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REVIEW

Coordination of microtubule acetylation and the actin cytoskeleton by formins

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Abstract

The acetylation of the lysine 40 residue of α-tubulin was described more than 30 years ago and has been the subject of intense research ever since. Although the exact function of this covalent modification of tubulin in the cell remains unknown, it has been established that tubulin acetylation confers resilience to mechanical stress on the microtubules. Formins have a dual role in the fate of the actin and tubulin cytoskeletons. On the one hand, they catalyze the formation of actin filaments, and on the other, they bind microtubules, act on their stability, and regulate their acetylation and alignment with actin fibers. Recent evidence indicates that formins coordinate the actin cytoskeleton and tubulin acetylation by modulating the levels of free globular actin (G-actin). G-actin, in turn, controls the activity of the myocardin-related transcription factor-serum response factor transcriptional complex that regulates the expression of the *α-tubulin acetyltransferase 1* (α-*TAT1*) gene, which encodes the main enzyme responsible for tubulin acetylation. The effect of formins on tubulin acetylation is the combined result of their ability to activate α-*TAT1* gene transcription and of their capacity to regulate microtubule stabilization. The contribution of these two mechanisms in different formins is discussed, particularly with respect to INF2, a formin that is mutated in hereditary human renal and neurodegenerative disorders.

Keywords Microtubules · Tubulin acetylation · Formins · INF2 · α-Tubulin acetyltransferase 1 · Actin homeostasis · Serum response factor · Myocardin-related transcription factor

Abbreviations

α-TAT1	α-Tubulin acetyltransferase
CMT	Charcot–Marie–Tooth
DAD	Diaphanous autoregulatory domain
DID	Diaphanous inhibitory domain
FH	Formin homology
HDAC6	Histone deacetylase 6
INF	Inverted formin
FSGS	Focal segmental glomerulosclerosis
MRTF	Myocardin-related transcription factor
MT	Microtubule
SRF	Serum response factor
WH2	Wiskott–Aldrich syndrome homology region 2

Introduction

Microtubules (MTs) are hollow biopolymers assembled by lateral interaction of protofilaments, which are polarized structures composed of α/β-tubulin heterodimers linearly aligned in a head-to-tail fashion. MTs are major constituents of the cytoskeleton and are organized as cytoplasmic centrosomal and non-centrosomal arrays and as an integral part of subcellular structures such as mitotic spindles and intercellular cytokinetic bridges, and of organelles, such as centrioles and cilia. In response to specific external signals, MTs rapidly remodel to adapt the cell for a large variety of functions, including adhesion, polarization and migration [1–4].

MTs are the subject of numerous posttranslational modifications. In addition to the modifications frequently found in other proteins, such as phosphorylation, acetylation, palmitoylation and ubiquitylation, MTs undergo other less common modifications such as detyrosination, polyglutamylation and polyglycylation [5, 6]. It has been proposed that, analogous to the histone code on chromatin [7], tubulin

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posttranslational modifications constitute a code that is important for cell functioning [8, 9].

Tubulin acetylation

Lysine acetylation, which consists of the covalent addition of an acetyl moiety to the ε-amino group of a lysine residue in a protein, was originally found to regulate the activity of histone and a variety of transcription factors, but now it is also known to be present in non-nuclear proteins [10]. Acetylation of tubulin on lysine was first reported in *Chlamydomonas* flagella [11]. Subsequently, the lysine 40 (K40) residue of α-tubulin was identified as the major acetylation site [12]. Mitotic spindles, intercellular cytokinetic bridges, centrioles, cilia and a subset of cytoplasmic MT arrays are heavily acetylated on the K40 residue of α-tubulin in mammalian cells (for an extensive review, see [13]). K40 acetylation appears to modulate intracellular transport by regulating the traffic of kinesin motors [14, 15], although this effect might be indirect [16, 17]. K40 acetylation accompanies epithelial cell polarization [18] and is important for cell adhesion and contact inhibition of proliferation in fibroblasts [19], mechanosensation [19–23], and neuron polarization [14, 24]. Polycystic kidney disease [25] on the one hand, and Alzheimer [26, 27], Huntington [15, 28], Parkinson [29, 30] and Charcot–Marie–Tooth (CMT) [15, 28] degenerative neuropathies on the other have been associated with increased and decreased levels of K40 acetylation, respectively. Cystic fibrosis [31], familial dysautonomia (a disease of the autonomic and sensory nervous systems) [32], and specific types of cancer [33–35] are some additional examples of diseases with altered tubulin acetylation (for an extensive review, see [36]).

