for the recruitment of ESCRT-III to the abscission site and thus successful abscission (Fig. 4). To our knowledge, this mechanism represents the first example of a role for oxidoreduction in cell division.

### **Mechanisms of recruitment and activation of MICAL1 during cell division**

Uncontrolled activation of MICAL family members leads to almost complete depolymerization of all F-actin pools in cells (Giridharan et al., 2012b; Grigoriev et al., 2011; Hung et al., 2010), implying that MICAL-mediated enzymatic activity and localization must be tightly regulated. In this section, we discuss the most recent findings we and other obtained with regard to the localization and activation of MICALs, in particular the emerging roles of Rab GTPases in both processes.

#### *Recruitment of MICAL1 to the intercellular bridge*

Previous studies have demonstrated that the conserved C-terminal region of MICAL proteins interacts with several Rab GTPases (Deng et al., 2016; Fukuda et al., 2008; Grigoriev et al., 2011; Rai et al., 2016; Weide et al., 2003). In particular, using yeast two-hybrid assays and co-immunoprecipitation experiments, MICAL1 has been shown to interact with Rab35 (Deng et al., 2016; Fukuda et al., 2008). Interestingly, like MICALs (Fig. 2), Rab35 is involved in many cellular functions requesting remodelling of the actin cytoskeleton (reviewed in Klinkert and Echard, 2016). In particular, Rab35 is localized in the intercellular bridge and is required for proper cytokinesis both in human and *Drosophila* cells by regulating actin dynamics (Cauvin et al., 2016; Chesneau et al., 2012; Dambournet et al., 2011; Klinkert et al., 2016; Kouranti et al., 2006). Amino acids 918-1067 of MICAL1 were found to be sufficient for its interaction with Rab35 (Fig. 1). Using isothermal titration calorimetry (ITC), we also demonstrated that recombinant MICAL1 interacts directly with

active, GTP-bound Rab35 with a dissociation constant in the  $\mu\text{M}$  range (Frémont et al., 2017). As expected, MICAL1 and Rab35 colocalize during cytokinesis at the midbody and at the abscission site. Importantly, the dominant-negative mutant Rab35<sup>S22N</sup> strongly reduces the recruitment of MICAL1 to the bridge, indicating that Rab35 promotes the recruitment of MICAL1 at the correct time during cytokinesis. Furthermore, the observation that Rab35 inactivation decreases but not completely abolishes the recruitment of MICAL1 to the intercellular bridge suggests that other proteins, likely other Rabs, also contribute to localization of MICAL1. Finally, a mutant of MICAL1 that lost its ability to interact with Rab GTPases failed to both localize to the bridge and to rescue the cytokinetic defects in MICAL1-depleted cells. Taken together, we concluded that MICAL1 recruitment to the bridge through Rab35 and possibly additional Rab GTPases is crucial for its function during cytokinetic abscission (Frémont et al., 2017).

#### *Interaction between MICALs and Rabs: new insights from structural studies*

The structural basis of the interaction between Rab GTPases and members of the MICAL family has been unknown until recently, but was believed to involve a predicted coiled-coil domain in the C-terminus of the MICAL proteins (Fukuda et al., 2008; Giridharan and Caplan, 2014). Recently, two independent studies including ours solved the structure of this MICAL1 domain by X-ray crystallography (Frémont et al., 2017; Rai et al., 2016). Surprisingly, the structure consists of a curved sheet of three helices, exposing two opposite flat surfaces and thus differs from most three helices folds, which usually form compact bundles. Moreover, biophysical measurements indicate that this domain is a monomer and thus does not form an elongated dimeric coiled-coil. This domain fold is conserved for other members of the MICAL family (Rai et al., 2016).

Interestingly, the crystal structure of MICAL1 in complex with Rab10 identified two distinct binding sites with different affinities on either side of the sheet consisting of three helices (Rai et al., 2016). In contrast, our ITC experiments shows that Rab35 binds only to the high affinity Rab-binding site of MICAL1 (Frémont et al., 2017). Our extensive mutagenesis analysis identified key residues essential for binding of MICAL1 to Rab35 as three single mutations (M1015R, I1048R, R1055E) in the C-terminal domain disrupted the interaction (Frémont et al., 2017). Consistent with the co-crystal structure of the Rab10-MICAL1 complex (Rai et al., 2016), the identified amino acids in MICAL1 are indeed positioned at the Rab-MICAL1 interface in the MICAL1-Rab10 structure. Interestingly, point mutations in the low affinity Rab-binding site do not perturb the localization of MICAL1 during cytokinesis, whereas point mutations in the high affinity Rab-binding site prevent MICAL1 localization to the intercellular bridge (Frémont et al., 2017). Future studies are needed to

better understand the differential roles of each Rab binding site in determining the localization and functions of MICAL proteins in cells.

