Actin–microtubule crosstalk in cell biology

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Abstract

The cytoskeleton and its components — actin, microtubules and intermediate filaments — have been studied for decades and multiple roles of the individual cytoskeletal substructures are now well-established. However, in recent years it has become apparent that the three cytoskeletal elements also engage in an extensive crosstalk, which is important for core biological processes. Actin-microtubule crosstalk is particularly important for the regulation of cell shape and polarity during cell migration and division, and the establishment of neuronal and epithelial cell shape and function. This crosstalk engages different cytoskeletal regulators and encompasses various physical interactions, such as crosslinking, anchoring and mechanical support. Thus, the cytoskeleton should be considered not as a collection of individual parts, but rather as a unified system, in which subcomponents co-regulate each other to exert their functions in a precise and highly adaptable manner.

[H1] Introduction

A large number of cellular processes depend on the cytoskeleton, a filamentous scaffold of proteins that pervades the cytoplasm and extends all the way from the plasma membrane to the nucleus. In animal cells, the cytoskeleton consists of three filamentous subsystems: microtubules, actin filaments, and intermediate filaments. All three subsystems contribute to the internal organization of the cytoplasm and stabilization against applied forces. Actin filaments and microtubules in addition actively generate forces to drive cell shape changes and motility. They are also substantially more dynamic than intermediate filaments, and these dynamic properties have important roles in many cell biological processes.

Microtubules self-assemble from tubulin dimers in the presence of the nucleotide guanosine triphosphate (GTP). They form relatively stiff hollow tubes consisting of 13 protofilaments and have the ability to switch between growing and shrinking phases in a process termed dynamic instability [G][1]. In many cells, microtubules are anchored through their minus end to an organizing structure such as a centrosome, leading to an astral arrangement with microtubule plus ends radiating outward to the cell periphery. A correctly organized microtubule cytoskeleton is responsible for the segregation of chromosomes during cell division, the transport and distribution of different cargoes,

44 such as intracellular vesicles and organelles, and the maintenance of polarity in migrating cells and in
45 epithelial tissues.
46   Actin monomers polymerise into double helical strands in the presence of the nucleotide
47 adenosine triphosphate (ATP), which are thinner and less stiff than microtubules. Dynamic actin
48 filaments are often cross-linked into bundles or networks to perform their function in cells[2]. The
49 actin cytoskeleton is important for cell migration, reinforces the membrane at the cell cortex **[G]**,
50 and drives cytokinesis at the final stages of cell division.
51   Both the cell biology and biophysics of the actin and microtubule cytoskeletons have been
52 studied extensively in the last decades, leading to a fairly complete description of their behaviour
53 and regulation in a large variety of cellular contexts. It is, however, becoming increasingly clear that
54 the two cytoskeletal systems often work together in core cellular processes, and that their functional
55 dynamic properties are often intimately intertwined. Here we review recent insights in the
56 mechanisms that underlie functional crosstalk between microtubules and actin with a focus on the
57 role of physical interactions mediated by associated proteins or protein complexes. We summarize
58 recent studies showing how different modes of crosstalk combine in the contexts of cell motility, the
59 control of shape and polarity in neurons and epithelial cells, and cell division. Finally, we discuss how
60 future experiments may help us address the gaps that remain in our understanding of functional
61 actin–microtubule interactions.
62

[H1] Means of crosstalk

64 One can distinguish between a number of different ways in which the actin and microtubule
65 cytoskeletons may "talk" to each other. Although there are often context-specific molecular players
66 involved (see Table 1), it appears that crosstalk between actin and microtubules can be distilled
67 down to a limited list of (physical) mechanisms that, with small variations, are found in very different
68 cellular contexts. These mechanisms vary from interactions mediated by molecular components that
69 provide direct physical crosslinks or regulate the dynamic behaviour of cytoskeletal filaments, to
70 shared regulators that affect the dynamic properties of both systems, to more indirect mechanisms
71 based on mechanical effects of one cytoskeletal system on the other.
72

73 [H2] *Actin–microtubule crosslinking and guidance of microtubule growth.*
74 One direct way of actin-microtubule crosstalk is provided by proteins that crosslink microtubules to
75 actin bundles. This physical linkage is mediated by large multi-domain proteins or protein complexes
76 with binding sites for F-actin and microtubules. Some of these crosslinking proteins also have the
77 ability to interact with microtubule plus-end-binding proteins (EB proteins), thereby acting both as
78 actin–microtubule cross-linkers and microtubule plus-end trackers **[G]** (often referred to as +TIPs)[3,4].
79 These proteins can thus provide dynamic links between the plus-ends of growing microtubules and
80 actin bundles **(Fig. 1a)**, which may result in the redirection of microtubule growth along actin
81 bundles. It was shown by *in vitro* reconstitution that this type of crosslinking does in fact provide a
82 sufficient mechanism to explain actin-mediated microtubule guidance and orientation of
83 microtubule growth along actin bundles[5].
84

85 *[H2] Actin-mediated anchoring and stabilization of microtubule ends.*
86 Another type of physical linkage occurs via the anchoring and stabilization of microtubule ends (both
87 plus and minus ends) by protein complexes associated with actin networks, as often observed at the
88 cell cortex **(Fig. 1b)**. This type of linkage may involve protein complexes that not only physically
89 capture microtubule ends, but also directly suppress the dynamic properties of microtubules,
90 leading to stable connections between actin networks and microtubule ends. Actin-mediated
91 anchoring of microtubule ends at the cell cortex may furthermore promote the exposure of
92 microtubules to cortical microtubule regulatory factors.
93

94 *[H2] Actin as a physical barrier for microtubule growth.*

95   Actin structures such as the actin cortex and actin in migratory protrusions may alternatively act as
96   an effective physical barrier that prevents growing microtubules from penetrating to the plasma
97   membrane **(Fig. 1c)**. A physical barrier impedes microtubule growth and subsequently promotes the
98   occurrence of catastrophes **[G]**, as was demonstrated *in vitro*[6]. As a consequence, the actin cortex
99   may block microtubules from reaching the membrane to interact with membrane-bound cortical
100 anchors[7] or to exert protrusive forces on the membrane[8]. The actin cortex may not only affect
101 microtubule behaviour by direct interactions, but also by controlling the shape of the cell. Myosin
102 motor activity in the actin cortex generates cortical tension in mitotic cells, which rigidifies and
103 rounds up the cell. Geometrical effects associated with cell rounding are for instance important for
104 microtubule spindle formation and positioning in dividing cells[9-11].
105
106 *[H2] Microtubule-mediated nucleation of actin filaments.*
107 There is evidence that microtubules may contribute directly to the localization of factors that
108 promote local actin polymerization[12] **(Fig. 1d)**. Actin nucleation and assembly is mediated by
109 formins, Ena/VASP, and Wiskott-Aldrich syndrome protein (WASP) family proteins that may
110 associate with microtubule ends both directly and indirectly via +TIPs[13,14]. For example, a recent *in*
111 *vitro* reconstitution study demonstrated that growing microtubule plus ends can directly stimulate
112 actin assembly via the association of a plus-end tracking factor (CLIP-170) with the formin mDia1
113 (also known as DIAPH1)[15]. In vivo, there is evidence of CLIP-170-mediated recruitment of mDia1
114 from studies in macrophages[16]. It is however unclear whether this type of actin-microtubule
115 interaction extends to other formins. Different formins appear to differ in their ability to interact
116 with actin and microtubules simultaneously and the interaction can be context-dependent[17-20].
117
118 *[H2] Crosstalk through shared regulators.*
119 Actin and microtubules also crosstalk via the regulators they share. Central players are members of
120 the Rho family of small GTPases. Rho GTPases act as molecular switches through their ability to cycle
121 between active (GTP-bound) and inactive (GDP-bound) states. They are activated by guanine
122 nucleotide exchange factors (GEFs) and inactivated by GTPase activating proteins (GAPs). Actin and
123 microtubules are coupled by Rho GTPase signalling in two ways: both systems are regulated by Rho
124 GTPases, and additionally, microtubules regulate the activity of Rho GTPases by interacting with
125 GEFs and GAPs[21], thereby contributing to the regulation of actin dynamics **(Fig. 1e)**.
126   The three best-characterized Rho GTPases are RHO, RAC and CDC42. RHO stimulates the
127 assembly of contractile actin arrays by promoting formin-mediated assembly of linear actin bundles
128 and activation of myosin II, whereas RAC and CDC42 promote the assembly of protrusive arrays by
129 promoting ARP2/3-mediated assembly of branched actin filaments. The action of Rho GTPases on
130 microtubules is primarily exerted at the microtubule ends and occurs through modulation of
131 microtubule-associated proteins (MAPs) such as stathmin, which fine-tunes microtubule dynamics
132 through modulating the pool of free tubulin[21].
133   Interestingly, recent studies have also indicated the centrosome as a shared regulator of
134 actin and microtubules. Actin and microtubule assembly may be coordinated at the centrosome
135 since the centrosome was shown to not only nucleate microtubules, but also directly promote actin
136 assembly[22].
137
138 *[H2] Mechanical cooperation.*
139 Microtubules are much stiffer than actin filaments, which is expressed by the persistence length — a
140 value that reflects the distance over which filaments remain straight under the action of thermal
141 forces. The persistence length of microtubules is a few mm, whereas it is only ~10 μm for actin. Since
142 microtubules are stiff polymers, they are capable of bearing considerable compressive loads[23-25], and
143 they are thought to be important in providing mechanical support against membrane retraction
144 during cell protrusion events **(Fig. 1f)**. Thus, microtubules mechanically collaborate with actin to
145 form actin-based protrusions. Note that although membrane protrusions are often driven by actin

146 polymerization, in some cases membrane protrusion can also be induced by microtubule pushing,
147 resulting from persistent polymerization[26] or from sliding antiparallel microtubules by the attached
148 motor proteins[26-30].
149

[H1] Crosstalk in cells

151 In the following sections, we review the functional significance of actin–microtubule crosstalk in the
152 context of different cellular processes. In each case, we summarize the known or suggested roles of
153 the different means of interaction described above, together with the specific molecular players
154 involved. It should be noted that direct evidence for the different means of interaction is inherently
155 more difficult to obtain in living cells than in isolated *in vitro* systems. This is due to the difficulty of
156 co-imaging the microtubule and actin cytoskeleton at sufficient spatial resolution. Moreover,
157 multiple molecular players are usually simultaneously involved in actin-microtubule interactions and
158 these molecular players often have many different functions. As a result, their manipulation may
159 have pleiotropic effects in cells. Evidence in cells is thus sometimes at best based on co-localization
160 of factors that are known to be able to mediate actin-microtubule crosstalk *in vitro*. In the future,
161 progress in super resolution imaging techniques in cells, combined with increased mechanistic
162 insight from *in vitro* experiments will likely improve this situation.
163