It is notable that K40 acetylation occurs in the MT lumen [37], while all other posttranslational modifications occur on the external surface [6, 38]. It has been proposed that tubulin acetylation could affect the binding of proteins that are transported through the interior of the MT [39–41]. K40 acetylation does not significantly change either the ultrastructure of MTs or the conformation of tubulin [42], but it does weaken lateral inter-protofilament interactions, endowing MTs with greater flexibility and resistance to mechanical stress [43–45]. MTs are highly dynamic and undergo continuous cycles of growth and shrinkage. During this phenomenon, known as "dynamic instability", tubulin subunits associate and dissociate rapidly from the growing end of the protofilaments [46]. Whereas most cytoskeletal MTs show this behavior and, therefore, have short half-lives (5–10 min), there is a subset that has a much longer half-life (> 1 h) [47, 48]. Notably, tubulin acetylation, rather than contributing to MT stability, accumulates in the stable MT subset because of their longevity [49, 50].

Tubulin acetylating and deacetylating enzymes

The level of acetylated tubulin is governed by the opposing reactions of acetylation and deacetylation [13] (Fig. 1). In mammals, α-tubulin acetyltransferase 1 (α-TAT1) functions as the major tubulin acetyltransferase in vivo, as demonstrated by the observation that deletion of the mouse gene leads to the almost complete loss of α-tubulin acetylation [19, 21–23]. α-TAT1 accesses the lumen of the microtubule via quick diffusion to acetylate the lumen-facing target residue K40 at a slow catalytic rate [51, 52]. The activity of α-TAT1 is regulated by autoacetylation [21]. The expression of α-TAT1 orthologues are found in all organisms with axonemal structures such as cilia and flagella, and is absent from organisms that subsequently lost cilia. This finding suggests that the primeval and ongoing cellular function of tubulin acetylation is linked to cilium biogenesis, function, and/or maintenance [23]. α-TAT1 is distantly related to the histone acetyltransferase GCN5 (general control nondepressible 5) [53, 54], which is a member of the GCN5-related *N*-acetyltransferase (GNAT) superfamily [55]. The crystal structure of the human α-TAT1/acetyl-CoA complex

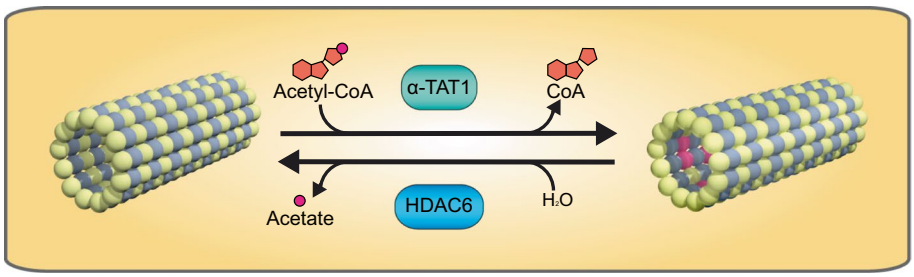

Fig. 1 Enzymes involved in tubulin acetylation and deacetylation. Tubulin acetylation refers to the transfer of the acetyl group from acetyl-coenzyme A to the K40 residue of α-tubulin. This modification is catalyzed by α-TAT1 in mammals, whereas its reverse reaction is catalyzed by the deacetylase HDAC6. Note that the K40 residue of α-tubulin is located within the microtubule lumen