#### *Activation of MICAL1*

In addition to the factors that determine the localization of MICALs, another key question is to understand how their enzymatic activity is activated to control F-actin disassembly at the right time and place. Several previous studies have demonstrated that the full-length MICAL1 is catalytically inactive as it adopts an auto-inhibitory state in the absence of stimuli (Giridharan et al., 2012b; Schmidt et al., 2008). The N- and C-terminal regions of the protein have been shown to physically interact and this likely inhibits the enzymatic activity (Schmidt et al., 2008). Indeed, overexpression of full length MICAL1 does not result in disassembly of cellular actin, but either deletion or mutation of the C-terminal region of MICAL1 triggers F-actin depolymerization to an extent that is similar to overexpression of only its monooxygenase domain (Giridharan et al., 2012b). It has thus been suggested that proteins interacting with the C-terminal region of MICALs might release the auto-inhibitory state and so induce enzymatic activity. In agreement with such a mechanism, previous studies of axon guidance in *Drosophila* revealed that the C-terminal region of MICAL interacts with the semaphorin receptor Plexin A, which has been proposed to induce enzymatic activity (Schmidt et al., 2008; Terman et al., 2002). This could explain how MICAL activity can be regulated by external cues (in this case semaphorins) that are received at the cell surface through Plexin receptors (Fig. 5).

However, MICAL1 activation during cytokinesis (or other cell autonomous processes) by extracellular ligands such as Semaphorins is unlikely. During cell division, Rab35 is a good candidate for activating MICAL1 because it colocalizes with MICAL1 and directly interacts with its C-terminal domain (see above). Consistent with MICAL1 taking on an intramolecularly folded conformation, we found that the C-terminal domain (amino acids 879-1067) and N-terminal half (the monooxygenase-CH-LIM domain) of MICAL1 directly interact with one another. Remarkably, the addition of GTP-bound Rab35 displaces the intramolecular interaction between the C- and N-terminal regions of MICAL1, suggesting that binding to Rab35 indeed regulates the enzyme activity. Using single actin filament assays, we confirmed that full length MICAL1 is inactive *in vitro*. Importantly, incubation of full-length MICAL1 with GTP-bound Rab35 relieves the auto-inhibition of MICAL1 and greatly enhances the depolymerization rates of actin filaments, which in fact, reached the values as when we exposed filaments to the fully active, N-terminal monooxygenase domain of MICAL1 (Frémont et al., 2017). Thus, binding of Rab35 to MICAL1 not only recruits MICAL1 to the cytokinetic bridge but also activates its redox activity, thereby allowing for the localized depolymerization of F-actin at the abscission site. The fact that other Rabs interact with the

C-terminal part of MICAL1 suggests that they might also be able to relieve the auto-inhibition of MICAL1 (Fig. 5). Our study thus provides a new mechanism for Rab-mediated activation of MICALs, which could be utilized at multiple locations on intracellular membranes where Rab GTPases are specifically localized (Stenmark, 2009).

### **Redox-independent regulation of cell division by other MICAL family members**

Recently, two studies reported the role of two further MICAL family members, MICAL3 and MICAL-L1 in cell division (Liu et al., 2016; Reinecke et al., 2015). However, the underlying mechanisms are likely to be completely different and do not involve any oxidation-reduction, as the redox activity of MICAL3 is not required for its role during cytokinesis and the monooxygenase domain is absent in MICAL-L1.

