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Tools for studying the cytoskeleton during plant cell division

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Highlights

- The cytoskeleton regulates fundamental biological processes in plants including cell division, cell elongation and stress responses.
- Mutation of cytoskeleton regulators, when not part of a large gene family, impacts cell division, but it is difficult to disentangle the direct effects induced by chronic cytoskeleton perturbations from indirect ones.
- Experimental systems can acutely manipulate cytoskeleton in cultured cells, transient assays or stably transformed plants. These include pharmacological and genetic strategies which work in relevant time frames for the studying of the cell division mechanisms.
- The development of fast and reversible optogenetic tools in animal cell biology field might in the near future, change our experimental design regarding plant cytoskeleton perturbations.

Outstanding question box

- Can we translate what is already described in cell culture to cells embedded in their tissues?
- Do the concentrations of drugs needed to generate an effect vary between model organisms?
- Can we find new molecules to perturb a given subpopulation of microtubules or actin cables?
- What are the effects of the cytoskeleton drugs on cell division progression at the subcellular level in time and space?
- Can we use the microfluidic technology to reevaluate the effects of drugs on cell division progression?
- For *in planta* analyses, can we upgrade the available genetic tools using the inducible-expression technology?
- How to generate a system to express a transgene only in dividing cells?
- How to ensure that an inducible genetic tool will be turned off?
- Can we produce conditional mutants impaired in plant cell division?
- How to develop new tools to perturb the cell division at specific steps?
- Can we already use optogenetic systems to perturb the division in plant cells?
- How easy would it be to translate the optogenetics to a green multicellular organism?

Glossary box

- **Actin depletion zone (ADZ):** ADZ: zone at the plasma membrane that was reported in some species
- **Actin Interacting Protein 1 (AIP1).** Conserved WD-repeat protein found in fungi, slime molds, nematodes, frogs, mammals, and plants. AIP1 caps AFs weakly and enhances the actin-severing activity of the Actin Depolymerizing Factor.
- **Amiprofos-methyl (APM).** The herbicide amiprofos-methyl (APM), completely depolymerized both cortical and mitotic MT arrays in cell culture.
- **Bisphenol-A (BPA).** Widespread environmental pollutant, reportedly harmful to living organisms.
- **BY2-cells.** Plant cell line established from a callus induced on a seedling of *Nicotiana tabacum* cv. BY-2
- **Carbamate herbicides.** N-Phenyl carbamate herbicides, such as propham chlorpropham, (N-(3-chlorophenyl)-carbamate, CIPC) and pronamide, bind to plant tubulin and directly affect MTs polymerization in cells.
- **Colchicine.** Alkaloid from the autumn crocus, binds to tubulin dimers in vitro and the formation of such tubulin–colchicine complex prevents MT assembly.
- **cortical division zone (CDZ):** zone at the cortex of the cell define early during division that mark the future division plane
- **CYCLASE ASSOCIATED PROTEIN 1 (CAPI).** Predicted plant protein of 476 amino acids highly homologous with the yeast CAPp which binds to actin in vitro.
- **Cytochalasin-D.** Mycotoxin produced by molds such as *Helminthosporium*, a potent inhibitor of F-actin polymerization.
- **Disassembly-promoting, encodable Actin tools (DeActs).** This system is based on the expression of the mono(ADP-ribosyl)transferase domain of the *Salmonella enterica* SpvB which induces the ADP-ribosylation of actin monomers. This process prevents the formation of AFs, and results in the disassembly of all dynamic AFs.
- **iLID system.** Light-induced dimers that can be turned on and off with high spatial and temporal resolution in living *systems*
- **Latrunculins.** Toxin by sponges, such as the *Latrunculia Magnifica Keller* from the red sea.
- **Light-Oxygen-Voltage-sensing (LOV) domain:** protein sensor responsible for *detecting* blue light
- **MG132 (carbobenzoxy-Leu-Leu-leucinal).** Potent, reversible, and cell-permeable proteasome inhibitor.
- **Optogenetic.** Optogenetic. Genetically-encoded system that allows light-stimulated manipulation of a biological pathway
- **Oryzalin.** Herbicide of the dinitroaniline class, which has a strong binding affinity for plant tubulins and acts through the depolymerization of MTs.
- **Paclitaxel/Taxol.** Toxin from the Pacific yew, Paclitaxel binds reversibly to MTs, with a stoichiometry approaching 1:1, relative to the tubulin heterodimer.
- **PHS1.** mitogen-activated protein kinase phosphatase containing an atypical kinase domain, with weak similarity to the slime mold Actin-Fragmin 311 kinase, adjoining the phosphatase domain.