[H1] Cell migration

165 Cell migration is essential for many processes, including embryogenesis, wound healing, and
166 immunity as well as cancer cell invasion. Cells adapt their mode of migration to the physical
167 properties of their microenvironment. Cells encountering flat tissue surfaces exhibit 2D crawling
168 motion driven by actin polymerization in the lamellipodium **[G]**[31]. Traction is provided by integrin-
169 based focal adhesions **[G]**, which anchor the actin cytoskeleton to the extracellular matrix (ECM).
170 Crawling cells typically adopt a polarized morphology with a leading edge **[G]** containing a
171 protrusive, branched F-actin network and a trailing edge **[G]** containing a contractile actin-myosin
172 network **(Fig. 2a)**. Microtubules are typically anchored at the centrosome or the Golgi by their minus
173 ends and extend their plus ends towards the cell cortex at the front and rear. Nascent focal
174 adhesions assemble near the leading edge, grow in size, and eventually release and disassemble
175 under the cell body and in the rear as the cell moves forward. Focal adhesion stabilization and
176 maturation is dependent on myosin-II-driven tension in the lamellar actin network and the
177 formation of actin stress fibres **[G]**[32]. The main role of microtubules is to maintain directional
178 migration through mechanical stabilization of the leading edge[26], polarized trafficking of integrins
179 and matrix proteases[33], mitochondria positioning to deliver ATP to fuel motility[34], and control of RHO
180 and RAC GTPases that signal to the actin cytoskeleton[35].
181   When cells encounter more complex environments such as interstitial collagen networks,
182 they switch to different motility modes, depending on the degree of confinement and
183 ECMadhesion[36]. Migration of mesenchyme cells bears some resemblance to 2D cell crawling, being
184 driven by actin polymerization at the front. However, cells adopt an elongated morphology in 3D and
185 the flat lamellipodia is replaced by long pseudopodia **[G]** supported by microtubules[37,38]. Elongation
186 of pseudopodia requires persistent growth of microtubules[39]. Highly confined cells with low
187 adhesion switch to amoeboid motion, driven by actin flows or membrane blebbing **[G]**[40]. The role of
188 microtubules in amoeboid migration is largely unexplored.
189

190 *[H2] Actin-microtubule crosstalk in 2D cell migration.*
191 In migrating cells, positive feedback between the actin and microtubule cytoskeletons is essential for
192 establishing and maintaining the polarized organization of the cytoskeleton. Interactions with actin
193 guide microtubule growth toward the leading edge[41], where in turn microtubules help promote actin
194 polymerization. A context in which actin–microtubule crosstalk is often studied, is the
195 spatiotemporal regulation of focal adhesion turnover. Pioneering live-cell imaging studies revealed
196 that growing microtubules selectively target early adhesion sites, and that their plus ends get

197 captured and stabilized at the actin cortex in the vicinity of these sites[42-44]. Microtubules anchored at
198 focal adhesions mediate localized delivery of matrix-degrading enzymes that cleave the cell-ECM
199 adhesions, thereby promoting focal adhesion turnover[45]. Notably, in this context microtubules
200 appear to interplay with actin, but the mechanisms of this crosstalk are poorly characterized[46].
201 Moreover, microtubules regulate focal adhesion turnover through control of integrin recycling and
202 signalling via MAP4K4 as well as RhoGTPases[47-51].

204 The principal molecular players that allow microtubules to reach and interact with focal adhesions
205 have recently been identified. Guidance of microtubules **(Fig. 1a)** towards focal adhesions was
206 shown to rely on physical crosslinking of growing microtubules to actin stress fibres by
207 spectraplakins[52-54] and GAS2 proteins[55-57], which act as actin–microtubule cross-linkers and as
208 +TIPs[3,4]. Once microtubule plus ends reach the actin cortex, they can get captured **(Fig. 1b)** by
209 micrometre-sized cortical patches referred to as cortical microtubule stabilization complexes
210 (CMSCs)[58]. Recent proteomic analysis showed that capture occurs selectively at CMSCs near active
211 (matrix-bound) integrins[59]. CMCs are assembled around the rim of focal adhesions by recruitment of
212 KANK1 (KN motif and ankyrin repeat domain-containing protein 1), which associated with focal
213 adhesions through talin[58] **(Fig. 2b)**. Additional core components of CMCs are LL5β (also known as
214 PHLDB2) and liprins, which are membrane-bound. CMCs interact with microtubule plus ends through
215 several +TIP proteins, including CLASPs and CLIP-170[58,60,61], ACF7[62,63], APC[13], IQGAP1[64], and through
216 formins[64]. Whether these interactions are concurrent, competing, or sequential and how the
217 interactors are precisely spatially arranged within these complexes is still poorly understood.
218 Currently, it appears that CLASPs[45,65] and ACF7[62] simultaneously contribute to microtubule capture.
219 The relative contributions of membrane-bound factors versus those associated with actin at cell
220 cortex to microtubule capture are also unclear. Association with CMCs influences microtubule
221 stability both directly through interactions between microtubule ends and CMC components and
222 indirectly through Rho signalling **(Fig. 1e)** and increased microtubule acetylation **[G]**[66,67]. Live cell
223 imaging showed that the actin cortex may alternatively act as a physical barrier **(Fig. 1c)** that
224 prevents interactions of microtubule plus tips with the membrane[56]. In addition, in the lamellipodia
225 of migrating cells actin undergoes retrograde flow, which is generated by actin polymerization at the
226 front combined with myosin II-based pulling at the back of the leading edge. This causes rearward
227 transport of microtubules[68,69] as well as microtubule buckling and breaking[70,71]. It has been proposed
228 that depolymerization of broken microtubules from their minus ends could supply tubulin dimers to
229 feed microtubule growth at the leading edge**.** Thus, the density of microtubules that make it into
230 areas of actin protrusion appears to depend on a balance between stabilization of growing
231 microtubules at the cortex and/or plasma membrane and the opposing effects of steric hindrance
232 and retrograde flow of the actin meshwork **(Fig. 2c)**.

234 In a reciprocal mechanism, microtubules regulate the protrusive activity of the actin network at the
235 leading edge by regulating actin nucleation and polymerization via multiple factors. Several formins,
236 which as discussed above promote actin nucleation as well as microtubule stability, interact with
237 microtubules directly[17,18], and/or indirectly, through binding to EB proteins and APC[13,14] **(Fig. 1d)**. For
238 example, APC is a +TIP that is transported along microtubules and forms clusters at the plus ends of
239 a subset of microtubules at the leading edge[72] and is known to form a complex with mDia1 [73].
240 Several WASP family members such as WHAMM, which promote actin assembly by activating
241 ARP2/3, also associate with microtubules[74]. Through these interactions, formins and WASPs recruit
242 polymerization-ready (profilin **[G]** -bound) actin to microtubules, likely providing a localized reservoir
243 of actin monomers for efficient nucleation[75,76]. Microtubules also stimulate actin polymerization
244 through RhoGTPase signalling [35,77] **(Fig. 1e)**. Microtubule ends captured by cortical ACF7 for instance
245 activate RAC and thus stabilize nascent membrane protrusions[63]. The relative importance of direct
246 interactions involving tip-bound actin nucleating complexes versus indirect signalling mechanisms
247 via Rho GTPases for actin regulation at the leading edge remains to be established.

248

249 *[H2] Cell migration in 3D.*

250 There are relatively few studies on the role of actin–microtubule crosstalk in 3D cell migration. While
251 pseudopodia extension is dependent on actin, microtubules likely have a supporting mechanical
252 function, whereby they prevent cell retraction by counteracting compressive forces from myosin-
253 driven retrograde actin flow[39,78] **(Fig. 1f)**. Mechanical stability of microtubules may in turn be
254 reinforced by the surrounding actin network, which can counteract buckling and consequent
255 microtubule breakage[25]. This type of mechanical cooperation is probably more important for cells
256 migrating in soft 3D extracellular matrices, where substantial substrate support is lacking, than on
257 2D substrates, where substrate adhesion provides mechanical support. In addition to this
258 mechanical role, microtubules likely exert similar regulatory functions in 3D as in 2D migration,
259 through regulating polarized trafficking and RhoGTPase signalling[26], although these functions remain
260 to be explored in the context of 3D migration[79].

261 There are only a few studies of the role of actin–microtubule crosstalk during amoeboid motion in
262 3D matrices. What is known is that in leukocytes that naturally tend to migrate in an amoeboid
263 fashion, microtubules are needed for directionality, but not for motility per se[80,81]. In addition, during
264 bleb-based motility, microtubules appear to negatively regulate bleb formation in a mechanism
265 involving inositol lipid metabolism[82].

266

[H1] Neuronal cells

268 Neurons have a polarized architecture specialized for signal transduction: a single long axon, which
269 protrudes from the cell body (soma) carries nerve signals away from the cell body and towards the
270 synaptic junction with another cell, whereas multiple shorter dendrites on the cell body receive the
271 signals **(Fig. 3a)**. These protrusions (collectively referred to as neurites) are mechanically supported
272 by bundles of non-centrosomal microtubules[83]. Axons contain bundles of microtubules crosslinked
273 by Tau, which have their plus ends oriented towards the axon tip. Dendrites instead contain a MAP2-
274 bound microtubule array of mixed polarity[83]. Axons are additionally supported by a periodic array of
275 cortical actin rings[84]. Developing and regenerating axons exhibit a growth cone at their tip that
276 drives neuronal path finding during neurite outgrowth. The actin organization in the growth cone
277 resembles that of a lamellipodium, consisting of an actin-rich leading edge studded with filopodia
278 **[G]**. Dendrites bear small protrusions along their shaft known as dendritic spines, which receive
279 input from axons[85]. Actin forms a ring at the base of these spines and dynamic patches within the
280 spine. Most of what we know about actin–microtubule crosstalk in neuronal morphogenesis comes
281 from studies in neurons cultured on rigid 2D substrates. But neurons are mechanosensitive and
282 often navigate through soft tissues (like the brain)[86]. With the advent of advanced 3D imaging
283 techniques, it now becomes possible to investigate cytoskeletal crosstalk in neurons within live
284 organisms[87].

285

286 *[H2] Axon specification*

287 Acquisition of neuronal cell shape is a well-studied cellular context for actin–microtubule
288 crosstalk[88,89], as it involves a series of events in which the actin and microtubule cytoskeleton
289 undergo coupled remodelling. The first key event is the formation of neurites from an initially
290 spherical cell. Neurite outgrowth is often thought to be initiated by polymerization of actin, although
291 there is recent evidence that kinesin-mediated microtubule sliding may contribute as well[28,90]. The
292 subsequent transformation of filopodial membrane protrusions into neurites with a characteristic
293 microtubule- rich shaft and an actin growth cone occurs through actin–microtubule crosstalk
294 mechanisms that resemble those found in motile non-neuronal cells (see previous section). Some of
295 the same molecules are involved in crosstalk (e.g. +TIPS EB1, EB3, ACF7, APC, CLASPs[91]), but there
296 are also neuron-specific molecules such as Tau and MAP2, which apart from bundling microtubules
297 also cross-bridge them to actin[92,82], and drebrin, which cross-bridges actin to EB3 at microtubules
298 plus ends[91].

It is thought that dynamic microtubules are first guided **(Fig. 1a)** into filopodia along filopodial actin bundles decorated with drebrin, which provides a link to the plus ends of the invading microtubules by binding to EB3[91,93] **(Fig. 3b, left)**. Crosslinking by ACF7 and Tau may contribute to invasion as well[92]. The invading microtubules are then thought to stabilize the nascent neurites by mediating transport of vesicles and organelles into the developing protrusion **(Fig. 3b, right)**. Moreover, they promote actin polymerization through RAC signalling **(Fig. 1e)** and possibly also through recruitment of actin nucleators to the microtubule plus tips **(Fig. 1d)**. Proteins of the Navigator family **[G]** have for instance been reported to track microtubule plus ends and interact with regulators of ARP2/3-mediated actin filament nucleation[94-96]. In turn, actin promotes bundling of the microtubules to form a nascent neurite shaft (engorgement and consolidation). Microtubule bundling and penetration into the nascent growth cone are further facilitated by the activity of ADF/Cofilin **[G]**, which severs actin filaments and thus facilitates microtubules entry[97].

