Leaving the mark: FMOs as an emerging class of cytokinetic regulators

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Abstract

Posttranslational modification of proteins plays a fundamental role in cell biology. It provides cells a means to regulate the signaling, enzymatic or structural properties of proteins without continuous cycles of synthesis and degradation, offering multiple distinct functions to individual proteins in a rapid and reversible manner. Modifications can include phosphorylation, ubiquitination or methylation, which are widespread and simple to detect using current approaches. More challenging to identify, one modification of growing significance is the direct oxidation of cysteine and methionine side chains. Protein oxidation has long been known to occur spontaneously upon the accumulation of cellular reactive oxygen species (ROS), but new data are providing insight into the targeted oxidation of proteins by flavin-containing monooxygenases (FMOs). Here, we review how oxidation of cellular proteins can modulate their activity and consider potential roles for FMOs in the targeted modification of proteins shaping cell division, with a particular focus on two families of FMOs: MICAL and OSGIN.

Introduction

The regulation of protein activity and homeostasis within the cell depends on various events such as transcription and translation, but also posttranslational modifications (PTMs) on one or more amino acid residues. Several types of PTMs can impact the properties of a given protein, including its enzymatic, signalling or scaffolding activity, its localization and/or its stability [1]. One such modification that has been well studied is phosphorylation – the addition of a phosphate group on a Ser, Thr or Tyr residue [2]. This modification is carried out by specialized enzymes known as protein kinases and can be reversed by another group of enzymes termed phosphatases, thus enabling a relatively dynamic means to impact the function of a protein. Other common PTMs include glycosylation, ubiquitination, acetylation and prenylation, which can also impact protein function (reviewed in [1,3]). One PTM that has gained growing attention is oxidation – the covalent attachment of an oxygen group to sulfur-containing (Cys, Met) or aromatic (Tyr, Phe) residues. Protein oxidation can be chemically achieved by cellular reactive oxygen species (ROS), but is also catalyzed by a group of enzymes known as flavin-containing monooxygenases (FMOs) [4]. In recent years, important roles for FMOs have been uncovered in the fundamental process of cell division, particularly cytokinesis.

Cytokinesis is the final step of cell division and separates a mother cell to properly distribute the chromosomes, organelles and cytoplasm into two daughter cells [5]. A key regulator of cytokinesis is the small GTPase RHOA which, when activated via GTP loading, coordinates the assembly of a contractile ring of actin and non-muscle myosin at the cell's equatorial cortex [6]. This ring guides a membrane furrow that ingresses between the two sets of separated chromosomes until only a thin intercellular bridge (ICB) remains, transiently connecting the two daughter cells. RHOA is crucial for stabilizing the ICB, facilitating the reorganization and maturation of the midbody - a specialized organelle that serves as a platform for the recruitment of machinery required to locally rearrange the plasma membrane and physically separate the daughter cells, a process termed abscission [7,8].

RHOA belongs to the Rho family of small GTPases. Like most GTPases, it cycles between inactive GDP- and active GTP-bound states in response to various upstream signals (Figure 1A-B). Positive stimuli result in GTP loading through the activity of guanine-nucleotide exchange factors (GEFs) [9], enabling interaction of GTPases with a plethora of effector proteins that relay intracellular signals to distinct pathways [10]. Small GTPases become inactivated when bound GTP is hydrolyzed to GDP, an enzymatic activity intrinsic to these proteins that is greatly accelerated by GTPase-activating proteins (GAPs) [11]. Rho family GTPases are also regulated by Rho GDP-dissociation inhibitors (RhoGDIs), which sequester these proteins away from the plasma membrane and maintain them in the inactive, GDP-bound state [12]. In addition to this canonical, switch-like activity, GTPases are subject to regulation by various PTMs, including oxidation [13,14]. Here, we review protein oxidation as a form of PTM and the role of FMOs as a class of enzymes catalyzing protein oxidation within cells, in particular their relevance as cytokinetic regulators. Additionally, we address known mechanisms of RHOA regulation by oxidation and discuss more specifically the molecular mechanisms that enable regulation of cytokinesis completion by two FMO families: MICAL and OSGIN.

Protein oxidation: a versatile post-translational modification to regulate protein activity

In the last decades, protein oxidation has emerged as an important PTM regulating a wide variety of cellular processes [15,16]. Initially viewed as a passive form of protein modification whereby mainly sulphur-containing cysteines and methionines (but also other amino acids) are oxidized by intracellular ROS (such as hydroperoxide, superoxide or hydroxyl radical; Figure 2A-B), it is now clear that under physiological conditions ROS-mediated protein oxidation plays an active role to ensure rapid signal propagation within cells, notably in response to stresses or extracellular cues [17]. Cellular ROS can be generated in a controlled manner by specialized enzymes and transported by carriers functioning as reduction-oxidation (redox) relays to spatiotemporally regulate protein function [17,18].