- **Propyzamide.** Benzamide herbicide which inhibited the polymerization of tobacco tubulin, while no effect was observed for animal tubulin.
- **PPB.** Microtubules array from before the mitosis presumably from the cortical microtubule network. The PPB function is unknown, but it marks the future cell division plane.
- **Phragmoplast.** Structure of MTs and AFs, formed during the cytokinesis, that drives the polar secretion toward the cell plate during its expansion and attachment.
- **splakinolide (JAS).** Macrocyclic peptide isolated from the marine sponge *Jaspis Johnstoni*, a potent inducer of actin polymerization in vitro by stimulating MFs nucleation.
- **Swinholide-A/bistheonellide (BA).** Found mostly in the marine sponge Theonella,
- **Trifluralin.** Herbicide, banned in the European Union since 2008, primarily due to high toxicity to aquatic life.
- **Vinblastine (VBL).** Binds to the tubulin dimer and causes depolymerization of MTs into free tubulin subunits.

Tools for Studying the Cytoskeleton during Plant Cell Division

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1 **Abstract**

2 The plant cytoskeleton regulates fundamental biological processes including cell division. How to
3 experimentally perturb the cytoskeleton is a key question if one wants to understand the role of both
4 actin filaments and microtubules in a given biological process. While a myriad of mutants is available,
5 knock-out in cytoskeleton regulators, when non-lethal, often produce little or no phenotypic perturbation
6 due to such regulators are often part of a large family, leading to functional redundancy. Here, alternative
7 techniques to modify the plant cytoskeleton during plant cell division are outlined. The different
8 pharmacological and genetic approaches already developed in cell culture, transient assays, or in whole
9 organisms are presented. Perspectives on the use of optogenetics to perturb the plant cytoskeleton are
10 also discussed.

11

12 **Studying cell division in plants**

13 Cell division is a tightly regulated mechanism that relies on crosstalk between the microtubule (MT)
14 and actin filament (AF) cytoskeletons, the nucleus, and vesicular trafficking [1]. The mother plant cell
15 divides into two daughter cells via a predefined cell division orientation pattern (Figure 1). The
16 reorganization of the MT into the Pre-Prophase-Band (PPB) marks the entry into mitosis in plants [2,
17 3]. The position of the PPB correlates with the future division plane, suggesting a role in cell division
18 orientation. However, recent studies, demonstrated that MTs at the PPB are not essential for the proper
19 orientation of cell division, and nuclear positioning appears unaffected by the loss of PPB MTs [4, 5].

20

21 As the PPB of MTs narrows, AF disappears from the cortical region occupied by the PPB, giving rise
22 to an actin-depleted zone (ADZ) [6, 7]. In BY-2 tobacco culture cells, the ADZ is surrounded by AF
23 enrichment on each side – termed « actin twin peaks ». However, AF rearrangement during this short
24 transition period is barely visible in plant cells embedded in their endogenous tissue [8-10].