It is a long-standing question what destines only one neurite to become an axon[98]. Two early events that precede axon specification are local microtubule stabilization[99] and the appearance of cytoplasmic flow **[G]** [100] in one neurite. It was recently shown that axon specification relies on positive feedback mechanisms between these two events[101]. Actin waves from the cell body to the tip of the neurites generate flows that transiently widen neurite shafts. This stochastically creates space needed for more microtubules to polymerize and create tracks for kinesin-based transport of axon-promoting proteins. The resulting dynamic multi-polar state is eventually stabilized in individual neurites in response to external cues.

[H2] Axon outgrowth and path finding.
To form the nervous system, developing axons need to seek out synaptic partners by directed outgrowth mediated by the growth cone. Axon outgrowth exhibits three characteristic phases known as protrusion, engorgement and consolidation, which mechanistically resemble the steps leading to neurite initiation described above. A tight coupling between microtubules and actin is essential for steering the direction of axon outgrowth[102].

The protrusion stage involves the advancement of the leading edge of the growth cone and axon elongation. The leading edge is advanced by actin polymerization, which is stimulated by dynamic microtubules that penetrate into the growth cone from the axonal shaft **(Fig. 3c)**. Tangential actomyosin contractile bundles at the rear of the lamellipodium[103] and backwards transport by retrograde flow of lamellopodial actin[102] block most microtubules, but a small population of 'pioneer' dynamic microtubules is nevertheless able to invade **(see also Fig. 2c)**. Crosslinking of microtubules to actin can hamper microtubule entry by enhancing retrograde microtubule motion together with actin, but microtubules can resist retrograde flow once they are crosslinked to filopodial actin bundles by ACF7–EB1[104,105], drebrin–EB3[91], and probably Tau[92] **(see also Fig. 3b)**. The microtubules that make it to the actin cortex are thought to promote growth cone extension by somehow locally promoting actin polymerization **(Fig. 1d)**. Microtubule growth is unlikely to have an important direct role in axon elongation, given that microtubule polymerization is ~10-fold slower than axon elongation. However, microtubules probably do contribute by providing mechanical resistance against retraction driven by myosin II-based tension in the actin cortex along the axon shaft **(Fig. 1f)**, similar to their recently discovered function in 3D cell migration[26]. Moreover, there is evidence that the main minus-end directed microtubule motor, dynein, when anchored to the membrane-bound actin cortex, promotes forward sliding of microtubules into the growth cone[106-108] which may mechanically promote axonal extension[109,110].

During the engorgement stage, filopodia move to the lateral edges of the growth cone and microtubules invade further into the growth cone where they deliver vesicles and organelles. Finally, the consolidation phase involves the formation of a new segment of axon shaft in the wake of growth cone advance. Myosin II contractility drives inward motion of contractile actin arcs from the sides to the centre of the growth cone neck, which pushes the microtubules closer together and

349 facilitates subsequent crosslinking into a stable microtubule bundle by MAPs such as doublecortin,
350 which also interacts with actin[111,112] (**Fig. 3d**).
351
352 *[H2] Maintenance of mature neurons.*
353 There is a strong need for axons to resist mechanical deformations, which is a challenge given their
354 length (hundreds of micrometres up to even 1 meter, depending on the type of neural cell and on
355 species). It has recently become apparent how the actin and microtubule cytoskeleton may work in
356 synergy to provide the required mechanical protection. Cortical F-actin in the form of a periodic
357 array of membrane-bound rings all along the shaft of the axon stabilizes microtubules within the
358 axon by promoting microtubule polymerization, via molecular mechanisms that are not yet
359 understood[113], and also by crosslinking the microtubules via spectraplakins[104,105]. Through these
360 mechanisms microtubules become much more resilient to catastrophes and can provide efficient
361 mechanical support throughout the lifetime of the axon.
362   The shape and mechanical resilience of the axon as a whole is governed by an intricate force
363 balance between the bending resistance of the microtubule bundle and longitudinal tension in the
364 actin–spectrin cortex[114]. A recent study additionally demonstrated the presence of circumferential
365 tension driven by actin and myosin, generating a compressive force on the microtubule bundle in
366 the axon interior[115] (**Fig. 3d**). This interplay between actin-based compression and the mechanical
367 resistance by the microtubule bundle determines the diameter of the axons, which is important for
368 regulating the velocity of impulse conduction along axons.
369   Dendritic spines are dynamic structures and these dynamics are closely linked to the function of
370 dendritic spines in memory storage and synaptic transmission. In mature dendrites, actin–
371 microtubule crosstalk is essential for these dynamics. Dynamic microtubules occasionally venture
372 from the dendrite shaft into the dendritic spines, where they regulate actin dynamics and spine
373 shape[116]. Microtubule entry into the spines, which requires substantial bending, is mediated via F-
374 actin-mediated guidance **(Fig 1a)** involving drebrin–EB3[117].
375

[H1] Cell polarity

377 Differentiated epithelial cells arrange into multi- or mono-layered sheets whose formation and
378 integrity relies on cell–cell adhesion and requires the maintenance of apico-basal polarity[118]. Within
379 the epithelium cells have a narrow and elongated, columnar shape with the apical membrane facing
380 either the outside of the body or the lumen of internal cavities, whereas the basal membrane is
381 often anchored to a specialized ECM known as the basal lamina. Loss of apico-basal polarity is a
382 hallmark of tumorigenic transformation. Microtubules in columnar epithelial cells are mainly non-
383 centrosomal[119], and organized in parallel arrays along the apico-basal axis with minus ends facing the
384 apical side and plus ends facing the basal side (**Fig. 4**). They also form networks of mixed polarity
385 underneath the apical and basal membrane[120]. The apico-basal microtubule array contributes to
386 polarity maintenance by providing tracks for directional transport and probably also by regulating
387 polarity of the actin cortex through physical and RhoGTPase-mediated signalling interactions. Actin
388 forms a membrane-bound cortex all along the cell with functional and molecular differences
389 between the basal, apical, and lateral sides. Additionally, actin forms a contractile belt known as the
390 zonula adherens encircling the cell just below its apical face (**Fig. 4**). Here, adjacent cells are
391 connected via their lateral membranes through E-cadherin junctions, which are linked via
392 cytoplasmic binding partners known as catenins to F-actin. Some epithelial cells, such as in the small
393 intestines, feature an apical brush border consisting of actin-based microvilli, which mediate nutrient
394 absorption and are anchored in a dense actin-based network called the terminal web.
395
396 *[H2] Actin–microtubule crosstalk in apico–basal polarity.*
397 In polarized epithelial cells actin–microtubule coupling is important both to generate[121] and maintain
398 the typical columnar shape of these cells. Geometrical effects of the columnar shape of an epithelial
399 cell on the intrinsic dynamic instability of microtubules may in principle be sufficient to generate an

400   aligned microtubule array along the apico-basal axis[122]. However, apico-basal polarity is in addition
401   maintained (and perhaps generated) by regulated interactions of the plus and minus ends of
402   microtubules with the actin cortex (**Fig. 1b**) at the basal and apical surface, respectively.
403   At the apical surface of epithelial cells, the non-centrosomal microtubule array faces the
404   actin cortex with its minus ends. A series of recent studies showed that the minus ends are tethered
405   to the cortex via minus-end targeting proteins **[G]** (-TIPs) of the calmodulin-regulated spectrin-
406   associated protein (CAMSAP) family[123-125]. CAMSAP proteins protect non-centrosomal microtubules
407   from depolymerisation. Mammals have three homologues: CAMPSAP1 dynamically tracks growing
408   minus ends, whereas CAMSAP2 and CAMSAP3 stably bind minus ends due to an additional
409   interaction with the microtubule lattice[126,127]. CAMSAP3 is the relevant –TIP in epithelial cells, where
410   it is recruited to the apical membrane by the spectrin repeats of ACF7 (**Fig. 4a**). ACF7 itself is
411   anchored to the actin cortex through its actin-binding domain[123,124] and also to cortical spectrins[128]. It
412   is unclear whether the microtubule lattice-binding functionality of ACF7 also contributes to
413   microtubule tethering. The apical CAMSAP3–ACF7 foci act as non-centrosomal microtubule
414   organizing centres by capturing and stabilizing microtubule minus ends, thus polarizing the apico-
415   basal microtubule array. Microtubule polarization is in turn required for polarized transport, for
416   example to deliver molecular determinants for the formation of actin microvilli at the apical
417   surface[128]. In isolated epithelial cells, the same CAMSAP3–ACF7 crosslinking module was recently
418   shown to enhance the directionality of cell migration[51]. Here, non-centrosomal microtubules are
419   anchored to tangential actin arcs (which are parallel to the leading edge) at the back of the
420   lamellipodium by their minus ends, facilitating alignment of microtubules perpendicular to the actin
421   arcs by retrograde actin flow. This process maintains microtubule plus ends in the correct
422   orientation for reaching focal adhesions and thus enhances their targeting to focal adhesions.
423   At the basal surface of epithelial cells, the microtubule array faces the actin cortex with its
424   plus ends. Here epithelial cells are anchored to the laminin-rich basal lamina through laminin
425   receptor integrins ($\alpha3\beta1$ and $\alpha6\beta4$). This contact provides a crucial extrinsic cue for apicobasal
426   polarity **(Fig. 4b)**. There is evidence that microtubule plus ends are captured near the integrin
427   adhesions through similar molecular mechanisms as described for microtubule anchoring at focal
428   adhesions in migrating cells. The full set of molecular players in epithelial cells is not yet known, but
429   it seems to involve some of the same molecules. A recent study, for instance, demonstrated that
430   membrane-bound LL5α and LL5β in the vicinity of active integrins anchor microtubule plus ends
431   through EB1–CLASP[129]. It remains to be determined whether microtubules regulate the cell–ECM
432   adhesions in similar ways as in migrating cells (through ECM remodelling). It also remains to be
433   determined whether, and how, other microtubule +TIPS present at the basal cortex, such as ACF7
434   and APC, influence cortical microtubule capture[129].
435
436   *[H2] Crosstalk at lateral cell-cell contacts.*
437   Adjacent epithelial cells are connected by apically-localized cadherin-based adherens junctions. It is
438   well-established that during interphase, microtubules target adherens junctions by both their plus
439   and minus ends[130,131], which means that at least some microtubules deviate from pure apico-basal
440   alignment. Microtubule minus ends are captured by the –TIP CAMSAP3, which interacts with p120-
441   catenin through PLEKHA7[132] **(Fig. 4c)**, whereas the plus ends are captured by +TIPS that interact with
442   adherens junctions through β-catenin and p120-catenin **(Fig. 4d)**. Demonstrated +TIPS involved in
443   microtubule capture are cortical dynein[133,134], p150^{Glued} (the largest component of the dynactin
444   complex)[135], CAP350[136], and CLIP-170 in concert with CLASP2[134,137,138]. The actin cortex may help
445   stabilize the localization of the CLASPs near adherens junctions since CLASPs can bind actin[138,139].
446   Microtubules are further crosslinked with actin near junctions through ACF7[140].
447   Cortical tethering through CLASPs and perhaps other +TIPs as well selectively stabilizes
448   microtubule plus ends at the cell cortex. Reciprocally, microtubules promote junction formation and
449   stability by facilitating targeted delivery of junction components[133,141] and by promoting local
450   recruitment and activation of myosin II, which in turn drives clustering of E-cadherin[137,142]. In