The systematic identification of proteins and pathways that can be impacted by oxidation has been hindered by technological challenges [19]. While relatively simple tools for the identification of general patterns of cysteine oxidation have been available for over a decade — including 2D gel electrophoresis techniques combined with reagent-based detection [20] or infrared maleimide dyes [21] — methods for identifying methionine oxidation remain limited. Similarly to the antibody-based detection of phosphorylated Ser/Thr and Tyr moieties, the availability of antibodies raised against oxidated methionine (known as methionine sulfoxide or MetO, [22]) should offer a simple means of detecting this modification, yet the specificity of available antibodies has been debated [23]. The addition of oxygen atoms to amino acid moieties can also be tracked by mass spectrometry, which in principle enables the quantitative monitoring of protein oxidative states. However, the use of this approach to detect MetO is complicated by the non-specific oxidation of methionines that typically occurs during sample preparation and thus complicates analyses [24,25]. One approach to overcome this limitation stems from recent studies that employ O^{18}-labelled H_2O_2-mediated oxidation [25,26], which can distinguish oxidation events that occur *in vivo* from those arising during sample processing. This promising approach could allow the systematic detection of MetO in various contexts, yet while it has been effective *in vivo* [27] it remains to be validated in other settings, such as *in vitro* enzymatic oxidation.

Despite existing challenges to unambiguously identify and characterize protein oxidation, there are increasing reports that a wide array of proteins can be regulated by ROS-mediated oxidation, impacting numerous intracellular processes [28]. For instance, ROS was found to promote the formation of a cysteine disulfide bridge in the antioxidant enzyme Thioredoxin, which prevents it from interacting with the mitogen-activated protein kinase (MAPK) regulator ASK1 [29]. This enables ASK1 dimerization and leads to activation of p38 MAPK signaling in the context of apoptosis [30], cell differentiation [31] and immune response [32]. ROS also promotes oxidation of the catalytic cysteine residue in JNK-inactivating phosphatases (MKP-1, -3, -5, and -7) to sulfenic acid, leading to their inactivation and thus prolonging JNK activity during programmed cell death [33].

Signalling from several small GTPases has also reported to be controlled by ROS, including Rho GTPases [13]. These G-proteins are a subclass of the Ras GTPase superfamily and comprise 20 members, including RHOA, RAC1 and CDC42 that participate in cytoskeleton regulation [14]. Like other proteins, Rho family GTPases are subjected to various types of PTMs that impact their localization or activity, including oxidation arising from intracellular ROS or reactive nitrogen species (RNS) [13]. Rho GTPases possess a conserved redox-sensitive cysteine residue located in the P-loop, an N-terminal motif essential for guanine nucleotide binding (GXXXXGK[S/T]C_{20}, RHOA numbering, Figure 1A). ROS- or RNS-mediated oxidation of this cysteine in RHOA, RAC1 or CDC42 was found to facilitate their activation by enhancing the intrinsic rate of GDP dissociation by 400-600 fold [34]. Interestingly, RHOA uniquely possesses a second cysteine residue within the P-loop (Cys_{16}) that, when oxidized, can form a disulfide bridge with Cys_{20} [35]. Whereas RHOA Cys_{20} oxidation increases the rate of GDP dissociation, the presence of a Cys_{16}-Cys_{20} disulfide bridge prevents GTP loading or association with regulatory GEFs, thus promoting RHOA inactivation (Figure 1C) [36]. Functional studies showed that H_2O_2-generated ROS resulted in RHOA-induced stress fiber formation in cultured cells, an effect that was reversible and dependent on the P-loop Cys_{16} and Cys_{20} residues of RHOA [37]. Thus, there must exist a fine balance between Cys_{16}-Cys_{20} oxidation to control signaling output to RHOA effectors. Such redox regulations of RHOA activity have not yet been implicated in the regulation of cytokinesis.

ROS-mediated regulation of RHOA and related GTPases is carried out through cysteine oxidation. While not yet implicated in small GTPase regulation, methionine residues also contain a sulphur atom susceptible to oxidation and thus have the potential to regulate protein function (Figure 2B) [38]. Both cysteines and methionines are modified with comparable rates by strong oxidants *in vitro*, but methionines generally exhibit a slower reaction rate when mixed with mild oxidants such as H_2O_2 [39,40]. Under oxidative stress, solvent-exposed methionines were proposed to act as oxidant scavengers that protect other susceptible amino acids from oxidation [38,41]. This view is supported by the labile nature of MetO that can be reversed by specialized enzymes called methionine sulfoxide reductases (MSRs). MSRs function in a stereo-specific manner, however, and cannot equally access every MetO residue in proteins [42]. This suggests that their efficacy in regulating protein oxidation could be somewhat limited in dynamic environments, such as oxidative stress. Moreover, as is the case with phosphorylation, reversible oxidation can act as switch between protein activation and inactivation [38], suggesting it can operate as a specific and controlled means of regulating protein function.