25 After chromosome separation via the metaphase and anaphase spindle and during symmetric cell
26 division, another structure specific to land plants and their close algal ancestors composed of MTs and
27 AFs called the phragmoplast directs the polar transport of vesicles toward the center of the cell for the
28 initiation of cytokinesis (Figure 1). The expansion of the phragmoplast is driven by repeated cycles of
29 MT depolymerization on the phragmoplast inner face and MT polymerization on the phragmoplast outer
30 face, leading to centrifugal expansion of the vesicle flow and, consequently, of the cell plate [8]. The
31 transition from the cleavage-type cell division found in simple algae to cell plate-type cell division found
32 in land plants and their immediate ancestors is marked by several intermediate strategies in the different
33 branches of the *phragmoplastophyta*. The phragmoplast is essential for the formation and the expansion
34 of the cell plate, while the PPB is not required. Consistent with this idea, in mutants lacking PPB MTs,
35 the CDZ-resident POK1 protein is still recruited to the CDZ (although recruitment is delayed) and
36 cytokinesis is appearing normal [5].

37 An increasing number of tools are available to visualize and modify the plant cytoskeleton [9-14].
38 Mutations in genes encoding cytoskeleton regulators are often either lethal or do not result in any visible
39 phenotypes due to redundancy [15, 16]. It is, therefore, important to complement such an approach with
40 other techniques to perturb the plant cytoskeleton. In this review, the different drugs and genetic systems
41 available to perturb both the MT and the AF cytoskeleton are presented. While still not currently
42 available for work *in planta*, perspectives on the possible use of optogenetics to perturb the cytoskeleton
43 are discussed.

44

45 **1. Pharmacological perturbation of the cytoskeleton during cell division**

46 Pharmacological approaches to disrupt the cytoskeleton were the prevalent method of perturbing cell
47 division for many years, before being largely abandoned with the rise of modern genetics. These
48 methods allow researchers to relatively easily disrupt or stabilize the MT and AF cytoskeletons. It is
49 inducible and amenable to both model and non-model organisms for which genetic tools are not
50 available.

51 Drugs targeting AFs may act on the stability of the cytoskeleton network in different ways, including:
52 (i) preventing AF polymerization, (ii) provoking depolymerization, (iii) inducing polymerization, and
53 (iv) targeting actin-binding proteins (Table 1). Cytochalasin D prevents AF polymerization. It does not

54 inhibit the formation of the PPB in BY-2 cells, but inhibits the narrowing process that defines the precise
55 CDZ [17]. This result is consistent with the absence of AFs in the PPB in Arabidopsis roots [18]. In
56 *Physcomitrium patens*, in which the division site is defined by active spindle-positioning mechanisms,
57 latrunculin-A abolishes basal spindle motility [19]. Disruption of AFs during maize root apex growth
58 increases the frequency of oblique cell plates, while the effects of latrunculin-A in cell plate orientation
59 in dicot plants have not yet been tested [20, 21]. Inhibition of AF polymerization by Swinholidide-
60 A/bistheonellide increases the time of phragmoplast extension, suggesting that AFs play a more
61 significant function at the later stage of cell plate elongation [22, 23]. The induction of AF
62 polymerization by Jasplakinolide (JAS), in wheat protoplasts, promotes AF bundling [24]. In BY-2 cells,
63 abnormal “actin peaks” around the cell center are observed after Jasplakinolide treatment, with the
64 formation of triple peaks also occurring in the spindle at metaphase [25]. The small molecular inhibitor
65 CK-666, which inhibits Actin-Related Protein 2/3 complex by blocking its activation, can also be used
66 to study the role of the actin cytoskeleton in a given mechanism *in planta* [26]. While this molecule has
67 not yet been applied to study of plant cell division, a recent study in tomato pollen tubes showed size
68 reduction of the mat of adhered cells formed *in vitro*, when treated with CK-666 for 3 h [27].

69 Pharmacological substances targeting MTs may also (i) act on their stability, (ii) prevent their
70 polymerization, or (iii) trigger depolymerization (Table 1). Stabilization of the MT cytoskeleton using
71 paclitaxel/taxol increases the accumulation of vesicles at the equatorial plane of the phragmoplast, while
72 simultaneously inhibiting phragmoplast expansion [28]. In Arabidopsis, mutation in *TANGLED* leads
73 to increased sensitivity to mild concentrations of taxol (0.5 and 1 μm), while other mutants such as *air9*,
74 and *tan1 air9* plants did not [29]. Oryzalin prevents the polymerization of MTs and can also be used to
75 assess their stability. MTs undergoing constant growth and shrinkage (e.g., highly dynamic MTs) are
76 more sensitive to oryzalin, whereas highly stable MT arrays remain largely unaffected. This drug is
77 widely used to study the dynamic MT cytoskeleton during interphase [30, 31]. Spindle assembly
78 checkpoint mutants are hypersensitive to oryzalin highlighting their role for both cell survival and
79 growth [32].