451 addition, the kinesin KIF17 localized at microtubule plus ends activates RHOA by a mechanism that is
452 not fully understood, and promotes accumulation of junctional actin, which also contributes to
453 adhesion stability[143] **(Fig. 4d)**. Yet, microtubules appear to also engage in negative feedback with
454 adherens junctions, whereby junctions impair microtubule polymerization while microtubules
455 destabilize junctions mediated by either N-cadherin[144] or vascular endothelial (VE)-cadherin. The
456 exact mechanisms underlying this crosstalk remain to be established, but it has been suggested that
457 increased microtubule polymerization might contribute to breaking the junctions and migratory
458 phenotype of endothelial cells by influencing actin polymerization[145]; by contrast, the actin mesh at
459 adherens junctions could interfere with robust microtubule polymerization, thereby limiting
460 microtubule penetration into the junctional area and safeguarding junctional stability.
461

[H1] Cell division

463 In dividing cells, the interphase microtubule and actin cytoskeletons undergo profound remodelling
464 to form the machineries that drive chromosome segregation and cell cleavage. The microtubules are
465 reorganized into a bipolar mitotic spindle by two centrosomes that move to opposite poles of the
466 cell. The mitotic spindle is formed of three distinct microtubule populations (**Fig. 5a**): kinetochore
467 microtubules attach to the chromosomes; antiparallel central spindle microtubules interdigitate at
468 the spindle midzone and push the spindle poles apart; and astral microtubules extend to the cell
469 membrane. In concert with chromosome segregation, the contractile actomyosin cortex first drives
470 mitotic cell rounding by building up a uniformly high tension, and then, after symmetry break, forms
471 distinct cortical regions at the cell poles and an actomyosin contractile ring near the spindle midzone
472 (**Fig. 5a**). Positioning of the spindle is mediated by pushing and pulling forces generated by plus ends
473 of the astral microtubules in contact with the cortex. Pushing forces are generated when
474 microtubules grow against the membrane[146], whereas pulling forces are generated when
475 microtubule ends are captured by membrane-bound dynein motors[147] and by viscous drag **[G]** on
476 organelles that are transported along astral microtubules[148]. Spindle orientation is in part controlled
477 through polarity factors that bias the recruitment of dynein to the cell poles[149]. In addition, the
478 balance of pushing and pulling forces makes the spindle position sensitive to cell size and
479 shape[147,150]. In elongated cells, the spindle tends to orient along the long axis according to Hertwig's
480 rule **[G]**[151]. But surprisingly, even cultured cells that round up for mitosis align their spindle according
481 to the shape they had in interphase[152], indicating the presence of intrinsic cues that guarantee shape
482 memory. These cues have been associated with the formation of actin-rich fibres, known as
483 retraction fibres **[G]** that anchor rounded cells to their substrates.
484

485 *[H2] Actin–microtubule crosstalk coordinates cell division.*
486 In cell division, microtubule-based spindle assembly and actin-based cytokinesis are often studied as
487 separate processes, but there is a growing recognition that close crosstalk between the actin and
488 microtubule cytoskeleton is required for the coordination of chromosome segregation with
489 cytokinesis and hence for error-free cell division[153,154]. The actin cortex influences the assembly of
490 the spindle and its position and orientation prior to cell division, while, conversely, the spindle
491 positions the actomyosin contractile ring prior to cytokinesis (see the following subsections). Thus,
492 actin–microtubule crosstalk controls the axis of the division plane, which sets the size and
493 developmental fate of the daughter cells. For example, in epithelia spindle orientation regulates
494 horizontal versus vertical positioning of the cleavage furrow with respect to the apical surface, which
495 can determine the fate of the daughter cells[155]. A vertical cleavage plane (whereby the spindle is
496 horizontal to the apical surface) allows planar cell division **[G]** that promotes the maintenance of
497 adherens junctions between the daughters, whereas orthogonal division (where the spindle is
498 perpendicular to the apical surface and the cleavage plane is horizontal) results in the loss of
499 adherens junctions between daughter cells and consequently can lead to cell delamination from the
500 tissue. Planar versus non-planar divisions can also influence the decisions between cell proliferation
501 and differentiation.

502   In this section we will focus on somatic cells, where spindle positioning is mediated by astral
503 microtubules and polarization of cortical microtubule anchors. Of note, oocytes use a completely
504 different mechanism for spindle positioning dependent on a cytoplasmic actin network, which exerts
505 forces on the spindle via myosin V motors[156].
506
507 *[H2] Actin-mediated control of spindle position and orientation.*
508 The actin cortex indirectly influences the spindle through its active control of cell geometry. Both
509 single cells and cells in epithelia stiffen and round up before mitosis as a consequence of myosin II-
510 driven contraction of the actin cortex. It was shown that mitotic rounding is essential to provide a
511 three-dimensional space in which microtubules can efficiently search and capture the chromosomes
512 and assemble the spindle[9,10]. Mitotic rounding furthermore promotes planar cell divisions in
513 epithelial tissues because it prevents geometry-induced alignment of the spindle along the long
514 (apico-basal) axis of the cells, which are columnar in interphase[11]. Within the plane of the
515 epithelium, spindle orientation is further guided by anisotropies in cortical contractility, which lead
516 to cell elongation and subsequent alignment of the spindle along the long axis[157]. A critical factor for
517 mitotic rounding is the stable anchoring of the actin cortex to the cell membrane, which is provided
518 by proteins of the ERM (ezrin/radixin/moesin) family[9,10]. ERM proteins have additional, less well-
519 understood roles in spindle positioning, including regulation of nuclear mitotic apparatus protein
520 (NuMA) localization, which is involved in dynein recruitment[158], and direct cortical microtubule
521 anchoring[159].
522   The actin cortex has also more direct effects on spindle orientation by providing cortical
523 anchors (**Fig. 1b**) for plus ends of astral microtubule [160]. In single adherent cells, astral microtubules
524 directly interact with the actin cortex in regions known as 'subcortical actin clouds', which are
525 associated with retraction fibres. Astral microtubules interact with the actin cortex through mitotic
526 interactor and substrate of PLK1 (MISP), which binds EB1 and p150(glued)[161,162] and through myosin-
527 10[163]. Myosin-10 motors actively pull on astral spindle microtubules, in parallel with cortical
528 dynein[163,164], which is also specifically recruited to cortical sites near retraction fibres (**Fig. 5b**)[165].
529   In epithelia, mitotic cells also round up but they maintain cell-cell junctions and their cell
530 division axis is influenced by their interphase shape. The interphase distribution of cell–cell junctions
531 plays a key role in this shape memory[166]. Planar cell divisions involve coupling of astral microtubule
532 plus ends to E-cadherin junctions that are associated with the actin cortex (see above). Two
533 junctional components, E-cadherin itself and afadin, are both able to directly bind Leu-Gly-Asn
534 repeat-enriched protein (LGN; also known as GPSM2), another protein involved in dynein
535 recruitment[167,168]. These interactions enrich LGN specifically at E-cadherin adhesions already in
536 interphase[167]. Upon nuclear envelope breakdown, NuMa is released into the cytoplasm and forms a
537 complex with both LGN and the membrane-associated G protein alpha i subunit $G\alpha_i$ at cell–cell
538 junctions, thereby promoting robust dynein recruitment and capture of astral microtubules[168] (**Fig.
539 5c**). It was shown that NuMa localizes to tricellular junctions (where three cells contact), which align
540 with mechanical stresses on the tissue.
541   An important challenge is to understand how membrane-bound microtubule plus end
542 anchors and the actin cortex interplay in spindle positioning. On the one hand, the actin cortex can
543 support the activity of membrane-bound anchors such as dynein motors, which contribute to spindle
544 positioning by capturing the ends of dynamic microtubules and mediating the transmission of pulling
545 forces to spindle poles. The actin cortex contributes to the efficiency of this process by helping to
546 recruit dyneins, by rigidifying the membrane[169], and by preventing side-ways microtubule sliding. On
547 the other hand, a dense actin cortex may also act as a physical barrier (**Fig. 1c**) and impede astral
548 microtubules from reaching membrane-bound anchors. The interplay of actin and microtubules in
549 spindle positioning is likely context-dependent, being sensitive to parameters such as cell size and
550 matrix rigidity for single cells[154] and the presence of adhesive cell–cell contacts and mechanical
551 stresses in epithelial tissue[155].

552 Note also that using early gastrula embryos of *Xenopus laevis* an additional mechanism for
553 planar positioning of the spindle in epithelia that does not depend on cell–cell junctions was
554 proposed, at least for early embryonic epithelia. This mechanism involves two opposing forces: a
555 basally directed force, dependent on coupling of astral microtubule plus ends to myosin-10 and an
556 apically directed force exerted by apico-basal actomyosin flows. These forces not only position the
557 spindle in a planar orientation with respect to the tissue but also, by counteracting each other, act to
558 determine the position of the spindle along the vertical, apico-basal axis of the cell[170].

560 *[H2] Microtubule-mediated control of cytokinesis.*
561 During cytokinesis, the contractile ring always forms in a position that precisely bisects the spindle.
562 Two subsets of microtubules, the central spindle and astral microtubules, control cytokinetic ring
563 assembly at the equator[171]. The central spindle microtubules promote ring assembly by localized
564 delivery of the Rho activator ECT2 by kinesin-6 motors associated with microtubule plus ends[172-174].
565 RHOA is the master regulator of the actomyosin contractile ring. It promotes unbranched actin
566 polymerization through activation of mDia formins and simultaneously activates non-muscle myosin-
567 2 filament formation and motor activity (**Fig. 5d**)[175]. Interestingly, this type of microtubule-mediated
568 signalling to actin was recently reconstituted in a cell-free system using egg extracts[176]. Active gel
569 theories for the actomyosin cortex predict that biasing myosin activity within an equatorial band
570 should be sufficient to drive the formation and ingression of a contractile ring[177]. But there are also
571 specific molecular feedback mechanisms that reinforce the division plane. RHOA also activates
572 anillin, which scaffolds the contractile ring by interacting with F-actin, myosin II, septins **[G]**, and the
573 plasma membrane. Anillin provides positive feedback on spindle midzone specification by recruiting
574 more RHOA. Anillin also binds microtubules, which may potentially crosslink the contractile ring with
575 the mitotic spindle[178,179](**Fig. 5d**).
576 Astral microtubules have a complementary role to central spindle microtubules because
577 they inhibit the accumulation of myosin at the cell poles. It was proposed that this inhibition is
578 achieved by removal of anillin from the polar cortex by astral microtubules[180] (**Fig. 5d**). Anillin
579 binding to microtubules competes with RHOA binding[179]. Thus, at the cell poles, where RHOA levels
580 are low, astral microtubules would efficiently bind anillin, clearing it off from the cortex and in
581 consequence inhibiting anillin-driven myosin accumulation and reducing cortical tension at the
582 poles. This mechanism, together with positive cues from central spindle microtubules restricts anillin
583 to the RHOA-rich equatorial cortex. Polar astral microtubules in addition promote the formation of
584 short, branched F-actin at the cell poles via RAC signalling[173], which contributes to maintaining a
585 lower cortical tension at the poles than at the equator.