Enzymatic oxidation of methionines

Whereas the oxidation of methionine residues by mild ROS is inefficient [19], it can be catalyzed by specific enzymes termed flavin-containing monooxygenases (FMOs) [4]. A principal function of these enzymes is to catalyze an NADPH-dependent oxidation of primarily intracellular metabolites and xenobiotics (including drugs) [43]. Several groups of conserved FMOs have been defined across species, based on structural features and the nature of catalyzed oxidative reactions [4,43-45]. Two groups of FMOs are predominant in humans: groups A and B (Table 1). Group A FMOs are characterized by the presence of a single Rossmann fold and mainly target hydroxyl-containing substrates; some of the best characterized members of this group are MICAL family proteins. Group B FMOs contain two Rossmann folds and are known to catalyze the transfer of oxygen atoms on compounds bearing carbon-bound nitrogen, sulphur, selenium, iodine or phosphorous [4,40]; the best studied members of this group are six canonical isoforms, termed FMO1-6, each with different tissue expression profiles and varying substrate specificities (Table 1) [4]. The catalytic activity of group A and B FMOs necessitates a flavin adenine dinucleotide (FAD) cofactor, which is reduced by the bound NADH/NADPH coenzyme (Figure 2C). Through an electron transfer reaction, the reduced flavin cofactor interacts with molecular oxygen to form a hydroperoxide intermediate and a flavin radical, which is thus primed to transfer the oxygen onto a target substrate [43].

FMO1-4 were previously found to catalyze the stereospecific sulfoxidation of free methionine (Table 1) [40,46]. FMO1 and FMO3 are also capable of oxidizing peptide-bound methionine residues, yet this reaction was observed exclusively on Met at the N-terminus of assayed peptides. This suggests a limited role for human FMOs in methionine oxidation of intact proteins [47]. In contrast, *Aspergillus nidulans* FmoB specifically oxidizes the transcription factor NirA on a methionine residue within its nuclear export sequence, influencing its interaction with the exportin KapK/CRM1 and consequently its intracellular localization [48]. The biological and physiological importance of methionine oxidation by human canonical FMOs remains unclear, with no *in vivo* protein substrate for these enzymes identified to date.

MICAL proteins regulate cytokinesis via methionine oxidation

MICAL proteins are non-canonical FMOs that act on methionine side chains in protein substrates [49]. They were named after their initial characterization as interactors of the signaling scaffold protein CasL (Molecule Interacting with CasL) and the intermediate filament protein vimentin [50]. Concurrently, *Drosophila* MICAL was found to interact with neuronal Plexin A (PlexA) and regulate repulsive axon guidance in a Semaphorin 1a-PlexA-mediated manner [51]. Archetypal FAD-binding motifs, characteristic of FMOs, were identified in MICAL (Figure 3A) and the protein was found to bind to FAD *in vitro.* Mutations that disrupt FAD binding compromised semaphorin-mediated axonal repulsion, supporting a role for the FMO activity of *Drosophila* MICAL in this context [51].

Vertebrates have three *MICAL* genes (*MICAL1*, *MICAL2* and *MICAL3*), each encoding a multidomain protein with a highly conserved monooxygenase (MO) domain located in the N-terminal region [52]. As is the case for canonical FMOs, the MO domain of MICAL proteins binds FAD and utilizes NADPH and diatomic oxygen to catalyze oxidation reactions [49,53], with the protein actin serving as the most prominent substrate [52,54] (Table 1). Actin monomers (G-actin) can assemble into filaments (F-actin) that play fundamental roles in the regulation of cell shape, including during cytokinesis where it scaffolds the contractile ring. Mass spectrometry measurements of intact monomeric actin after incubation with *Drosophila* MICAL revealed a 32 Da increase in its total mass, accounting for the addition of two oxygen atoms of 16 Da and thus establishing actin as a MICAL substrate [55]. Mass measurements of MICAL-treated small peptide fragments further revealed that this enzyme catalyzes the stereospecific (*R*-isomers) addition of oxygen on the sulfur atom of two methionine residues (Met_{44} and Met_{47}) present in the so-called D-loop of actin, decreasing the affinity between actin monomers and leading to F-actin disassembly [55,56]. Furthermore, MICAL-oxidized G-actin monomers exhibit a filament reassembly rate approximately three times slower than that of unoxidized monomers, resulting in shorter and less stable filaments [57,58]. Thus, regulated actin oxidation by MICAL induces F-actin depolymerization and prevents *de novo* actin polymerization.