80 A crucial consideration with the use of pharmacological perturbations to study cytoskeletal dynamics
81 and function during cytokinesis is the length of any given treatment. Treatments for days may lead to
82 longer-term developmental consequences of repeatedly abnormal cell divisions such as micronuclei
83 formation [33]. Short treatments (less than 14hrs) are more desirable in most cases. Different phenotypes
84 have been reported in the literature for molecules theoretically all affecting MTs depolymerization. For
85 example, treatment with propyzamide arrests the cell cycle at metaphase in tobacco BY-2 cells by
86 preventing the formation of microtubule-kinetochore attachments [34]. Defects in cytokinesis are not
87 often observed with this treatment. In contrast, when using N-Phenyl carbamate herbicides such as
88 chlorpropham at a concentration allowing the complete disappearance of all MTs, Y-shaped MF-

89 containing phragmoplasts and branched cell plates were reported [35-38]. The differences in these
90 responses might depend on the timing of the application of the drug, and on when it will reach the
91 cytoplasm in terms of timing (or whether it will cross the cell wall and the plasma membrane at all) to
92 be incorporated to the dividing cell.

93 When targeting both MTs and AFs using drugs such as Bisphenol-A (BPA), malformed and
94 discontinuous emerging cell plates have been reported. This defect seems to be temporary, for only 1–
95 2 min, after which the discontinuous areas of the cell plate were filled in so that it reached a normal
96 appearance [22]. Other small molecules such as Small Molecule Inhibitor of Formin FH2 (SMIFH2)
97 inhibits both the ability of the formins to promote AF polymerization and to bind to MTs [39]. Since
98 formins represent a large multigenic family, this inhibitor has been useful to study their role in a wide
99 range of systems including cytokinesis [40-43]. In BY-2 cells, treatment with SMIFH2 slows down
100 phragmoplast expansion and causes cell plate swelling, suggesting that formins promote MT
101 polymerization and AF nucleation at the cell plate [44].

102 Caffeine has been widely used to study cell plate expansion, even though its molecular target is still
103 unknown in plants [45-48]. In *Arabidopsis*, BUB3.1 and BUB3.2 localize to the phragmoplast midzone,
104 interacting with the microtubule associated protein MAP65-3 [32, 34, 49]. Low-dose of caffeine
105 abolishes MAP65-3 localization from the midzone in the hypersensitive *bub3* mutants, further
106 substantiating BUB3's function in cytokinesis, possibly by promoting MAP65-3 microtubule bundling
107 [49].

108 The growing interest in microfluidic devices in the plant community will allow us to test the effect of
109 cytoskeleton perturbations using pharmacological approaches in a more controlled environment than
110 has traditionally been possible. Previously, one of the bottlenecks of this approach was the generation
111 of the microfluidic chips, which were not reusable. The plants needed to be well positioned to allow the
112 growth of the root in the channel, making such work extremely costly and time consuming. The recent
113 design of a closable single-layered PDMS silicone chip sculptured by micromachining, paves the way
114 for easier and likely more accessible work in this field [50]. The effect of pharmacological perturbations
115 on *Arabidopsis* plants also needs to be addressed further in the context of cell division orientation. In
116 particular, most previous studies were performed in cell cultures, where the orientation of the cell
117 division might encounter different physical constraints and molecular cues compared to cells in their
118 endogenous tissues. Combining vertical confocal microscopy at high resolution and root tracking
119 approaches may help to give a clearer view of the impacts of modifying the cytoskeleton during plant
120 cell division in vivo.