587 # [H1] Conclusions and perspective
588 Here we gave an overview of basic (physical) mechanisms through which the actin and microtubule
589 cytoskeleton may regulate each other's dynamics and organization. We furthermore gave a detailed
590 discussion of how these different types of mechanisms appear to conspire in a number of different
591 contexts to drive cellular function, including cell migration, neuronal shape and function, cell polarity
592 and cell division. However, actin–microtubule crosstalk has also been proposed in other contexts
593 such as growth and polarity of fission yeast cells[12], in plant cells[181], and in immune responses[182], and
594 likely there are many other examples to be explored.
595 To dissect in more detail the physical principles of actin–microtubule crosstalk, it will be
596 interesting to design coupled experiments in cells and in reconstituted systems. Reconstitution
597 experiments are ideally suited for deducing the minimal requirements for specific types of
598 interactions[5,15,176] and for disentangling the contributions of physical interactions and biochemical
599 signalling, while cell experiments can test the physiological relevance of the identified mechanisms.
600 In the future, it will in addition be interesting to use reconstitution experiments to address how
601 physical confinement affects the self-organization of coupled cytoskeletal systems. We previously
602 showed that confinement strongly affects individual cytoskeletal systems, for example by promoting

603 actin filament alignment and guiding positioning of microtubule asters[147,183-185]. In living cells, recent
604 advances in 3D imaging techniques that can be applied to 3D cell culture systems, organoids and
605 living organisms, coupled to advances in functional and super-resolution imaging using fluorescent
606 biosensors, optogenetics, and automated image analysis will probably rapidly bring more detailed
607 insights in the role of actin–microtubule crosstalk in different cellular contexts[186-188].
608

609 ## Acknowledgements

614
615

Figure Captions

Figure 1. Mechanisms of actin–microtubule crosstalk

a) *Guidance of microtubule growth*. Actin–microtubule crosslinking proteins that associate with growing microtubule ends via microtubule plus-end trackers (+TIPs) provide dynamic links between microtubules and actin bundles, which can redirect microtubule growth along actin bundles. **b)** *Anchoring and stabilization of microtubule ends*. Protein complexes associated with cortical actin networks can capture both the plus and minus ends of microtubules, leading to stable connections between the two cytoskeletal systems. In the example shown, a plus end is stabilized by a complex involving a motor protein, but the composition of the complex may vary depending on the context. **c)** *Actin as a physical barrier for microtubule growth*. The actin cortex may act as a physical barrier that prevents growing microtubules from penetrating to the plasma membrane by blocking growth and inducing catastrophes. **d)** *Nucleation of actin filaments at microtubule ends*. Actin nucleation factors such as formins may associate with growing microtubule ends, which leads to microtubule-mediated local stimulation of actin polymerization. **e)** *Shared regulators of actin and microtubule dynamics.* Members of the Rho family of small GTPases regulate both actin and microtubule dynamics via their interaction with both actin and microtubule associated proteins. In addition, microtubules may contribute to the local regulation of actin dynamics via their influence on Rho-GTPase activity. **f)** *Mechanical cooperation in membrane protrusions*. Stiff microtubules may provide mechanical support against membrane retraction in events of membrane protrusion driven by actin polymerization. This leads to cooperative behaviour of the actin and microtubule cytoskeletons in cell motility. GAP, guanine activating protein; GEF, guanine nucleotide exchange factor; MAP, microtubule associated protein.

Figure 2. Actin–microtubule crosstalk in cell migration

a) *Overview of the microtubule and actin cytoskeletons in migrating cells.* Cells migrating on 2D surfaces have a leading edge containing a protrusive, branched F-actin network and a trailing edge containing a contractile actin-myosin network. Microtubules are anchored at the centrosome by their minus ends and extend their dynamic plus ends towards the cell cortex. The cells are anchored to the substrate via integrin-based focal adhesions that are connected to actin stress fibers. **b)** *Actin–microtubule crosstalk at focal adhesions*. Guidance of microtubules towards focal adhesions relies on physical crosslinking of growing microtubules to actin stress fibres by spectraplakins and GAS2 proteins. At the actin cortex, microtubule plus ends get captured by cortical patches referred to as cortical microtubule stabilization complexes (CMSCs). Multiple factors associate with these complexes (see text). **c)** *Actin–microtubule crosstalk in the leading edge*. The probability that microtubules enter areas of actin protrusion depends on a balance between cortical stabilization of microtubule ends and the opposing effects of steric hindrance and retrograde flow of the actin meshwork. EB, end-binding protein; GAS2: growth-arrest-specific protein 2; KANK1, KN motif and ankyrin repeat domain-containing protein 1.

Figure 3. Actin-microtubule crosstalk in neuronal cells

a) *Overview of the microtubule and actin cytoskeleton in neuronal cells.* Neurons have a cell body with one long axon and multiple shorter dendrites, which are mechanically supported by bundles of non-centrosomal microtubules. Axons additionally contain a periodic array of cortical actin rings. Growing axons also exhibit a growth cone at their tip with an actin-rich leading edge studded with filopodia**.** Dendrites bear small protrusions along their shaft known as dendritic spines. Actin forms a ring at the base of these spines and dynamic patches within them. **b)** *Actin–microtubule crosstalk in neurite outgrowth*. Actin can act as a barrier for microtubule entry, but actin bundles can also guide

growing microtubules into filopodia by crosslinking between drebrin and end binding protein 3 (EB3) **(left)**, actin cross-linking family protein 7 (ACF7), and tau. Microtubules then stabilize the nascent neurites by mediating transport of vesicles and organelles into the developing protrusion, promoting actin polymerization through RAC signalling, and possibly also through recruitment of actin nucleators to the microtubule plus tips**. (right). c)** *Actin–microtubule crosstalk in growth cones*. Tangential actomyosin contractile bundles at the rear can physically block microtubules, but a small population of dynamic microtubules invades the growth cone. The axonal microtubule bundle splays out on entering the growth cone. Microtubule penetration is determined by a balance between forwards polymerization and backwards transport by retrograde flow of lamellopodial actin. Microtubules resist retrograde flow by becoming crosslinked to filopodial actin bundles by ACF7–EB1, debrin–EB3, and probably tau (not shown). Microtubules likely contribute to axon elongation by providing mechanical resistance against membrane retraction. **d)** *Actin–microtubule crosstalk in axonal shafts*. During the formation of a new segment of axon shaft, myosin II contractility drives inward motion of contractile actin arcs from the sides to the centre of the growth cone neck, which pushes the microtubules closer together and facilitates their subsequent crosslinking into a stable microtubule bundle. Similar mechanisms may also operate in mature neurons to regulate the width of the axon.

Figure 4. Actin–microtubule crosstalk in cell polarity
Epithelial cells have a columnar shape with an apical membrane and a basal membrane. Microtubules are organized in parallel arrays along the apico-basal axis with minus ends facing the apical side and plus ends facing the basal side. Actin forms a membrane-bound cortex all along the cell, as well as a contractile belt encircling the cell just below its apical face. Microtubule minus (a) and plus (b) ends are anchored at the basal and apical cortex, respectively. Minus ends are anchored by complexes involving actin cross-linking family protein 7 (ACF7) and the microtubule minus end tracker calmodulin-regulated spectrin-associated protein 3 (CAMSAP3). Plus ends are anchored by membrane-bound LL5α/β/ in the vicinity of active integrins through end-binding proteins and CLAPs (cytoplasmic linker associated proteins). A large set of molecular components is involved in actin-mediated stabilization of both microtubule minus (c) and plus ends (d) near cell–cell adhesions (see text). Microtubules promote junction formation by facilitating targeted delivery of junction components and promoting local recruitment and activation of myosin II, which drives clustering of E-cadherin to the junction. PLEKHA7, pleckstrin homology domain-containing family A member 7.

Figure 5. Actin–microtubule crosstalk in cell division
a) *Overview of the microtubule and actin cytoskeleton in cell division.* The mitotic spindle is organized by two centrosomes and contains three distinct microtubule populations. Kinetochore microtubules attach to the chromosomes, antiparallel central spindle microtubules interdigitate at the spindle midzone, and astral microtubules extend to the cell membrane. The contractile actomyosin cortex drives mitotic cell rounding and then breaks symmetry, forming distinct cortical regions at the cell poles and an actomyosin contractile ring near the spindle midzone. **b)** *Actin–microtubule crosstalk in spindle positioning*. Astral microtubule ends interact with the actin cortex through mitotic interactor and substrate of PLK1 (MISP) (which binds end-binding protein 1 (EB1) and p150(glued)) and myosin-10. Myosin-10 motors actively pull on astral spindle microtubules, in parallel with cortical dynein, which is specifically recruited to cortical sites near actin retraction fibres that connect the rounded cell to the substrate. **c)** *Actin–microtubule crosstalk in dividing epithelial cells*. Spindle positioning in planar cell division involves dynein-mediated coupling of astral microtubule plus ends to E-cadherin junctions. E-cadherin and afadin directly bind Leu-Gly-Asn repeat-enriched protein (LGN). Upon nuclear envelope breakdown, nuclear mitotic apparatus protein (NuMa) is released into the cytoplasm and forms a complex with LGN and G protein alpha i subunit (Gαi) at cell–cell junctions to

recruit dynein. **d)** *Actin–microtubule crosstalk in cytokinesis*. Central spindle microtubules promote cytokinetic ring assembly by delivery of the Rho activator ECT2 by kinesin-6 motors. RHOA promotes activation of mDia formins and simultaneously activates non-muscle myosin II filament formation and motor activity. RHOA also activates anillin, which scaffolds the contractile ring by interacting with F-actin, myosin II, septins and the plasma membrane. Anillin provides positive feedback on midzone specification by recruiting more RHOA. Astral microtubules inhibit the accumulation of myosin at the cell poles. It was proposed that this inhibition is achieved by removal of anillin from the polar cortex by astral microtubules, as anillin also binds microtubules.