Regulation of the actin cytoskeleton by MICAL proteins impacts numerous cellular processes including axonal guidance, cell migration, angiogenesis, endo- and exocytosis and transcription (reviewed in [59]). Moreover, the FMO-dependent actin depolymerization activity of MICAL1 is crucial for the completion of cytokinesis, as its inhibition causes delays in the final cellular abscission [54]. To fulfill this function, MICAL1 is recruited to the ICB in late cytokinesis through a direct interaction with the small GTPase RAB35. Interaction with activated, GTP-bound RAB35 relieves an autoinhibitory conformation, stimulating MICAL1 enzymatic activity by releasing intramolecular contacts between the FAD-CH-LIM domains and the C-terminal region. This activation drives MICAL1 to locally oxidize F-actin, promoting its disassembly and clearance from the ICB (Figure 3B). In turn, clearing of F-actin from the ICB enables the recruitment of ESCRT-III regulators to the abscission site, favoring membrane scission and cytokinetic completion [54]. The two Met residues in actin that are targeted by MICAL are highly conserved across species and among actin isoforms, suggesting that their regulation via oxidation may be a fundamental process for controlling the completion of cytokinesis [55]. The MICAL-family proteins MICAL3 and MICAL-like protein 1 (MICAL-L1) were also found to play roles in cytokinesis, albeit in a manner seemingly independent of oxidative reactions [60,61].

Interestingly, MICAL1-dependent actin oxidation is counteracted by the methionine sulfoxide reductase MSRB2, which is required to delay abscission when chromosomes have undergone errors in segregation [62]. Chromatin trapped in the ICB activates the so-called abscission checkpoint, which delays abscission and prolongs ICB stability until the problem is resolved [63-65]. MSRB2 participates in this process by reducing oxidized methionines on G-actin monomers, favoring their re-incorporation into actin filaments to prolong F-actin stability at the

ICB [62]. These studies have established the importance of actin oxidation reactions in the regulation of cytokinetic abscission and support the notion of methionine oxidation as a highly versatile and precise means of regulating protein function.

The OSGIN family of FMOs as new cytokinesis regulators

Recently, the oxidative stress-induced growth inhibitor (OSGIN) family proteins were identified as non-canonical FMOs that regulate ICB stability and cytokinesis completion in both *Caenorhabditis elegans* and human cervical cancer (HeLa) cells [66]. The gene encoding *C. elegans* OSGN-1 was initially uncovered as a regulator of stable ICB in primordial germ cells [67]. Sequence analyses demonstrated that OSGN-1 has putative orthologs in several animal species, with *Drosophila* being an exception having no recognisable ortholog. Humans encode two poorly characterized OSGIN-family proteins, termed OSGIN1 (also known as OKL38 or BDGI) and OSGIN2 (also C8orf1/hT41). Human *OSGIN1* was first identified as a gene differentially expressed during pregnancy and involved in the differentiation of breast epithelial cells [68]. Its expression was also shown to inhibit the growth of breast cancer cell lines and was associated with a reduction in tumor growth *in vivo* [69]. Additionally, expression of the genes encoding OSGIN1 and OSGIN2 was reported to be increased in response to oxidative stress downstream of the stress-response regulator NRF2 [70-72]. While the cellular function of OSGIN2 has yet to be elucidated, OSGIN1 was reported to interact with TUBB3, DYRK1A [73], and p53 [74] and has been implicated in senescence, cell cycle arrest [75,76], apoptosis and autophagy [77]. This supports claims that OSGIN1 could function as a tumor suppressor [69], though the precise mechanisms by which it constrains cell growth and proliferation are still unknown.

Sequence analysis of OSGIN-family proteins revealed that they contain two Rossmann folds, signature motifs for FAD-binding (GXGXXG) and NAD(P)H-binding (GXSXXA) (Figure 4A). While these domains are often present in classical FMOs, they were found to flank an uncharacteristic monooxygenase domain, lacking conserved residues that typically define the catalytic cleft (FXGXXXHXXXF/Y, Figure 4B) [78]. Despite this, *C. elegans* OSGN-1 and human

OSGIN1 both bind FAD and catalyze oxidation of the FMO artificial substrate methimazole *in vitro* in an NADPH-dependent manner [66]. These findings establish the orthologs OSGN-1 and OSGIN1 as *bona fide* FMOs (Table 1). It remains unclear whether OSGIN2 has FMO activity *in vitro*.