During cytokinesis, MICAL3 directly interacts with the centralspindlin component MKLP1 and recruits the adaptor protein ELKS, which tethers Rab8A-positive vesicles to the midbody (Liu et al., 2016). Importantly, Rab8A vesicles are known to transport important molecules, yet to be identified, that promote late cytokinetic steps (Guizetti et al., 2011; Kaplan and Reiner, 2011; Schiel et al., 2012). As a consequence, the depletion of either ELKS or Rab8A leads to abscission defects. Consistent with a function that is independent of its redox activity, MICAL3 does not regulate actin levels during cytokinesis. Overall, the authors proposed that MICAL3 acts as a midbody-associated organizer for vesicle targeting, which is crucial for the maturation of the intercellular bridge and thus for abscission (Liu et al., 2016). While MICAL3 can oxidize and disassemble F-actin *in vitro* (Lundquist et al., 2014), its function during cytokinesis (Liu et al., 2016) and in interphase cells (Grigoriev et al., 2011) both rely on vesicle tethering rather than on actin depolymerization. Thus, despite having an overall common structural organization (Fig. 1), MICAL1 and MICAL3 appear to exert different and non-redundant functions, at least during cytokinesis.

MICAL-L1, is another Rab35 effector (Giridharan et al., 2012a; Kobayashi et al., 2014b) and also plays a role in cell division (Reinecke et al., 2015). Depletion of MICAL-L1 leads to an abnormal spindle length, lagging chromosomes and binucleated cells. These effects are associated with a defective transport of Rab11A-positive endosomes along cytokinetic bridges, which are important for bridge stability (Montagnac et al., 2008; Schiel et al., 2013; Wilson et al., 2005). However, how exactly MICAL-L1 regulates spindle morphology and function, and whether the lagging chromosomes could contribute, at least in part, to the observed cytokinetic defects remain to be determined.

Taken together, a number of recent studies have identified roles for three members of the MICAL family together with their associated Rabs in critical, yet distinct aspects of

cytokinetic abscission: MICAL1, through its effect on F-actin depolymerization, MICAL3, which acts as a midbody-associated scaffold for vesicle targeting, and MICAL-L1 through its role in membrane trafficking (Fig. 6).

## Conclusion and Perspectives

Oxidoreduction is a fundamental process in living organisms and in particular has essential roles in metabolic reactions. However, excess of cellular oxidation can result in oxidative stress, which contributes to aging by non-specific oxidization of proteins, nucleic acids and lipids (Stadtman, 2006). In contrast to the deleterious effects of oxidation, recent work has begun to highlight the critical role of controlled actin oxidation in cytoskeleton dynamics during cell division, both in *Drosophila* and mammalian cells. Here, the Rab35 GTPase, by binding to the tail of MICAL1, not only contributes to localizing the enzyme at the right time and place, but also activates its redox activity by relieving the intramolecular auto-inhibition.

MICAL1 depletion only delays abscission in half of the cells, suggesting that additional mechanisms are involved in the clearance of F-actin from intercellular bridges. For instance, other members of the MICAL family (e.g. MICAL2) or other actin-depolymerizing proteins such as cofilin might contribute to the full disassembly of the actin cytoskeleton at the abscission site. Indeed, a recent study demonstrated that F-actin filaments oxidized by *Drosophila* Mical are more sensitive to cofilin-induced severing *in vitro* (Grintsevich et al., 2016). Future studies are needed to reveal whether there is indeed synergy between MICALs and cofilin. Furthermore, additional mechanisms have been shown to contribute to limiting actin polymerization in the bridge that are controlled by the small GTPases Rab35 and Rab11. Indeed, Rab35 controls the localization of its effector, the PtdIns(4,5)P<sub>2</sub> phosphatase OCRL-1 (also known as inositol polyphosphate 5-phosphatase, INPP5F), at the membrane of the intercellular bridge, which promotes PtdIns(4,5)P<sub>2</sub> hydrolysis and prevents F-actin accumulation (Dambournet et al., 2011). Indeed, PtdIns(4,5)P<sub>2</sub> is a key signaling molecule that controls a number of cellular pathways that activates actin polymerization (reviewed in Cauvin and Echard, 2015). In parallel, endosomes positive for Rab11A and Rab11 family of interacting proteins 3 (FIP3) transport the GTPase-activation protein (GAP) p50RhoGAP to intercellular bridges, where it limits F-actin accumulation by inactivating Rho and Rac GTPases, which promote actin polymerization (Schiel et al., 2012). Thus actin polymerization is prevented by p50RhoGAP-mediated inactivation of Rho and Rac and by Rab35-OCRL-1. In addition, F-actin is actively disassembled through the Rab35/MICAL1 pathway during late stages of cytokinesis, thereby ensuring successful abscission (Fig. 6).