722 **Table 1:** Molecular players known to mediate physical coupling between the actin and
723 microtubule cytoskeleton

724

Biophysical mechanism	Coupling protein[a]	Molecular interaction partners	Cellular functions
Actin–microtubule crosslinking	Plectin	• Actin • Microtubules	Cell stiffness and contractile force generation
	Tau	• Actin • Microtubules • EBs	Axon organization
	MAP2c	• Actin • Microtubules	Neurite formation
	Abelson non-receptor tyrosine kinase	• Actin • Microtubules • Cortactin • CLASP2	Lamellipodial protrusions
	Pod1 [Dpod1][b]	• Actin • Microtubules	Axon guidance
	Doublecortin	• Actin • Microtubules	Axon organization
	Septins	• Actin • Microtubules • EBs	Axon collateral branching
Guidance of microtubule growth by actin bundles	ACF7 (also known as MACF) [Shot]	• Actin • Microtubules • EBs • Dynein–dynactin • CMCs • ELMO	• Directional cell migration • Axonal outgrowth • Epithelial closure
	G2L1 [Pigs]	• Actin • Microtubules • EB proteins	Actin-microtubule co-alignment
	IQGAP1 with CLIP-170	• CLIP-170 binds EB and microtubules • IQGAP1 binds actin	Dendrite morphology
	CLASP [Orbit]	• Actin • EBs • IQGAP1 • CLIPs • Focal adhesions • p120-catenin	• Actin–microtubule co-alignment • Microtubule–contractile ring interactions in dividing cells • Regulation of cell–cell junctions and cell–matrix adhesions in cultured cells and epithelia • Axon elongation

	Drebrin	• Actin • EBs	• Neuritogenesis • Axon branching • Growth cone & dendrite formation • Neuronal migration • Epithelial cell shape
	APC	• Microtubules • Actin • EBs • mDia1 • IQGAP1	• Directional cell migration • Growth cone steering
Anchoring of microtubule plus ends by cortical actin networks	Afadin [Canoe]	• Actin • LGN [Pins] • Adherens junctions	Mitotic spindle positioning in single cells and epithelia
	ERM proteins [Moesin]	• Actin • Microtubules • Plasma membrane	Mitotic spindle positioning
	Myosin10	• Actin • Microtubules • Integrins • Plasma membrane	Spindle positioning in single cells and epithelia
	MISP	• EBs • Dynein–dynactin • Actin	Spindle positioning in single cells
	Anillin	• Actin • Microtubules • Myosin-II • Septins • RHOA • RacGAP50C • Plasma membrane	Polarization of mitotic actin cortex by astral microtubules
Anchoring of microtubule minus ends by actin networks	ACF7 [Shot or Shortstop or Kakapo]	Microtubule -TIP protein CAMPSAP (also known as Nezha) [Patronin]	• Apico-basal cell polarity in epithelia • Epithelial cell migration • Tight junction regulation in intestinal epithelia
Actin nucleation and elongation from microtubule plus ends	Complex of EB1, CLIP-170 and formins (demonstrated *in vitro* for mDia1, mDia2, Daam1, INF1 INF2)	• Microtubule plus ends • Actin monomers	Dendritic branching in neurons
	Navigator protein [Sickie]	• Microtubule +TIPs • Actin nucleators	Neuronal outgrowth

725

726 [a]Note that this table is not exhaustive and some entries are not referred to in the text. Only proteins
727 for which direct interactions with the listed interaction partners have been documented are included
728 (for associated references see the fully referenced version of this table in the Supplemental

729   Information (Supplementary Table S1)). [b]Names are provided for the mammalian/human genes, with

730   the names of their *Drosophila melanogaster* orthologs (where applicable) between brackets.

731   ACF7, Actin crosslinking factor 7; APC, Adenomatous polyposis coli; CLASP, cytoplasmic linker

732   associated protein; CLIP-170, cytoplasmic linker protein 170; CLIP, CAP-Gly Domain Containing Linker

733   Protein; CAMSAP, Calmodulin Regulated Spectrin Associated Protein; CMCs, Cortical microtubule

734   stabilization complexes; Daam, Dishevelled-Associated Activator of Morphogenesis; EB, end-binding

735   protein; ELMO, engulfment and motility proteins; ERM, Ezrin-radixin-moesin; G2L1, Growth Arrest

736   Specific 2-Like 1; GAP: GTPase-activating protein; INF, Inverted formin; IQGAP1, 'IQ'motif-containing

737   GTPase-activating protein 1; LGN, Leu-Gly-Asn repeat-enriched protein; MACF, Microtubule-actin

738   crosslinking factor; MAP, Microtubule-Associated-Protein; mDia, mouse Diaphanous related formin;

739   MISP, mitotic interactor and substrate of PLK1; Pod1, polarity osmotic defective-1.

740

741

Glossary

Dynamic instability

A process of dynamic alternation between growing and shrinking states that is characteristic of microtubules and driven by the GTPase activity of tubulin.

Actin cortex

A thin (~100 nm) filamentous meshwork of actin filaments and actin-binding proteins including myosin motors, which is tightly associated with the plasma membrane via proteins of the ezrin-radixin-moesin family. The cortex protects the mechanical integrity of the cell membrane and has a central role in cell shape control.

Microtubule plus-end-trackers (+TIPS)

Structurally diverse proteins that bind to the plus ends of growing microtubules. At least 20 different families of +TIPS exist. End-binding (EB) proteins are +TIPs that autonomously recognize growing microtubule ends. Other +TIPs bind to EB proteins through SxIP, Cap-Gly, or LxxPTPh recognition motifs. +TIPS control microtubule dynamics and connect microtubules to various cellular structures including the actin cortex, stress fibres and filopodial actin bundles.

Catastrophe

The switch to rapid depolymerisation triggered by the loss of the GTP cap at the plus end of the microtubule.

Lamellipodium

A sheet-like membrane protrusion that spans 2-4 µm from the leading edge of migrating and spreading cells and of neuronal growth cones. It contains a dense, branched network of actin filaments that polymerize at their plus ends near the leading edge and depolymerize at the back. The part of the leading edge directly behind the lamellipodium contains a more stable network of unbranched actin filaments and is enriched in myosin II.

Focal adhesions

Adhesive junctions between cells and the extracellular matrix (ECM), which are mediated by transmembrane proteins integrins, whereby Integrins interact with the ECM on the extracellular side and with actin bundles via adaptor and signalling proteins through their intracellular tails. Focal adhesions can contain over 100 different proteins, collectively referred to as the integrin adhesome. Cells modify the size and composition of focal adhesions in response to changes in the molecular composition and dimensionality (2D or 3D) of the matrix and physical forces.

Leading edge

The front of a migrating cell. It is characterized by actin polymerization and the formation of nascent adhesions.

Trailing edge

The rear end of a migrating cell. It is characterized by stable actin bundles and the release and disassembly of adhesions.

780 <u>Stress fibres</u>

781 Bundles of 10-30 actin filaments crosslinked by α-actinin and often containing myosin II. There are 4
782 distinct types of stress fibres. Ventral stress fibres connect focal adhesions close to the cell edge to
783 adhesions behind or near the nucleus. They are contractile and drive tail retraction and cell shape
784 changes in migrating cells. Dorsal stress fibres are noncontractile but transmit contractile forces to
785 the substrate via connections to focal adhesions. Transverse arcs are curved bundles behind the
786 lamellipodium that are not connected to focal adhesions. They have been implicated in actin
787 retrograde flow. The perinuclear actin cap is an ensemble of stress fibres that is anchored to the
788 nucleus and controls its shape.

789 <u>Pseudopodium</u>

790 A type of membrane protrusions that contributes to crawling-like cell migration of amoeba and of
791 mammalian cells in 3D extracellular matrices, and in white blood cells, enables capturing and
792 engulfing antigens. Pseudopodia are extended by the polymerization of a dense network of
793 branched actin filaments at the leading edge and are supported by microtubules.

794 <u>Blebbing</u>

795 A process associate with the formation of blebs, which are round protrusions of the cell membrane
796 caused by contraction of the actomyosin cortex in conjunction with a local rupture in the actin
797 cortex or a transient detachment of the cortex from the cell membrane. Bleb expansion is driven by
798 intracellular pressure generated in the cytoplasm while bleb retraction is driven by reformation of an
799 actin cortex followed by myosin-driven contraction. Blebbing occurs during apoptosis, can drive 3D-
800 motility of confined cells and acts as a pressure valve in dividing cells.

801 <u>Microtubule acetylation</u>

802 A posttranslational modification associated with long-lived microtubules whereby the Lys40 residue
803 of α-tubulin in the microtubule lumen is enzymatically modified by tubulin acetyltransferase.
804 Acetylation confers resilience against repeated mechanical stresses, thus protecting long-lived
805 microtubules from mechanical ageing.

806 <u>Profilin</u>

807 Profilin is a regulatory protein that promotes actin assembly by sequestering monomeric actin,
808 converting ADP-actin monomers into ATP-actin monomers, and collaborating with actin nucleators
809 such as formin to promote actin filament elongation.

810 <u>Filopodia</u>

811 Thin (60-200 nm) membrane protrusions that extend from the leading edge of lamellipodia in
812 migrating cells, neuronal growth cones and in epithelial sheets. They contain parallel bundles of 10-
813 30 actin filaments crosslinked by fascin and fimbrin. Filopodia form focal adhesions with the
814 substrate and sense the extracellular environment at their tips using cell surface receptors. In
815 neurons, filopodia serve as precursors for dendrites.

816 <u>Navigator family</u>

817 The navigator family comprises microtubule-associated proteins that are expressed predominantly
818 in the nervous system.

819 <u>ADF/Cofilin</u>

820 ADF/cofilin is a family of actin-binding proteins which disassembles actin filaments by disassembly at
821 the minus end and by severing.

822 Bulk cytoplasmic flow

823 Cytoplasmic flow refers to the movement of cytoplasm driven either by actomyosin contractility or
824 by microtubule-based organelle movement. It is most common in plants and algae, but it also occurs
825 during oogenesis in the fruit fly and embryogenesis in Caenorhabditis elegans.

826 Microtubule minus-end-trackers (-TIPS)

827 -TIPS are proteins that specifically bind to the minus-end of non-centrosomal microtubules. The
828 best-characterized proteins of the CAMSAP/Patronin/Nezha family protect minus ends from
829 depolymerisation and connect them to various cellular structures including the actin cortex at the
830 apical surface of epithelial cells.

831 Viscous drag

832 The frictional force that opposes the motion of an object in a viscous fluid. The viscous drag force is
833 proportional to the velocity of the object, the fluid velocity, and the object's size, as expressed by
834 Stokes's law.

835 Hertwig's rule

836 A rule introduced by the German zoologist Oscar Hertwig in 1884 based on observations of the
837 orientation of divisions of frog eggs upon controlled compression stating that a cell divides along its
838 long axis.

839 Planar cell divisions

840 Symmetric cell divisions within the plane of an epithelial tissue. Planar alignment of the mitotic
841 spindle is mediated by cortical cues, cell shape and mechanical tension. Coordinated planar cell
842 divisions serve to elongate growing epithelial tissues while maintaining tissue cohesion.

843 Retraction fibres

844 Thin membrane tubes filled with actin filaments that maintain cell adhesion during mitotic rounding.
845 They confer a memory of the cell–ECM adhesion geometry during interphase, allowing cells to orient
846 their mitotic spindle.