CRISPR/Cas9-mediated deletion of *OSGIN1* or *OSGIN2* in HeLa cells revealed that a significant proportion of mutant cells display furrow regression in late cytokinesis, leading to binucleation in this subset of dividing cells, a phenotype similar to that observed in *C. elegans* germ cells depleted of OSGN-1. Human OSGIN1 and OSGIN2 are 58% identical at the amino acid level and both proteins have ~34% sequence similarity with *C. elegans* OSGN-1, largely in the FAD- and NADPH-binding domains. Notably, expression of *C. elegans* OSGN-1 in HeLa cells rescues the late cytokinetic defects of *OSGIN1* mutant cells, but not those of *OSGIN2* mutants. Furthermore, the rescue mediated by OSGN-1 could only be achieved with a catalytically-active enzyme, supporting the premise that *C. elegans* OSGN-1 and human OSGIN1 are functional FMO orthologs. This further implies that the two human OSGIN-family proteins have non-redundant roles in the regulation of late cytokinesis.

Regulation RhoA activity by OSGIN proteins

While the cellular substrate(s) of *C. elegans* OSGN-1 and human OSGIN1 remain unknown, the observation that RhoA activity (measured using two distinct biosensors) is decreased at the ICB of OSGN-1-depleted *C. elegans* germ cells and OSGIN1-mutant HeLa cells suggests that OSGIN promotes the activation of RhoA (Figure 4C) [66]. As RhoA itself is redox sensitive, one possibility is that OSGIN acts directly to oxidize RhoA and promote signaling. However, FMOs characteristically oxidize methionines, making it unlikely that OSGN-1 and OSGIN1 impact RhoA activity *via* oxidation of the known redox-sensitive Cys_{16} and Cys_{20} residues. It is possible that the targets may be one or both methionines conserved between the *C. elegans* and human RhoA proteins, Met_{82} and Met_{134} (Figure 1A). Another, non-mutually exclusive possibility is that OSGIN proteins regulate RhoA activity by oxidizing one of its regulators, for instance locally increasing the activity of a RhoGEF or decreasing the activity of a RhoGAP or RhoGDI. Finally, as activated

RhoA signals are transduced by downstream effectors in a retro-feedback manner [79,80], OSGIN proteins could oxidize one or more RhoA effectors to sustain their activity at the ICB. Identifying the molecular mechanism by which OSGIN proteins promote RhoA activity in late cytokinesis promises to shed light on a novel class of enzymes that impact a core regulator of intracellular signaling.

Conclusion

Increasing reports implicate oxidative reactions in the regulation of cellular protein activities, demonstrating that these types of PTMs are prevalent and important factors in cell physiology. Recent discoveries that two families of FMOs, MICAL and OSGIN, participate in the regulation of late cytokinesis events makes it likely that several other cellular processes are also subjected to targeted regulation by FMOs. The detection and identification of oxidized amino acids in intact proteins, especially methionines, represents a significant challenge for conventional methods, in part due to the limitation of specific labelling reagents and the instability of these modifications during sample processing [19]. As such, technical improvements of existing approaches and/or the development new techniques are highly desirable, as identifying FMOs and their substrates could uncover novel, actionable targets that can be exploited to manipulate cell physiology in both normal and pathological contexts.