reveals an overall fold similar to that of GCN5, but features a relatively wide substrate-binding groove and a unique structure involved in α-tubulin-specific acetylation [53]. Histone deacetylase 6 (HDAC6) is the main tubulin deacetylase in cultured cells [56] and in mice, since global α-tubulin hyperacetylation occurs in *HDAC6* gene knockout mice [57]. In addition to the α-tubulin K40 residue, HDAC6 deacetylates other acetylated lysine residues of α- and β-tubulin [58] and of other proteins, including the molecular chaperone Hsp90 (heat-shock protein 90 kDa) and the cytoskeletal protein cortactin [59]. SIRT2 was identified as a NAD^+-dependent α-tubulin deacetylase in vitro and in cultured cells [60]. However, the deletion of the *SIRT2* gene does not affect the level of tubulin acetylation in vivo [61, 62]. SIRT2 may function under special conditions, such as in murine macrophages, where SIRT2, but not HDAC6, is the responsible α-tubulin deacetylase during inflammasome activation [63].

Formins as dual regulators of the actin and microtubule cytoskeletons

Formins are a widely expressed family of proteins whose primary function is to nucleate and polymerize monomeric globular actin (G-actin) into linear actin filaments [64–66]. Humans exhibit 15 formins, classified into 8 phylogenetic groups [67]. The defining feature of all of them is the 350–400-amino acid formin homology (FH)2 [68], which catalyzes actin nucleation and forms a head-to-tail, doughnut-shaped dimer that encircles the growing end of the actin filament during elongation [69]. Immediately upstream, the FH2 domain has a proline-rich FH1 domain that binds profilin, which binds G-actin for provision to the FH2 [70]. In addition, most formins have an autoregulatory domain in their carboxyl half, known as the diaphanous autoregulatory domain (DAD), which is separated by the FH1 and FH2 domains from the diaphanous inhibitory domain (DID), which is present in the amino-terminal half. In the formin mDia 1, and probably in most formins, the DAD collaborates with the FH2 domain in actin nucleation [71]. The DAD has an important role in regulating formin activity by interacting with the DID to close the formin molecule in an inactive state [72]. In the case of the diaphanous-related formin group, which includes formins mDia1-3, the binding of a specific Rho GTPase to a zone encompassing part of the DID and a short amino-terminal extension, releases the DID–DAD interaction and opens up the molecule to transform it into its active form [73] (Fig. 2).

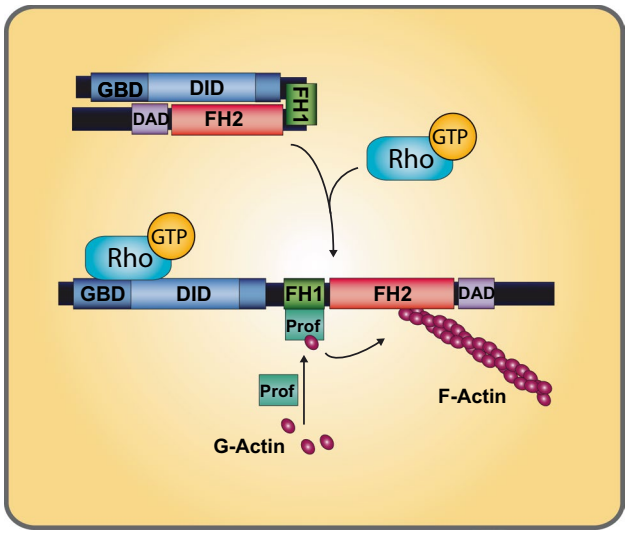

Fig. 2 Regulation of diaphanous-related formins and domain organization of distinct formins. The autoinhibitory effect of the DID–DAD interaction in the diaphanous-related formin group is released through binding of a specific Rho-family GTPase in its active GTP-loaded form. In the open conformation of formins, the FH1 domain recruits profilin (Prof), which, in turn, brings actin monomers close to the FH2 domain for actin polymerization