847 Septins
848 Septins are a family of guanine nucleotide binding proteins present in the cell as hetero-oligomeric
849 complexes. They form higher-order filamentous structures that can interact with actin,
850 microtubules, and lipid membranes.
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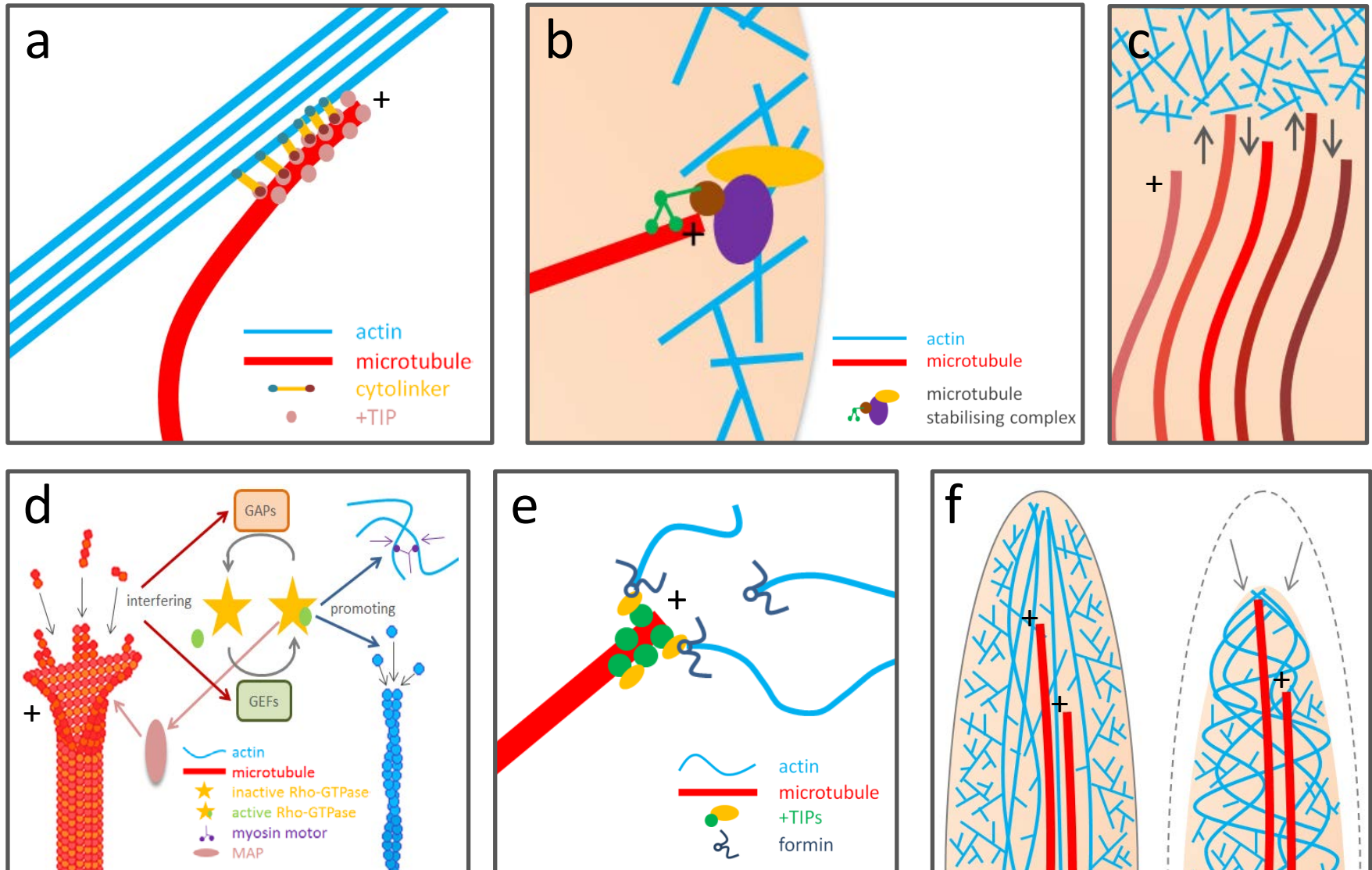
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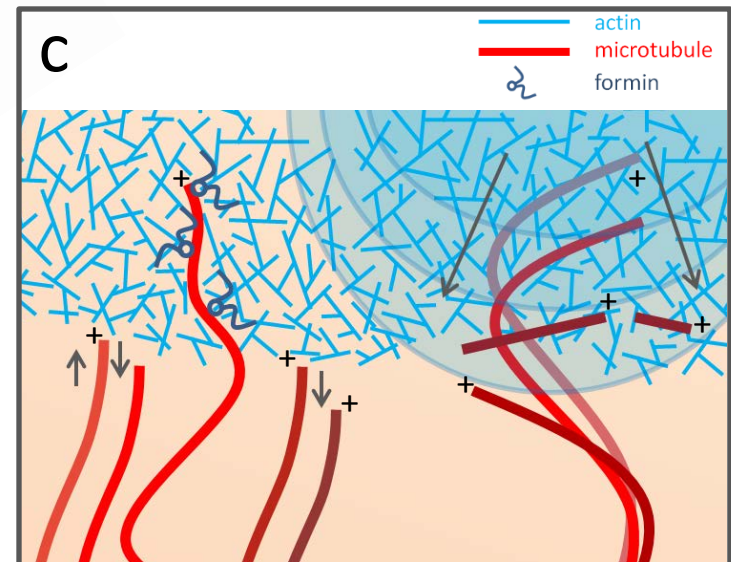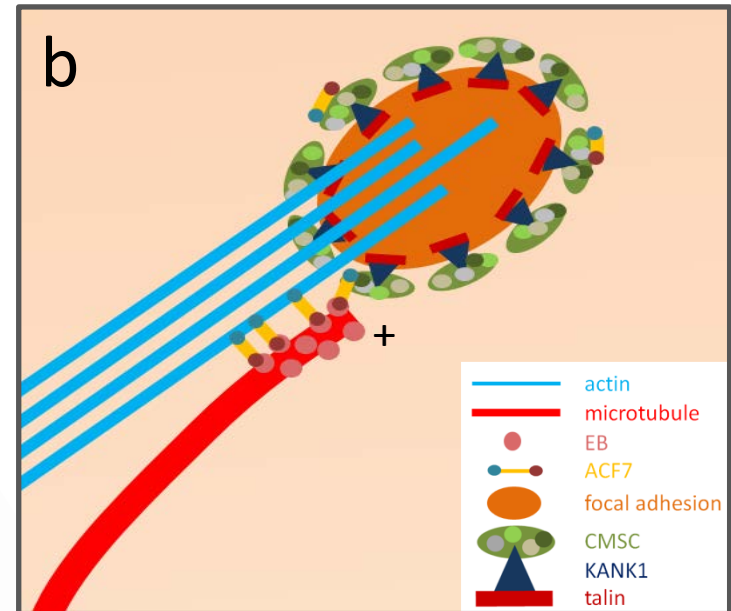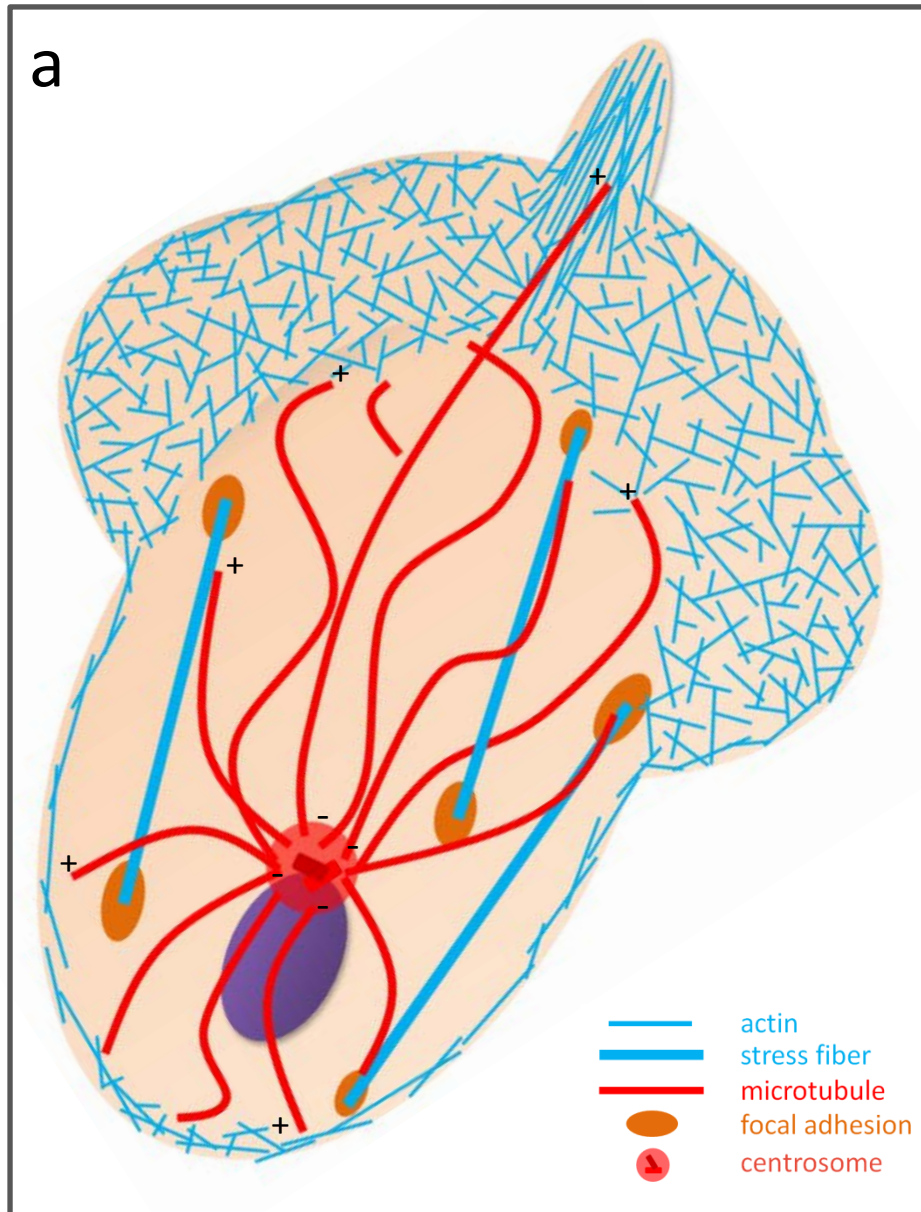
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Figure 1: Actin-MT crosstalk mechanisms

a — actin, microtubule, cytolinker, +TIP

b — actin, microtubule, microtubule stabilising complex

c

d — GAPs, interfering, promoting, GEFs, actin, microtubule, inactive Rho-GTPase, active Rho-GTPase, myosin motor, MAP

e — actin, microtubule, +TIPs, formin

f

Figure 2: cell migration

a

b

Legend (panel b):
- actin
- microtubule
- EB
- ACF7
- focal adhesion
- CMSC
- KANK1
- talin

c

Legend (panel c):
- actin
- microtubule
- formin

Legend (panel a):
- actin
- stress fiber
- microtubule
- focal adhesion
- centrosome