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Figures and Legends

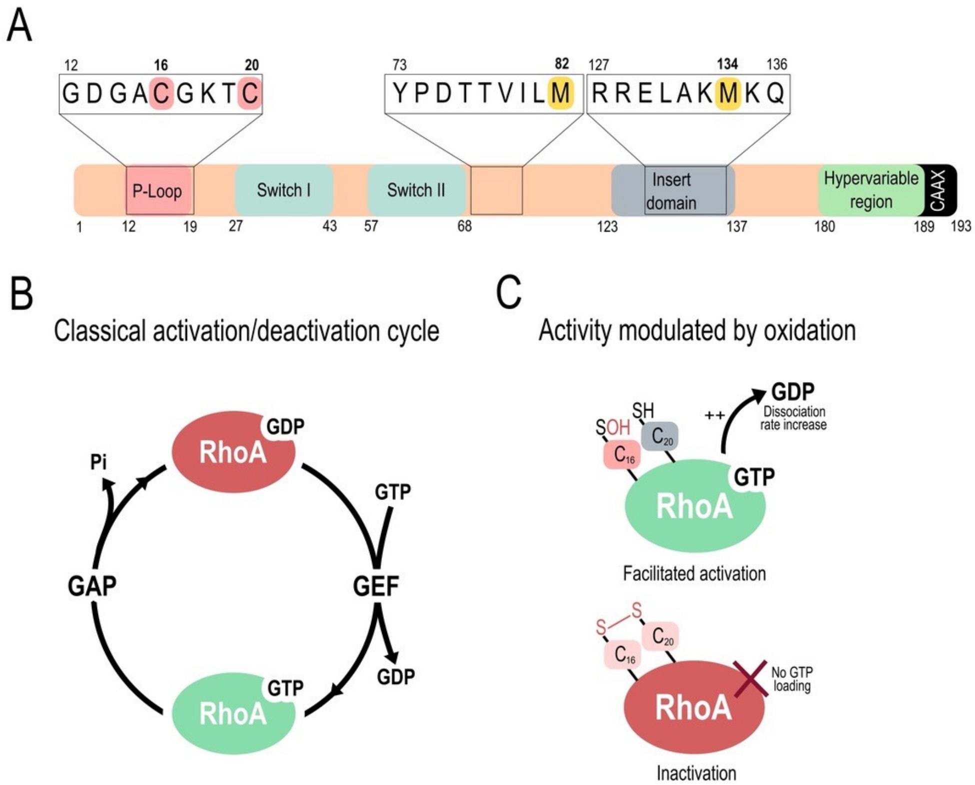

Figure 1. The small GTPase RHOA is regulated by oxidation. (A) Schematic representation of the domain organization of RHOA, highlighting the two redox-sensitive cysteine residues (Cys_{16} and Cys_{20}) and the two conserved methionine residues (Met_{82} and Met_{134}). (B) Schematic depiction of the GTPase activation (GTP-loading) and inactivation (GTP hydrolysis to GDP) cycle of RHOA by GEF and GAP proteins. (C) Schematic representation of the impact of cysteine oxidation on RHOA nucleotide states.

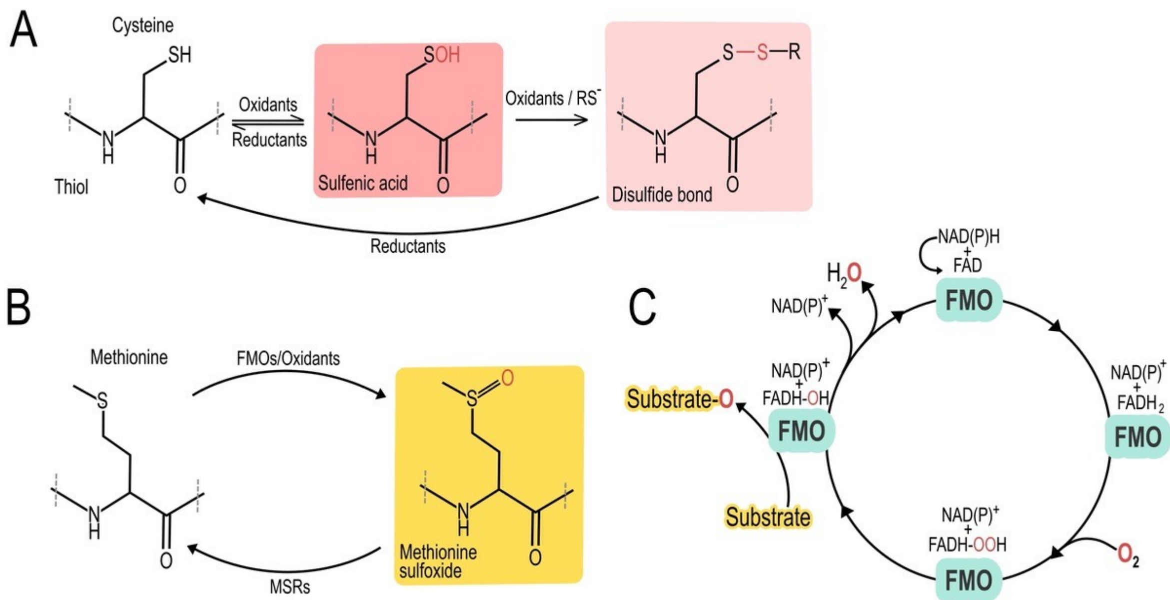

Figure 2. Cysteine and methionine residues are sensitive to oxidation. (A) Cysteine residues exposed to oxidants can lead to reversible sulfenic acid and disulfide bond formation. (B) Methionine residues can be oxidized to methionine sulfoxide, which can be reduced by MSR enzymes. (C) Depiction of the enzymatic reaction catalyzed by FMOs, resulting in the addition of an oxygen atom onto a substrate.