Formins bind and stabilize microtubules

The formins mDia1-3, Fmn1, INF1 and INF2 colocalize with MT or bind directly to MTs [74–76]. In addition to their role in actin nucleation, mammalian formins control the alignment of MTs with actin filaments, MT stabilization, and MT acetylation [74, 77–79]. The capping of the MT ends inhibits MT dynamics and causes MT stabilization, as shown by the lack of tubulin subunit turnover from the ends of the stabilized MTs [75, 80–82]. In the cases of mDia2 and INF2, the binding region and the stabilization activity include the FH2 domain [75, 82–84], both of which events are independent of the actin polymerization activity of the FH2 domain [75, 80, 85]. MT stabilization involves interactions among different formins and regulators of the MT cytoskeleton, including MT plus-end-tracking proteins EB1 and APC and the scaffolding protein IQGAP1, in a process that is regulated positively by active Rho GTPase and negatively by the kinase GSK3β [80, 82, 86–89]. INF2 interacts with mDia1-3 [90] and acts downstream of mDia2 in the process of MT stabilization [80]. Actin-capping protein [91], which is the main high-affinity barbed-end actin terminator [92], establishes a crosstalk between the actin and MT cytoskeletons by antagonizing the actin activity of mDia1 and facilitating its effect on MTs [93]. Therefore, formin-mediated MT stabilization appears to result from the capping of the MT ends by a large protein machinery complex or "MT stabilisome", which consists of formins and a large number of other proteins, whose components are hierarchically organized [80]. Since acetylated tubulin accumulates in long-lived MTs

[49, 94], the effect of formins on MT longevity clearly contributes to the extent of MT acetylation.

Formins control the actin–MRTF–SRF transcriptional circuit and induce tubulin acetylation

Serum response factor (SRF) is a widely expressed transcription factor of mammals [95, 96]. The myocardin-related transcription factor (MRTF) forms a complex with SRF and regulates its activity. At high G-actin concentrations, MRTF forms a reversible complex with G-actin via its RPEL domain and is held in an inactive state in the cytoplasm. At low G-actin concentrations, G-actin-free MRTF exposes a nuclear import signal, enters the nucleus and associates with SRF to direct transcription of target genes [97, 98]. SRF recognizes 10-bp DNA elements called CArG and CArG-like boxes [99, 100]. Many of the nearly 1000 genes with CArG elements whose transcription is regulated by the MRTF–SRF complex encode proteins involved in actin dynamics, cell adhesion, and extracellular matrix synthesis and processing [101, 102].

Overexpression of deregulated FH1FH2-containing fragments from different formins induces MRTF–SRF transcriptional activity to different extents due to the G-actin content decrease caused by their relative actin polymerization activity [79, 103–106]. mDia3, Fmnl1 and INF2 are the strongest inducers, FHOD1, FHOD3 and Fmn1 induce poorly, and the other formins analyzed show intermediate inducer activity [79]. Actin polymerization activity is essential for this effect, as demonstrated by the formin mDia1 [104]. Analysis of the *α-TAT1* gene has revealed the presence of functional CArG elements, as demonstrated by their response to activators of the actin–MRTF–SRF circuit or to transfection of active forms of MRTF [107]. Therefore, transcriptional activation of the *α-TAT1* gene contributes to the extent of tubulin acetylation promoted by formins.

The effect of formins on tubulin acetylation is a combined result of their microtubule stabilization activity and their capacity to activate *α-TAT1* gene expression

Formins affect tubulin acetylation by stabilizing MTs (Fig. 3) and by activating the transcription of the *α-TAT1* gene, but one of the mechanisms dominates the other, depending on the specific formin. For instance, in the case of endogenous INF2, the effects on *α-TAT1* gene transcription and on MT stabilization are both important and, as in most formins, the two require the presence of the FH2 domain [75, 107]. In formin INF1, however, they are segregated into different domains. The amino-terminal half containing the FH1 and FH2 domains exhibits intermediate MRTF–SRF-dependent