Figure 3: neurons

a — Dendritic shaft
- mixed polarity microtubules
- MAP2

Axonal shaft
- cortical actin ring
- polar microtubules
- tau

Growth cone

Dendritic spine
- actin
- microtubule

b
- actin
- microtubule
- drebrin
- EB3
- ACF7
- tau
- actin
- microtubule
- EB3
- actin nucleator
- Rac
- motor + cargo
- ADF/cofilin

c
- actin
- microtubule
- drebrin
- EB3
- ACF7

d
- cortical actin ring
- polar microtubules
- myosin

Figure 4: polarity

Figure 5: cell division

a

▬▬	central spindle microtubule
▬▬	kinetochore microtubule
▬▬	astral microtubule

b

▬▬	actin	▬	MISP	♪	myosin-10
▬▬	microtubule	●	EB1	♪	cortical dynein
▬▬	retraction fiber	⬮	p150		

c

～	actin
▬▬	microtubule
▭	E-cadherin
●	afadin
◣	LGN
▬	NuMa
♪	Dynein
▲	Gα

d

～	actin
▬▬	microtubule
★	Ect2
★	RhoA
♪	kinesin-6
～	mDia
♪	myosin-2
●●	anillin
⬮	septin
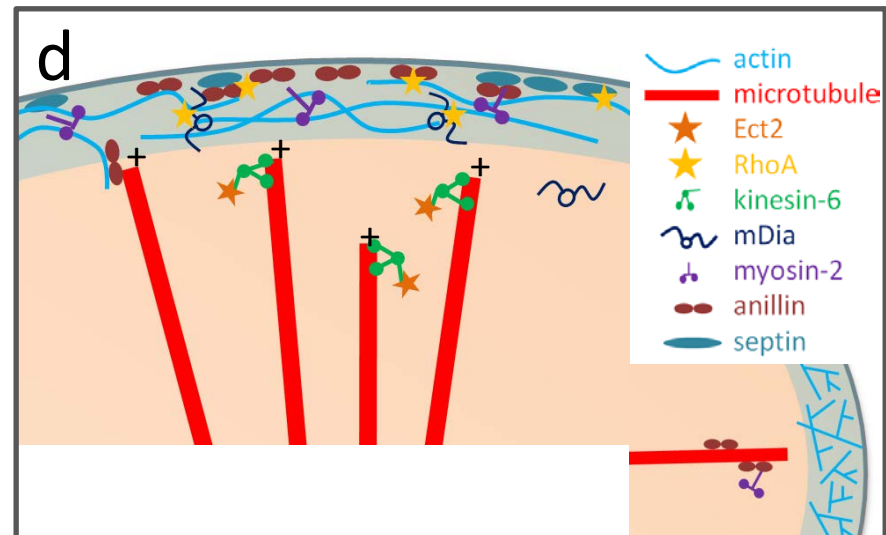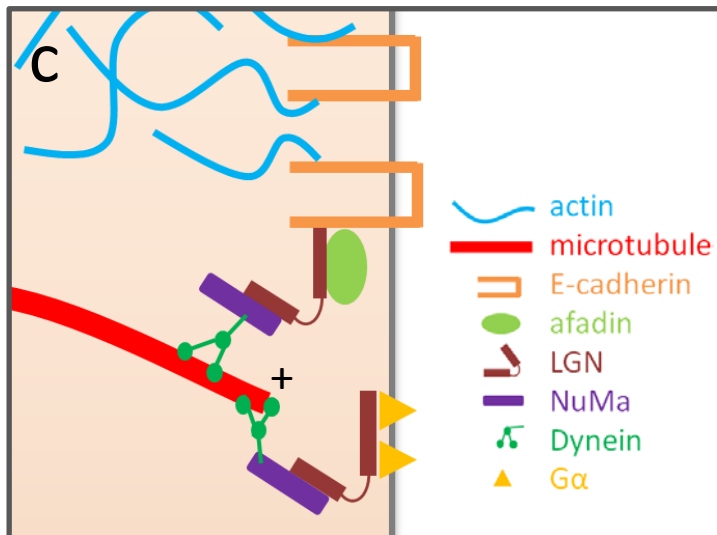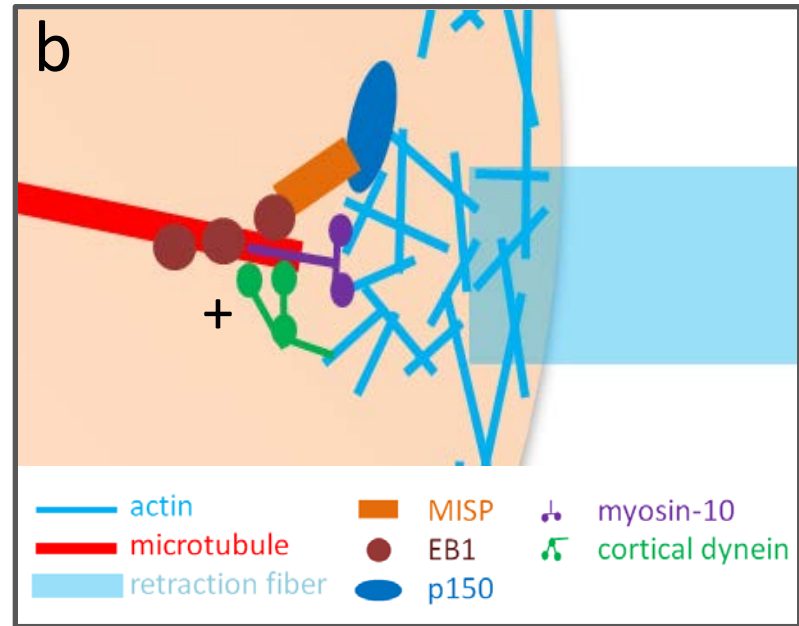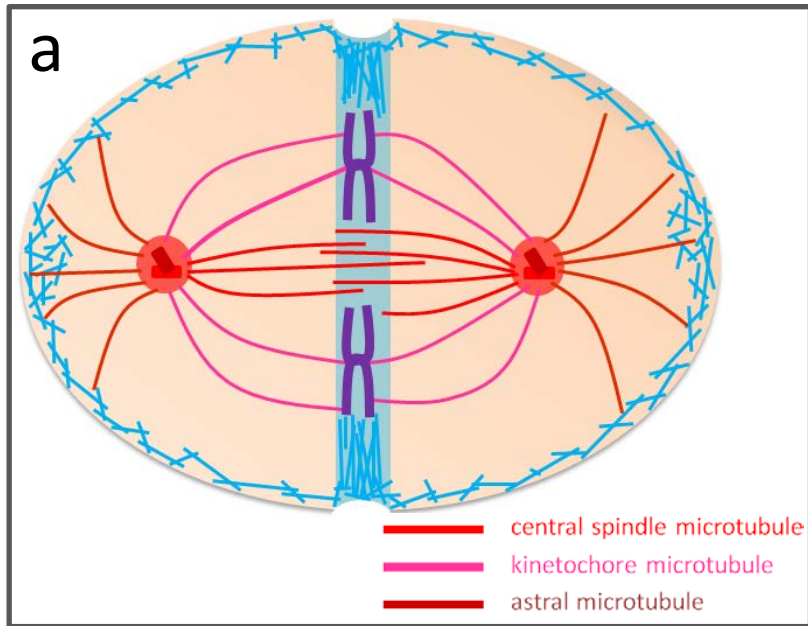
Supplementary Table S1

Overview of molecular players known to mediate physical coupling between the actin and microtubule cytoskeleton ordered by their physical mechanism of interaction. Only proteins are included for which direct interactions with the listed interaction partners have been documented. Names are provided for the mammalian/human genes, with the names of their *Drosophila melanogaster* orthologs (where applicable) between square brackets. Note that this table is not exhaustive and some entries are not referred to in the main text.

Biophysical mechanism	Coupling protein	Molecular interaction partners	Cellular functions
Actin-microtubule crosslinking	Plectin	Actin [1] and microtubules [2]	Cell mechanics [3]
	Tau	Actin and microtubules [4], EBs[1] [5]	Axon organization [4]
	MAP2c[2]	Actin and microtubules [6]	Neurite formation [6]
	Abelson (Abl) non-receptor tyrosine kinase	Actin[7], microtubules [7], cortactin [8], CLASP2[3] [9]	Lamellipodial protrusions [7]
	Pod1 (polarity osmotic defective-1) [Dpod1]	Actin and microtubules [10]	Axon guidance [10]
	Doublecortin	Actin and microtubules [11, 12]	Axon organization [12]
	Septins	Actin [13], microtubules [14], EBs [15]	Axon collateral branching [14]
Guidance of microtubule growth by actin bundles	ACF7[4]/MACF[5] [Shot]	F-actin [16-18], microtubules [19, 20], EBs [16, 17, 21, 22], dynein–dynactin [23], cortical microtubule stabilization complexes [24, 25], ELMO (engulfment and motility proteins) [25]	Directional cell migration [26, 27]; Axonal outgrowth [28-30]; Epithelial closure [31]
	G2L1[6] (GAS2-like proteins) [Pigs]	F-actin [32], microtubules [32], EB proteins [33, 34]	Actin-microtubule co-alignment [33, 34]
	IQGAP1 ('IQ'motif-containing GTPase-activating protein 1) with CLIP-170 (Cytoplasmic Linker protein 170)	CLIP-170 binds EB and microtubules [35-37], IQGAP1 binds actin [38]	Dendrite morphology [35]
	CLASP (Cytoplasmic Linker Associated Proteins) [Orbit]	EBs [39], actin [9, 40], IQGAP1 [41], CLIPs [41], focal adhesions [42, 43], p120-catenin [44]	Actin-microtubule co-alignment [40]; Microtubule–contractile ring interactions in dividing cells [45]; regulation of cell-cell junctions [44] and cell-

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[1] End-binding proteins
[2] Microtubule associated protein 2c
[3] Cytoplasmic Linker Associated Protein
[4] Actin crosslinking factor 7
[5] Microtubule-actin crosslinking factor
[6] Growth Arrest Specific 2 Like 1

			matrix adhesions in cultured cells [42] and epithelia [46]; Axon elongation [9, 47]
	Drebrin	Actin [48], EBs [49]	Neuritogenesis [49], axon branching [50], growth cone&dendrite formation [49, 51]; Neurokinesis [52]; epithelial cell shape [53]
	APC (Adenomatous polyposis coli)	EBs [21, 54], microtubules [54, 55], F-actin [55, 56], actin nucleation, alone and with mDia1 [56-59], IQGAP1 [60]	Directional cell migration [59]; growth cone steering [61]
Anchoring of microtubule plus ends by cortical actin networks	Afadin [Canoe]	Actin [62, 63], LGN[7] [Pins] [63], adherens junctions [62]	Mitotic spindle positioning in single cells and epithelia [63]
	Ezrin-radixin-moesin [Moesin]	Actin [64], microtubules [65], plasma membrane [66]	Mitotic spindle positioning [65, 67]
	Myosin10	Actin [68], microtubules [69], integrins [70], plasma membrane [71]	Spindle positioning in single cells [69, 72] and epithelia [73]
	MISP (mitotic interactor and substrate of Plk1)	EB (end-binding) proteins [74], dynein-dynactin [74, 75], actin [76]	Spindle positioning in single cells [74, 75]
	Anillin	Actin [77], myosin [78], microtubules [79], septins [80], RhoA [81], RacGAP50C [82], plasma membrane [83]	Polarization of mitotic actin cortex by astral microtubules [79, 84]
Anchoring of microtubule minus ends by actin networks	Spectraplakin family protein ACF7/MACF [Shot or Shortstop or Kakapo]	Microtubule –TIP protein CAMPSAP (Calmodulin Regulated Spectrin Associated Protein)/Nezha) [Patronin] [16-18, 85, 86]	Apicobasal cell polarity in epithelia [86, 87]; epithelial cell migration [85]; tight junction regulation in intestinal epithelia [88]
Actin nucleation and elongation from microtubule plus ends	Complex of EB1, CLIP-170[8] and formins (demonstrated *in vitro* for mDia1[9], mDia2, Daam1[10], INF1[11], and INF2)	Microtubule plus end and actin monomers [89, 90]	Dendritic branching in neurons [89]
	Navigator protein [Sickie]	Microtubule +TIPs and actin nucleators [91][92]	Neuronal outgrowth [91][92]

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[7] Leu-Gly-Asn repeat-enriched protein
[8] CAP-Gly Domain Containing Linker Protein
[9] Diaphanous Related Formin
[10] Dishevelled Associated Activator Of Morphogenesis
[11] Inverted formin

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