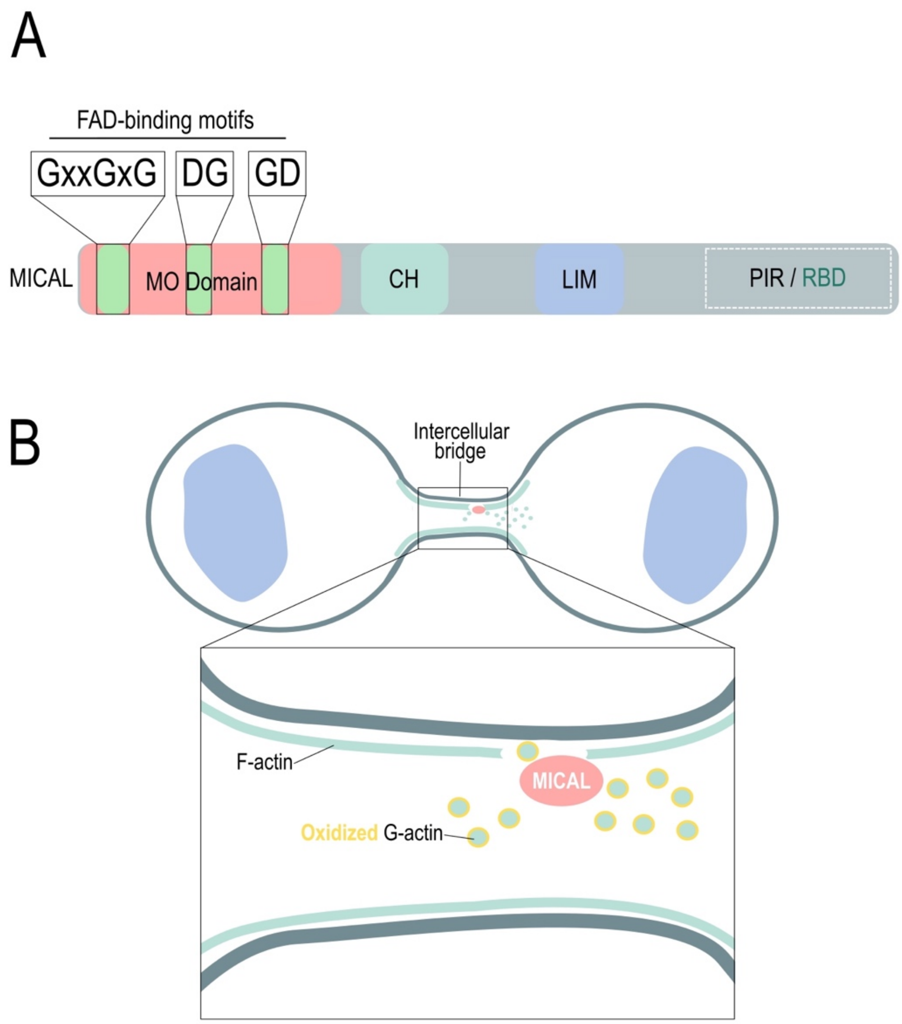

Figure 3. MICAL actin-depolymerizing activity regulates cytokinesis completion. (A) Schematic representation of the domain organization of MICAL proteins, highlighting the monooxygenase (MO) domain with FAD-binding motifs, a calponin homology (CH) domain, a LIM domain and a plexin-interacting region / RAB-binding domain (PIR/RBD). (B) Schematic depiction of the actin-depolymerizing activity of MICAL at the intercellular bridge during cytokinesis.