121

122 **2. Genetically-encoded systems to perturb the microtubule cytoskeleton**

123 The development of genetically-encoded inducible systems to perturb the plant cytoskeleton is
124 emerging. Such strategies allow targeted perturbations of either MTs or AFs with increased spatial and
125 temporal resolution in planta. While these methods are not often used to study plant cell division, such
126 tools could yet be deployed in the near future in the context of mitosis. As a possible example, we can
127 cite the kinesin superfamily member kinesin-13. Kinesin-13 does not move along MTs and instead
128 destabilizes MTs, promoting their depolymerization [51]. By using cell-type-specific promoters, we can
129 capitalize on this Kinesin-13 property to trigger MT depolymerization in a given tissue. Such approaches
130 allow a more spatially targeted perturbation than pharmacological inhibitors. The transient expression
131 of *AtKinesin-13A* was reported to induce a partial fragmentation of MTs ([52], Figure 2). In non-
132 differentiating *Arabidopsis* cells in vitro, disruption of MTs by the transient expression of *AtKinesin-*
133 *13A* impacted the localization of the plant-specific MT-binding protein MIDD1 domains, although
134 inactive *AtKinesin-13A* did not [53]. It is not currently clear whether this approach can be made
135 applicable to dividing cells, as it is uncertain whether *AtKinesin-13A* is active or stable in dividing cells.
136 However, there is some cause for optimism since functional analyses of the kinesin-13 three paralogs in
137 *Physcomitrium* implicated them in mitosis [54].

138 Another system already successfully deployed to study plant cell division is based on the MT
139 depolymerizing enzyme PHS1 [55]. The kinase domain of PHS1 directly phosphorylates α -tubulin at
140 Thr349, and phosphorylated α -tubulins cannot be incorporated into MTs [55]. Expression of *PHS1*
141 induces complete MT disassembly, mimicking the effect of oryzalin [56] (Figure 2). This pioneering
142 work inspired the development of synthetic systems to induce tissue-specific depolymerization of MTs
143 [57]. In *Arabidopsis* lines that stably express a phosphatase dead version of PHS1 (*PHS1AP*) specifically
144 in lateral root founder cells, MTs are effectively depolymerized. In turn, lateral root initials swell,
145 showing that MT integrity is required for lateral root founder cells to expand asymmetrically [57]. This
146 system is of particular interest for studies of cell division progression or orientation in a specific cell
147 type, using a cell type-specific promoter. Using cell division-specific promoters to perturb the
148 cytoskeleton only during mitosis is difficult. Indeed, most promoters active in mitosis leads to residual
149 expression throughout the meristem and not specifically in dividing cells [58]. To by-pass this limitation,
150 it is possible to fuse the protein of interest to a destruction box, which degrades the chimeric protein at
151 the metaphase/anaphase transition. However, such strategy might not allow studying late events of
152 cytokinesis.

153

154 **3. Inducible genetic systems to perturb the actin cytoskeleton**

155 The actin cytoskeleton is tightly regulated by actin-binding proteins that bind AFs to control their
156 dynamics via polymerization/depolymerization and severing [59]. These proteins are also used as tools

157 to perturb in an inducible manner AFs *in planta*, such as the overexpression of the actin monomer
158 binding protein *CYCLASE ASSOCIATED PROTEIN 1 (CAPI)* under a Dex-inducible promoter in BY-
159 2 cells, which induces the depolymerization of AFs and prevents cells from undergoing mitosis [53].

160 The *Actin Interacting Protein 1 (AIP1)*, a conserved WD repeat protein, caps AFs weakly and enhances
161 the actin-severing activity of the Actin Depolymerizing Factor [60, 61] (Figure 3). In *Physcomitrium*,
162 the knock-out of AIP1 leads to the accumulation of AF bundles and a lack of AF dynamics, as well as a
163 reduction in the frequency of severing [62]. Ethanol-inducible *HA-tagged AIP1* overexpression in
164 *Arabidopsis* leads to finer AFs with a larger variation in orientation [60]. Moreover, in ethanol-induced
165 *AIP1* RNAi lines, the number of actin bundles was larger and the degree of alignment with the
166 longitudinal axis of the root hair seemed reduced ([63]).