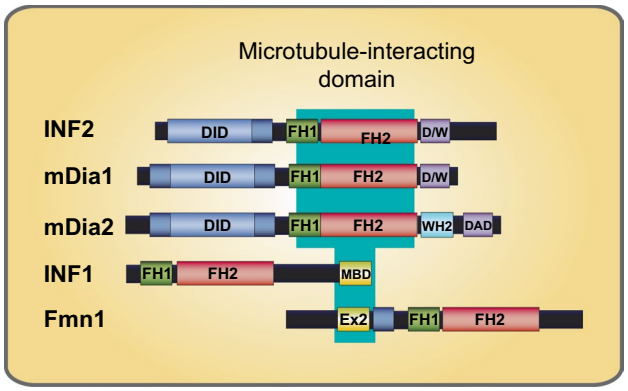

Fig. 3 Domain organization of the indicated formins. The domain involved in MT stabilization is indicated. The molecules are not drawn to scale

transcription activation and, hence, contributes to *α-TAT1* gene transcription. In addition, since it neither binds nor stabilizes MTs, the contribution of this part of the molecule to promoting microtubule stabilization is almost negligible. However, a bipartite MT binding domain at the carboxyl-terminal of the FH2 of INF1 mediates binding to MTs and strong MT stabilization [79, 106]. Therefore, the carboxyl-terminal part is the main contributor to tubulin acetylation by the INF1 molecule. A similar case could be that of formin Fmn1, which also has a bipartite MT-binding region but, unlike that of INF1, is at the amino-terminal of the FH2 domain [108].

By controlling their actin polymerization activity, formins modulate the levels of G-actin and, through the effect of G-actin on MRTF–SRF activity, coordinate the actin cytoskeleton with the levels of acetylated tubulin via *α-TAT1* gene transcription. Since the concentration and regulation of formins probably vary between cell types and under different physiological and pathological conditions, the global effect of formins on the level of tubulin acetylation depends on the sum of the individual contributions to MT stabilization and MRTF–SRF activation (Table 1) and, consequently, to *α-TAT1* gene expression in each type of cell.

INF2 regulates actin homeostasis and tubulin acetylation in a cell type-dependent manner

INF2 is an atypical formin that, in addition to polymerizing actin, as other formins do, causes severing and disassembly of actin filaments. The latter two activities require the DAD, which in INF2 contains a Wiskott–Aldrich syndrome homology region 2 (WH2) domain that binds G-actin [109]. INF2 regulates vesicular transport [110, 111], mitochondrial fission [85, 112], podosome formation [113], and prostate

Table 1 Summary of the effect of some formins on the MT cytoskeleton and MRTF–SRF activity

Formin	MT binding	MT interacting domain	MT stabilization	MRTF–SRF activation (FH1FH2)	MT acetylation (FH1FH2)	References
INF2	Yes	FH1FH2	Yes	Yes	Yes	[79, 80, 84, 85]
mDia1	Yes	FH1FH2	Yes	Yes	Yes	[79, 84, 104]
mDia2	Yes	FH1FH2	Yes	Yes	Yes	[75, 79]
INF1	Yes	MBD	Yes	Yes	Yes	[79, 106]
Fmn1	Yes	Ex2	n.d.	No	No	[79, 108]

cancer cell migration and invasion [114]. Mutations in the DID of INF2 cause autosomal-dominant focal segmental glomerulosclerosis (FSGS), a degenerative kidney disease, with or without associated CMT neuropathy [115].

The control of actin homeostasis by formins depends on the formin that has the most prominent role in actin polymerization in each type of cell. For instance, in RPE-1 cells silenced for INF2 expression, the G-/F-actin ratio increases, and subsequently MRTF–SRF activity, *α-TAT1* gene transcription and tubulin acetylation decrease. DIA1 silencing, however, does not have any significant effect [107]. This observation indicates that INF2 is more important than DIA1 for actin homeostasis and tubulin acetylation in RPE-1 cells. However, this is not true in Jurkat T cells, in which INF2 silencing does not affect tubulin acetylation [107, 116]. Therefore, INF2 controls actin homeostasis and tubulin acetylation in a cell type-specific manner.