Although the MICAL family members have clearly emerged as essential regulators of actin dynamics in many cellular functions, several important questions need to be addressed. At the structural level, it remains to understand the significance of the two Rab-binding sites with different affinities for the intracellular localization of MICALs, as well as the structural basis of the intramolecular inhibition between the N-terminal and C-terminal parts of MICALs and how Rab GTPases precisely regulate this interaction. At the functional level, it has to be investigated whether the balance between oxidases (MICALs) and reductases (MsrBs) are locally regulated, and to be discovered which mechanisms could compensate for the inactivation of MICALs *in vivo*. In addition, in cells, filaments are disassembled by MICAL-induced oxidation even though their ends are unlikely to be free to depolymerize and oxidation alone is insufficient to sever filaments. This raises interesting questions, such as whether oxidation favor uncapping and whether oxidation is enough to make otherwise harmless amounts of cofilin sever filaments instantly.

As a newly discovered Rab35 effector, MICAL1 could potentially be involved in other processes, in which Rab35 and actin remodelling have been implicated (reviewed in Klinkert and Echard, 2016), such as in endocytic recycling (Cauvin et al., 2016), phagocytosis (Egami et al., 2011), neurite outgrowth (Kobayashi and Fukuda, 2012; Villarroel-Campos et al., 2016), immunological synapse formation (Patino-Lopez et al., 2008), or the establishment of apical polarity in 3D organoids (Klinkert et al., 2016). The fact that each MICAL protein interacts with multiple Rabs (Fukuda et al., 2008) suggests that other Rabs than Rab35 could potentially activate and thus locally regulate the actin-depolymerizing activity of MICAL family members in the cell. These perspectives open a new area of study directed towards the precise control of actin remodelling via oxidation, which emerges at the heart of many cellular processes that are controlled by Rab GTPases.

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## -Figure Legends

### **Fig. 1: The MICAL family members mediate direct oxidation of actin filaments.**

(A) **Domain architecture of the three human MICAL proteins.** Human MICAL proteins contain an N-terminal monooxygenase domain (red), a Calponin Homology (CH)-domain (blue) and a LIM (Lin-11, Isl-1 and Mec3)-domain (green). MICAL1 and MICAL3 contain a C-terminal RBD or Rab binding domain (cyan) that interacts with Rab GTPases. The RBD has two distinct Rab binding sites with different affinities.

(B) **MICAL-mediated oxidation of actin.** In the presence of the coenzyme NADPH and dioxygen, MICAL catalyzes the oxidation of actin.

**Fig. 2: Reported cellular functions of MICAL proteins.** Note that most cellular functions involve actin remodelling. When known, the specific involvement of each member of the human MICAL family (MICAL-L1, MICAL-L2, MICAL1, MICAL2 and MICAL3) and of *Drosophila* Mical family (dMical and dMical-I) has been indicated.

**Fig. 3: Mechanism of MICAL-mediated actin depolymerization.** MICAL binds to F-actin and oxidizes the actin filament. Actin oxidation by MICAL weakens longitudinal interactions between actin subunits within the filament, leading to the filaments become more fragile and thus enhanced depolymerization from both ends.

**Fig. 4: Model of the function of MICAL1 during cytokinetic abscission.** Oxidation of F-actin by MICAL1 is critical for local F-actin depolymerization at the intercellular bridge. After F-actin clearance, ESCRT-III components are recruited to the abscission site and drive successful abscission.