167 Disassembly-promoting, encodable actin tools (DeActs) are universal tools for studying the actin
168 cytoskeleton in cell culture, tissues, and multicellular organisms [64]. This system is based on the
169 expression of the mono(ADP-ribosyl)transferase domain of the *Salmonella* SpvB, which induces the
170 ADP-ribosylation of actin monomers ([65], Figure 3). This process prevents the formation of AFs, and
171 results in the disassembly of all dynamic AFs [65]. It was adapted to plant research initially for the study
172 of lateral root emergence in *Arabidopsis*, demonstrating that AFs are important for the early steps of the
173 asymmetric cell division that eventually leads to the formation of the lateral root [57, 64].

174 The tobacco protein WLIM1 binds directly to AFs *in vitro* and is associated with AFs during both
175 interphase and mitosis in BY-2 cells [66]. Expression of *WLIM1-GFP* in *N. benthamiana* modifies the
176 normal actin cytoskeleton architecture by promoting the recruitment of AFs into thick actin bundles and
177 cables [66]. This approach was later extended with a Dex-inducible promoter (1 μ M Dex for 12-24h) to
178 investigate the potential role of AFs for membrane permeability induced by nanosecond pulsed electric
179 fields [67].

180

181 **4. Towards optogenetic control of the plant cytoskeleton**

182 Optogenetics is a technology that combines genetic (adapted from tools described in the previous
183 section) and optical methods, allowing for rapid perturbations (second-scale) with subcellular precision
184 [68]. With the development of optogenetic tools, the animal cell biology field has benefitted from rapid
185 and reversible systems to perturb cellular processes and machinery, including the cytoskeleton [69-76].
186 Although these technologies are not yet available in plants, some of the following examples might, in
187 the future, introduce new possibilities for experimental cytoskeleton perturbations in plants.

188

189 **(i) The photo-uncaging of actin depolymerization factor and tubulin acetylase**

190 In photo-uncaging approaches, the activity of a given protein that is repressed in the dark and can be
191 released after being exposed to a specific wavelength of light. In this context, the active site of an MF-
192 associated protein can be fused to the photo-uncaging actuator and expressed in cells in the absence of
193 light. By using a photomanipulation module, the activity of such proteins on the cytoskeleton can be
194 induced in specific regions of interest via light-dependent uncaging of the active site. The light-induced
195 photo-uncaging system is based on the protein A Z-domain-derived affibody, Zdk1, that binds to the
196 oat phototropin 1 (LOV2) domain with high affinity in the dark, but dissociates in blue light [77]. The
197 innovative Z-lock system is based on such affibody and is a reversible, light-controlled system that
198 allows steric inhibition of both AFs and MTs in animal cells [70]. In Z-lock, the LOV2 domain and Zdk
199 are attached to each extremity of the protein of interest (Figure 4). In this chimeric construct, the active
200 site of the protein of interest is blocked in the dark by the dimerization of LOV2 with Zdk. Upon light
201 induction, LOV2 changes conformation and releases Zdk, exposing the active site of the protein of
202 interest [77].

203 The Z-lock strategy allowed a reversible, light-controlled steric inhibition of cofilin, an actin-binding
204 protein. This system was validated *in vitro* and *in vivo*, in which uncaging using a single illumination
205 pulse resumed the severing activity of cofilin. The same approach was also used to modify MTs, using
206 an active fragment of the tubulin acetylase α TAT which, in this system, acetylates tubulin after light
207 induction [70]. Since ADFs/cofilins are relatively conserved proteins between kingdoms, it is
208 conceivable that such a system may also be applicable to plant cells. Some engineering might be required
209 to adapt the system at a molecular level for use *in planta*, using either plant endogenous candidates or
210 ADFs/cofilins already characterized in other species.