An important feature of INF2 not found in other formins is that the in vitro binding of G-actin to the WH2/DAD releases INF2 from its autoinhibitory state, thereby activating actin polymerization [117]. If this property also applies in vivo, given the low affinity of the INF2 DID–DAD interaction [118] and the high affinity of the INF2 WH2/DAD for G-actin [117] (Fig. 4), the actin polymerization activity of INF2 might be regulated by small fluctuations in the cytosolic pool of G-actin in such a way that an increase or decrease in the levels of G-actin increases or decreases the activity of INF2 [117]. Other human formins have WH2-like motifs within their DAD (e.g., mDia1) or outside it (e.g., FMNL2-3, DAAM1) [119]. The mDia1 DAD binds G-actin with micromolar affinity whereas the affinity of its DID–DAD interaction is in the submicromolar range (Fig. 4) [71]. Consequently, mDia1 remains autoinhibited in vitro in the presence of

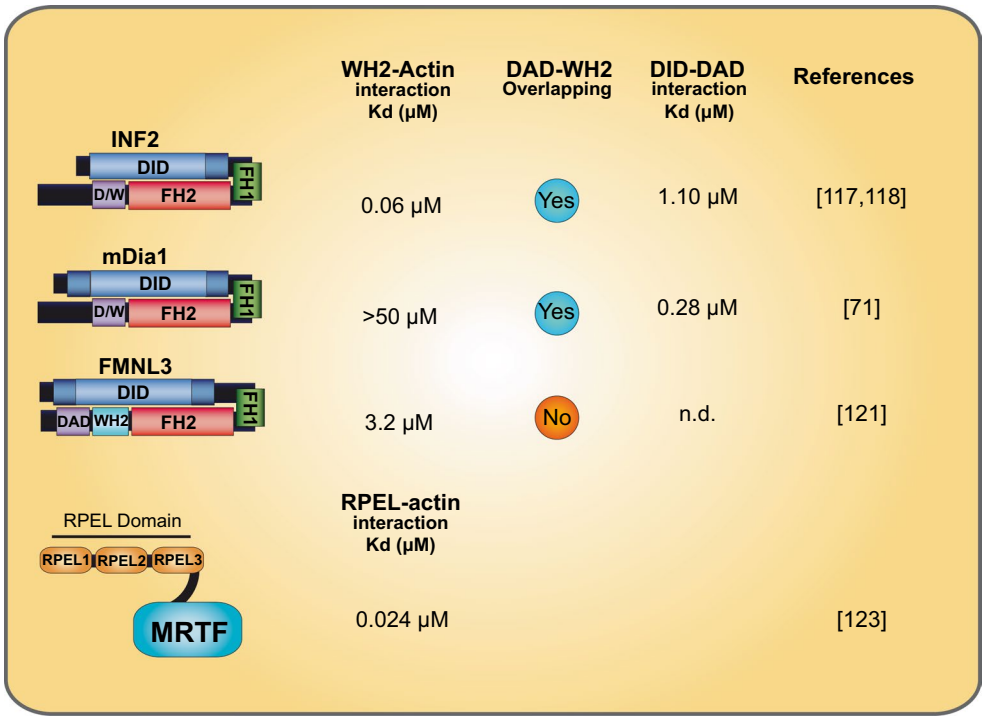

Fig. 4 Affinity constants of MRTF and different formins for G-actin. Compilation of the dissociation constants of MRTF and of the DAD/WH2 of INF2, mDia1 and FMNL3 for G-actin, and dissociation constants for the DID–DAD interaction in these formins

free G-actin under conditions in which INF2 is activated [117]. Despite its low affinity for actin, exogenous mDia1 can be activated in response to high G-actin cytosolic concentrations in cultured cells [120]. The formins FMNL2, FMNL3 and DAAM1 also contain WH2-like sequences that, in the case of FMNL3, bind G-actin with an affinity intermediate between those of INF2 and mDia1 (Fig. 4). It is likely that the binding of G-actin to these three formins does not interfere with their DID–DAD interaction, since their WH2-like sequence is not embedded in their DAD [119, 121, 122]. In conclusion, the high-affinity binding of the INF2 WH2/DAD to actin and the activation of its actin polymerization activity by G-actin make INF2 a formin especially well suited to act as a sensor of subtle physiological oscillations in the levels of cytosolic G-actin, and to respond to them by fine-tuning its actin polymerization activity, thereby controlling actin homeostasis.