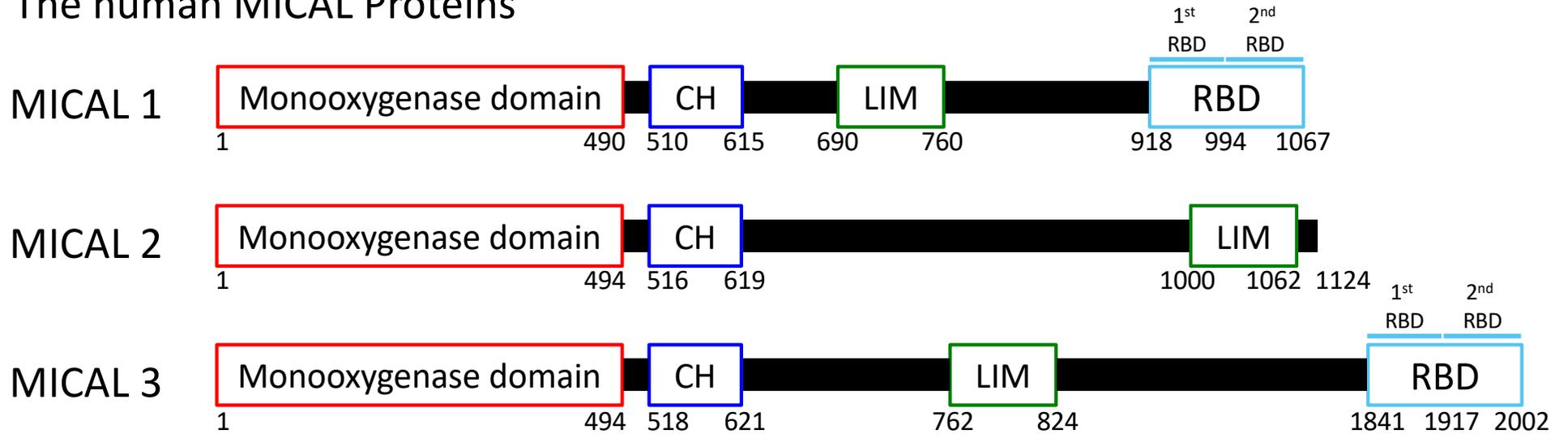
**Fig. 5: Model for activation of the redox MICAL enzymes.** *Drosophila* Mical is activated upon interaction with Plexin A in response to Semaphorin signaling (left) and human MICAL1 upon interaction with the GTPase Rab35 during cytokinetic abscission (right). The MICAL domains are indicated as in Fig. 1. Actin is in green.

**Figure 6: Functions of several MICAL family members during cytokinetic abscission.**

Rab35 GTPase recruits and activates MICAL1, thus enabling oxidization and F-actin disassembly (green) in the intercellular bridge. Actin clearance is essential for normal ESCRT-III (orange) recruitment at the abscission site and for successful abscission. Additional mechanisms contribute to limit actin polymerization in the bridge and depend on the GTPase Rab35, through the recruitment of the PtdIns(4,5)P<sub>2</sub> phosphatase OCRL, and on the GTPase Rab11, through the recruitment of p50RhoGAP. MICAL3 interacts with the centralspindlin component MKLP1 and recruits the adaptor protein ELKS, which tethers Rab8A-positive vesicles to the midbody, in a redox-independent manner. MICAL-L1 promotes the transport of Rab11A-positive endosomes along cytokinetic bridges, which is important for bridge stability.