211

212 **(ii) Light-induced recruitment systems**

213 To create a reversible recruitment of proteins to MT plus ends, the light-induced dimerization system
214 iLID (iLID/SspB) was developed. In this system, the iLID module is fused to an EB-binding SxIP motif,
215 a canonical motif that localizes many proteins to growing MT plus ends. When exposed to blue light,
216 SxIP-iLID recruits SspB-tagged proteins to MT plus ends [78]. This system can be adapted to answer a
217 variety of questions pertaining to the regulation of MT network organization and dynamics, particularly
218 in the context of cell division. To advance the understanding of how cross-linking affects cytoskeletal
219 dynamics and network organization in animal cells, this system was further developed to crosslink MTs
220 and AFs under light control [78]. A similar approach was also used to address the role of the dynein-
221 mediated pulling forces exerted on astral MTs during spindle positioning [74]. In this system, light

222 induction activates the artificial anchoring of the dynein to the plasma membrane. Results from this
223 experiment indicated that the motor being anchored at the cell periphery is not enough for it to pull on
224 microtubules. Instead, it was shown that dynein needs to be activated by another component of the
225 complex, to control the location of the spindle and, therefore, of the cleavage plane [74]. Spatially
226 precise photoactivation is difficult during mitosis because the dividing cells are generally small and the
227 mitotic figures are highly dynamic. Moreover, anchors targeting plant-specific structures such as the
228 CDZ or ADZ still need to be developed. Yet, with the increased knowledge of plant-specific molecular
229 players during mitosis that has arisen in recent years, potential candidate domains are now emerging [5,
230 16, 79].

231

232 (iii) Photo-dissociation of EB1

233 Using the Z-lock technology described above, an acute and reversible blue light-mediated
234 photodissociation of the conserved MT plus-end tracking End Binding protein 1 (EB1) was recently
235 obtained [80]. The EB1 protein consists of two functional domains, the amino-terminal CH domain
236 which recognizes growing MT ends, and the carboxy-terminal EBH domain which recruits other
237 members of the MT tip-tracking protein (+TIPs, [81, 82]). The LOV2-Zdk1 module was introduced into
238 the intrinsically disordered linker between the N-terminal and C-terminal halves of EB1 [80]. Because
239 EB1 requires dimerization for efficient plus-end tracking, an additional GCN4 leucine zipper was
240 inserted between the CH and LOV2 domains to retain dimerization and plus-end tracking of the N-
241 terminal half alone. The inactivation of EB1 by light in a knockout mutant leads to the production of a
242 conditional mutant controlled with high spatial and temporal accuracy [80].

243

244

245 (iv) Reversible knock-sideways of PRC1/MAP65 from the spindle in metaphase

246 Optogenetics can also be used for the fast and reversible removal of a protein of interest, using
247 techniques derived from the light-inducible dimer (iLID) system [83, 84]. This approach was developed
248 to control the recruitment of MAP65/PRC1 to the spindle midzone during chromosome attachment and
249 segregation [72]. The first part of this system is composed of a fluorescent-tagged version of PRC1 fused
250 to the bacterial protein SspB. This construct was used in a complementation analysis to ensure that the
251 chimeric PRC1 protein was functional. The second part of the system, the iLID, contains the bacterial
252 peptide SsrA and the LOV2 domain targeted to the plasma membrane by a short CAAX peptide. While
253 the MAP65/PRC1 is fulfilling its function in the dark, the light-induced dimerization of SsrA and SspB
254 induces the translocation of MAP65/PRC1 from the spindle to the plasma membrane [72, 73]. This
255 method is reversible - after cessation of exposure to blue light, PRC1 localization on the spindle was

256 restored [72]. In *Arabidopsis*, there are eight homologs of MAP65/PRC1, including some members with
257 well-characterized roles in plant cell division [11, 85]. These could, therefore, be good candidates for
258 the translation of such an approach to *in planta* work.

259

260 The development of optogenetic tools in plants will likely, however, be challenging, and will require
261 complex protein engineering strategies to circumvent technical issues that might affect their
262 functionality or create undesirable side effects. These include efficient expression and localization, as
263 well as diminishing any interference between these plant-derived tools and endogenous light signaling
264 pathways in plants.