The affinity of the WH2/DAD of INF2 for G-actin is in the same range as that of the actin-binding RPEL domain of MRTF (Fig. 4) [123]. Consequently, given the regulation of INF2 actin polymerization activity by G-actin, the WH2/DAD can sense the levels of free G-actin and, depending on the levels of G-actin, promote the assembly of actin filaments to control the nuclear localization of MRTF. Consequently, changes in INF2 activity can control actin homeostasis and modulate the expression of *α-TAT1* and a large number of other genes related to cytoarchitecture that are regulated by the actin–MRTF–SRF transcriptional circuit [97, 101] (Fig. 5). Therefore, INF2 could act as a G-actin sensor that controls not only tubulin acetylation but

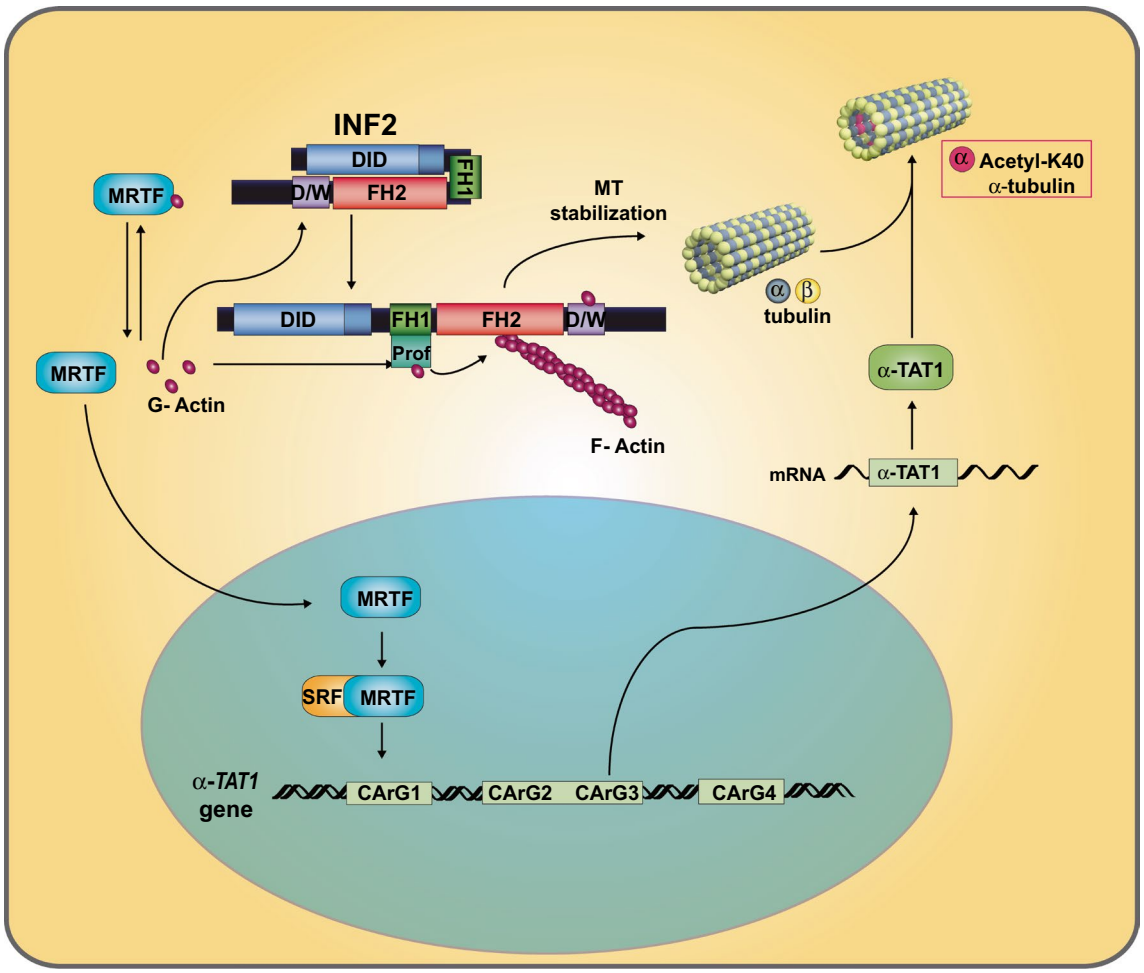

Fig. 5 Schematic of the proposed model of INF2 function on microtubule acetylation. Given that the affinity of the WH2/DAD (W/D) of INF2 for G-actin is much higher than for the DID, INF2 may sense the increase of the levels of G-actin better than other formins. Since, in the case of INF2, the binding of G-actin to the DAD/WH2 releases INF2 from autoinhibition, increased levels of free G-actin result in INF2-mediated actin polymerization and, consequently, a decrease in free G-actin. This decrease allows MRTF to enter the nucleus and associate with SRF to direct the transcription of the *α-TAT1* gene and other target genes. The increased levels of α-TAT1 mRNA produce more α-TAT1 and, subsequently, MT acetylation on the K40 residue of α-tubulin augments. In addition, INF2 contributes to tubulin acetylation via MT stabilization by forming part of a large protein complex that stabilizes MTs in an active Rho GTPAse-dependent manner

also more extensive cytoskeleton remodeling [124, 125]. This role could be particularly relevant in cellular processes involving INF2 in which the cytoskeleton globally remodels as during podosome formation [113, 125], in cell migration and invasion of cancer cells [114], and in the response to acute stress conditions such as the application of mechanical force [124] and Ca^{2+} influx [126].

Studies performed in *C. elegans* with disease-associated INF2 mutations point to an effect of mutant INF2 on both MTs and actin [127]. The role of INF2 in controlling the transcription of MRTF–SRF target genes, including the *α-TAT1* gene, may help explain the molecular basis of INF2-related disease [115] and the alterations in tubulin acetylation noted in other human disorders [36].

Concluding remarks and outstanding questions