A

### The human MICAL Proteins



B

### MICAL-mediated Oxidation of Actin

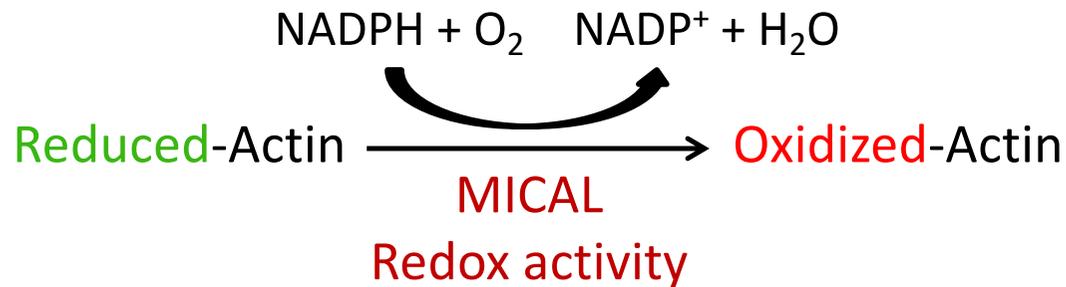


Figure 1

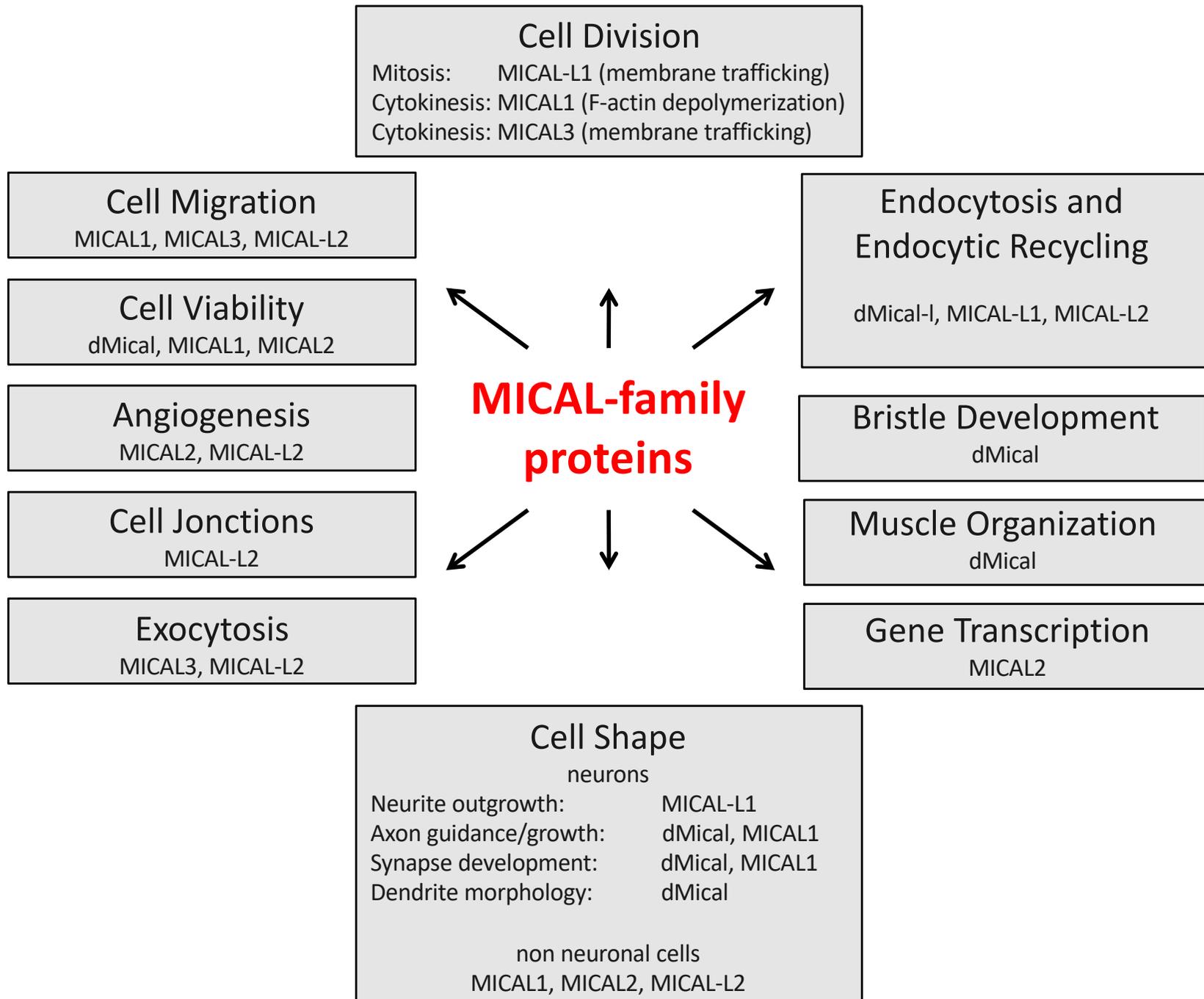


Figure 2

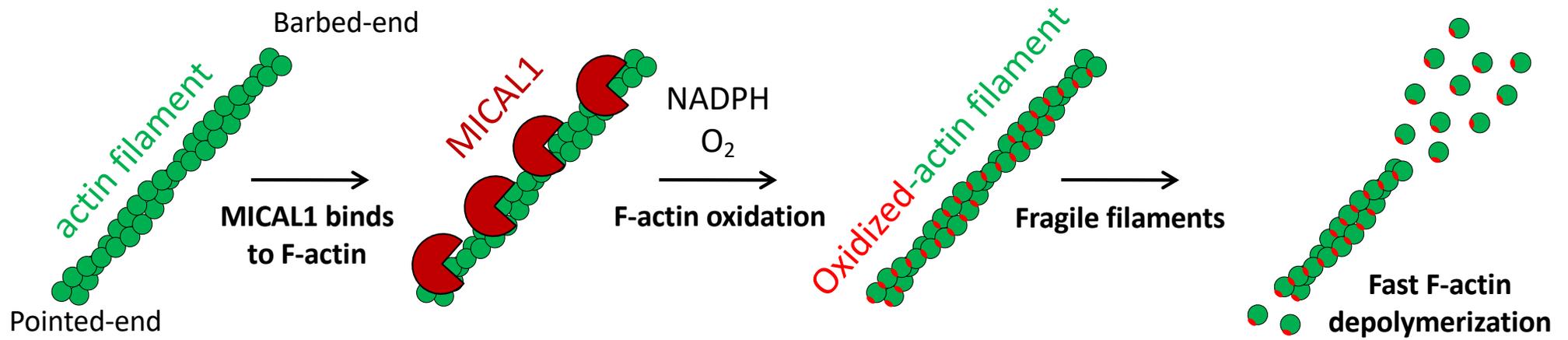