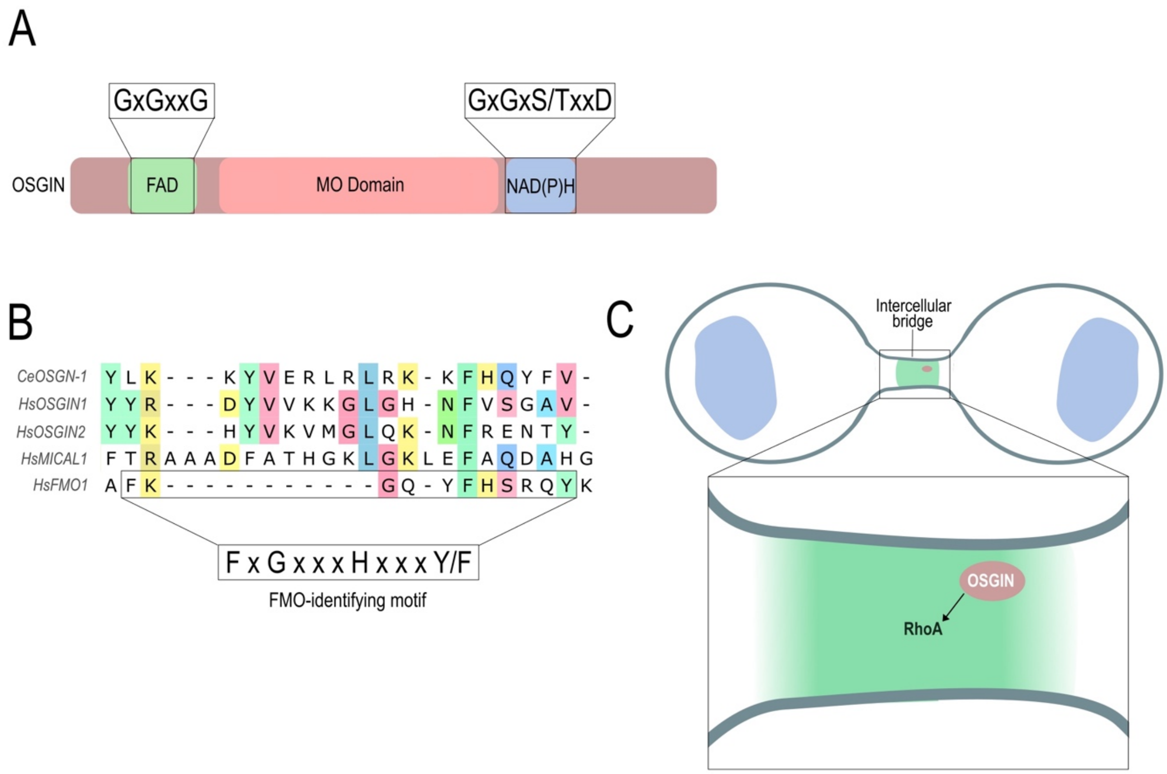

Figure 4. OSGIN proteins are FMOs regulating cytokinesis completion. (A) Schematic representation of the domain organization of OSGIN proteins, highlighting the FAD- and NADPH-binding motifs and an uncharacteristic monooxygenase (MO) domain. (B) Multiple sequence alignment of the FMO-identifying motif of *C. elegans* OSGN-1 (CeOSGN-1), human OSGIN proteins (HsOSGIN1 and HsOSGIN2), human MICAL1 (HsMICAL1) and human canonical FMO1 (HsFMO1). The motif is not conserved in OSGIN family or MICAL1 proteins. (C) Schematic depiction of the regulation of RHOA activity at the intercellular bridge in late cytokinesis by OSGN-1/OSGIN1 enzymes.

Table 1. Classification and features of relevant human FMOs.

FMO Group[a]	Name[b]	Enzyme Commission number (EC)[c]	Expression	Homologs[d]	*In vivo* substrate(s)[e]	Cellular process(es)	References
A	MICAL1	1.14.13.225	Ubiquitous	*Dm*Mical *Ce*EHBP-1[g]	S-oxidation of (R)-Methionines 44 and 47 of Actin (*Dm* and *Hs*)	Actin cytoskeleton reorganization, cytokinesis	[49,54,55,81]
	MICAL2		Ubiquitous			Actin cytoskeleton reorganization, myogenic differentiation, tumorigenesis	[55,81-84]
	MICAL3		Ubiquitous			Actin cytoskeleton reorganization, cytokinesis	[55,60,81,85]
B	FMO1	1.14.13.8	Fetal liver, kidney, adult liver, adipose tissue, small intestine	*Ce*FMO-1-5 *Dm*Fmo-1-2	Methionine, cysteamine, *S*-cysteine conjugates	Taurine biosynthesis, cell metabolism[h]	[86,87]
	FMO2		Adult liver, oesophagus, lungs		Methionine, cysteamine	Cardiac remodeling, tryptophan metabolism	[87-91]
	FMO3		Adult liver		Trimethylamine	Glucose and lipid metabolism	[87,92]
	FMO4		Kidney, liver		Unknown	Cell metabolism	[93-95]
	FMO5		Liver, skin, small intestine		Unknown, capable of C- or S-oxidation *in vitro*	Cell metabolism	[96,97]
	FMO6P[f]		Liver		Unknown	Unknown	[98,99]
Not yet ascribed (likely B)	OSGIN1	Unknown (most likely 1.14.13)	Liver, kidney, ovary	*Ce*OSGN-1 No *Dm* ortholog	Unknown, capable of S-oxidation *in vitro*	Cytokinesis, apoptosis, autophagy	[66]

[a]Groups are based on the CATH (Class, Architecture, Topology and Homologous superfamily) classification of their flavin binding domain [44,45]. [b]Homo sapiens. [c]All 1.14.13 enzymes are classified as FMOs-subclass. [d]*Ce*: *Caenorhabitis elegans*, *Dm*: *Drosophila melanogaster*. [e]Most common/known endogenous substrates. A wide variety of N- and S-containing drugs and xenobiotics agents can be oxygenated by FMO1-3 (reviewed in [100]) and FMO5 [101]. [f]FMO6P is a pseudogene in humans. [g]Whereas no ortholog of MICAL exists in *C. elegans*, EHBP-1 has a similar organization to the non-FMO portion of MICAL [102,103]. [h]FMO1-5 are involved in several metabolic pathways [104].