Crosstalk between the actin cytoskeleton and the MT network is essential for critical cellular processes, including formation of the leading edge and focal adhesions during cell migration, and the assembly of the intercellular bridge during cytokinesis [128]. Since the function of formins affects the actin and MT cytoskeletons [74, 78], formins make excellent candidates for coordinating the two structures. The function of the acetylation on the K40 residue of α-tubulin is intriguing. Although relationships between formins and MT stabilization, and between MT stabilization and accumulation of acetylated tubulin were established some time ago, no other mechanism linking formins and tubulin acetylation was known until recently. The finding that tubulin acetylation is controlled by formins also through the regulation of *α-TAT1* gene transcription represents a new mechanism of coordination in the actin and MT cytoskeletons [107].

It is puzzling that INF2 is critical to microtubule acetylation in some types of cells (e.g., epithelial RPE-1 and ECV304 cells) but not in others (e.g., Jurkat T cells) [107, 116]. It would be interesting to identify the tissues and cell types in which INF2 is crucial, and to investigate how the actin and MT cytoskeletons coordinate in the rest of tissues. Unfortunately, the mutations in the WH2/DAD of INF2 known to interfere with G-actin binding also affect its binding to the DID [109] and activate actin polymerization, just as the binding of G-actin to the INF2 WH2/DAD does [117]. Although it is not straightforward to examine, the possible function of INF2 as a sensor of free G-actin in certain cell types deserves more detailed study. It is of note that mechanosensation has been previously related to MT acetylation [19–23] and to INF2 [126]. It would be interesting to analyze INF2 knockout animals to investigate whether the effect of INF2 on mechanosensation is mediated by the lack of MT acetylation. INF2 controls MRTF–SRF transcriptional activity in RPE-1 cells and probably in other types of cell [107]. Therefore, in addition to *α-TAT1* gene expression, INF2 can regulate the expression of other genes regulated by MRTF–SRF, including a large number of genes encoding regulators of the cytoskeleton, transcription, and cell growth and metabolism. Mutations in INF2 cause FSGS and other nephropathies as well as FSGS with associated CMT disease [115]. Some of the mutations occur even in contiguous amino acids but produce either FSGS alone or FSGS plus CMT, as is the case of the L76P and L77P mutations, respectively [129]. Therefore, a greater knowledge of both INF2 structure and the role of INF2 in coordinating the actin cytoskeleton and MT acetylation may help us understand the molecular basis of human INF2-related pathologies [115] and of other disorders featuring altered levels of tubulin acetylation [36].

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