Figure 3

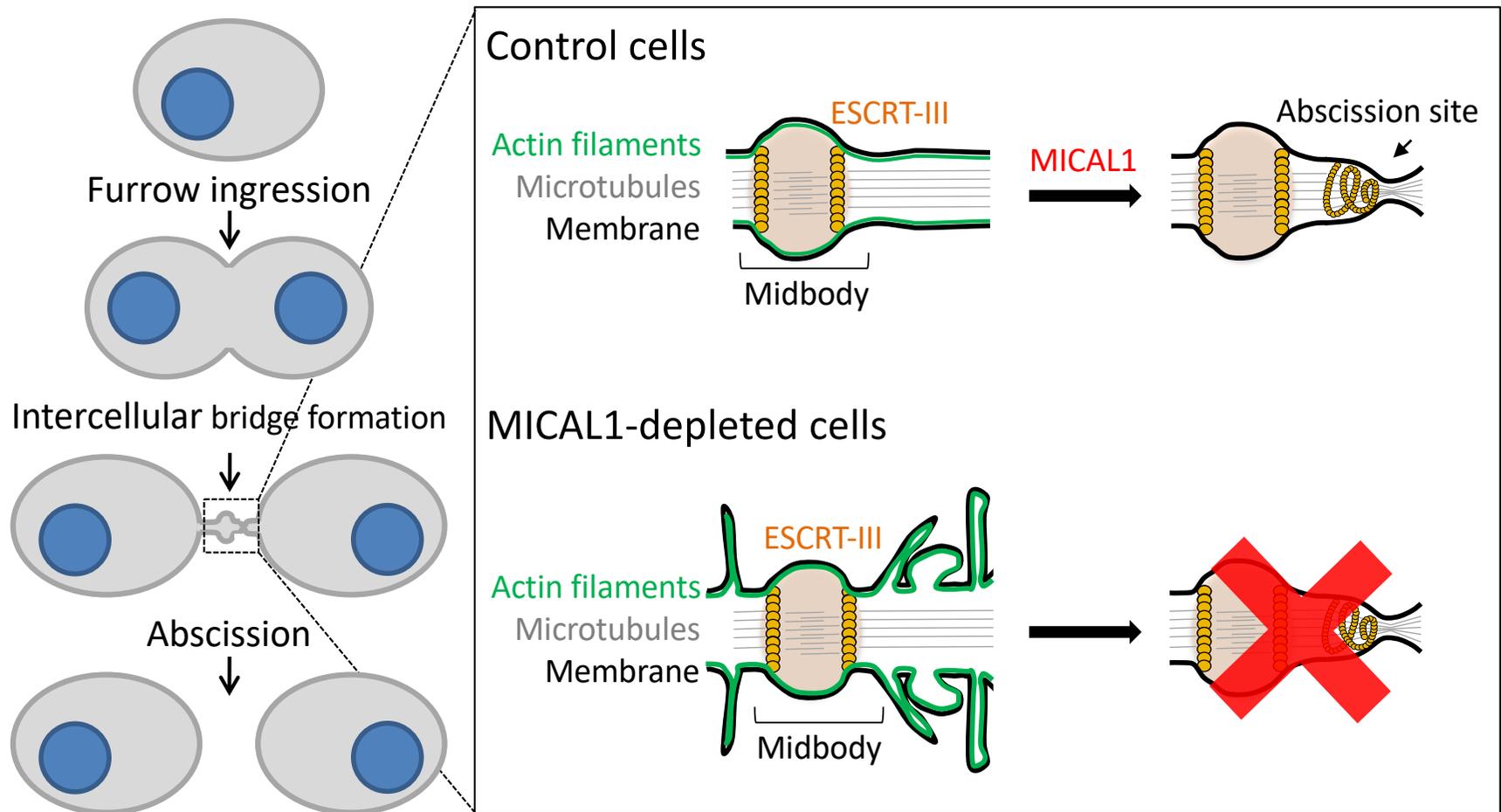


Figure 4

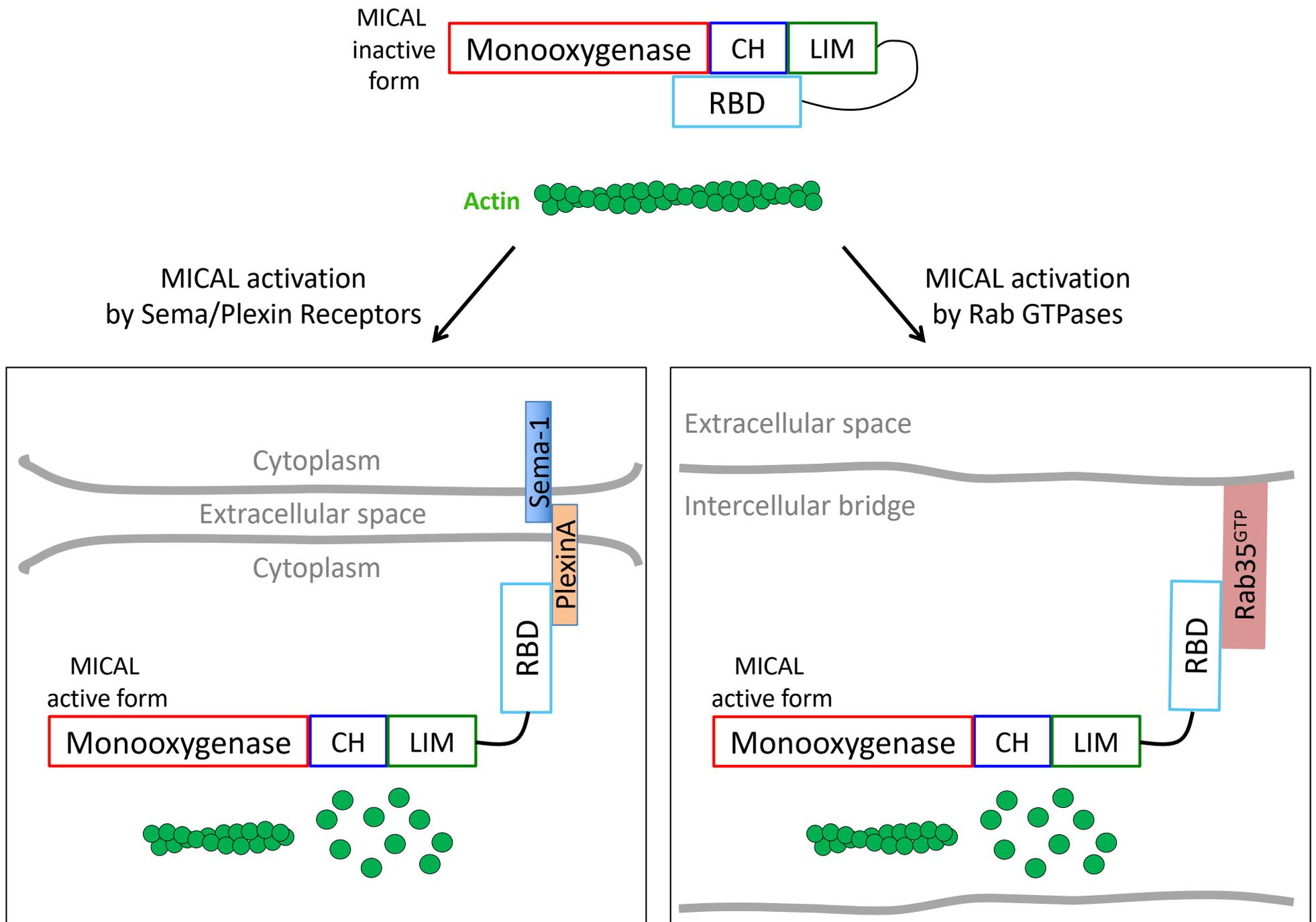


Figure 5