265

266 **Concluding remarks and future perspectives**

267 The use of drugs to perturb the plant cytoskeleton, while extremely common in the late 20th century, has
268 reduced concomitantly with the explosion of genetic tools. The recent development of microfluidic
269 technologies [50, 86] allow treatments with a given chemical compound with high temporal-controlled.
270 Using such a device, pharmacological perturbation of the cytoskeleton may now regain popularity as a
271 way to perturb MTs, AFs, or both. To prevent adverse consequences that can be associated with long
272 treatments, pulse treatments should be prioritized. Such work will also likely benefit from recent
273 advances in long-term time-lapse acquisition with root tracking systems to capture for example the
274 effects of pulse treatment on the overall cell cycle [87, 88]. By analyzing root architecture and, at the
275 subcellular level, cytoskeleton organization using fluorescent dyes [89], this approach allows the effect
276 of the drug to be tracked as soon as it is administered. Monitoring of the effects of pharmacological
277 perturbation over time can now be achieved with a high range of time points and in a quantitative
278 manner. The adaptation for plant research of already available optically controlling cytoskeleton
279 inhibitors might be the way to solve the problem of specificity, since light can be applied with high
280 spatiotemporal precision [90-92].

281 Currently, genetic perturbation of the cytoskeleton is by far the most selective system available and they
282 have limited off-target effects. The available perturbation systems can be adapted for use in different
283 plant models, for example by changing the expression vector. Moreover, the recent development of a
284 cell division system in *N. benthamiana* leaf epidermis [93], will allow studying cell division in a
285 transient expression system in conjunction with genetic cytoskeleton perturbation. However, contrary
286 to drugs where a washout might be sufficient to reset the system, genetic tools are often irreversible.
287 Reversibility is one of the major advantages of optogenetics, already widely used in the field of animal
288 cell biology. This technology has yet to be substantially adopted in plants, but interest is now growing
289 in the community towards adapting such powerful tools perturbation tools [94]. In animal and yeast

290 cells, optogenetics has been used to modulate the function of conserved regulators of cytoskeleton
291 dynamics. It is, therefore, conceivable that such approaches could be translated to work on the plant
292 cytoskeleton. To date, use of optogenetic work in plants has been published only couple of times, and
293 was used to modify the transcription of genes, enhance stomatal kinetics and to mimic drought
294 conditions[95-97]. This technological breakthrough might be the first step towards the wider adaptation
295 and adoption of optogenetic technology to perturb plant cells.

296

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301

302 **Figures**

303 **Table 1.** Examples of drugs available to perturb AFs and MTs during plant cell division. The
304 concentration/concentration ranges generally used in published studies are presented.

305 **Figure 1.** Stages of plant cell mitosis. MTs are represented in green, AFs in red. Before mitosis, the Pre-
306 Prophase-Band (PPB), a specific MT structure forms. The cortical division zone (CDZ) formed early during
307 cell division marks the site of the eventual division plane. During metaphase, the acentrosomal spindle
308 positions the chromosomes at the cell equator. At the metaphase/anaphase transition, the spindle assembly
309 checkpoint verifies that all the chromosomes are attached to the MTs, allowing the progression of the
310 mitosis. After chromosome separation, the phragmoplast composed of MTs and AFs directs the transport
311 of vesicles toward the center of the cell for the initiation of cytokinesis. The expansion of the phragmoplast
312 from solid state to ring state is driven by repeated cycles of MTs depolymerization on the phragmoplast
313 inner face and MTs polymerization on the phragmoplast outer face, leading to centrifugal expansion of the
314 vesicle flow and, as a result, the cell plate. At the end of cytokinesis, the cross wall is formed after cell plate
315 attachment at the center of the earlier defined CDZ, named the cell plate fusion site.

316 **Figure 2.** Genetic perturbation of MTs. MTs are made up of protofilaments, themselves forming from
317 dimerization of α - and β -tubulins. “Structural” Microtubule Associated Proteins (MAP) such as the
318 PRC1/MAP65 family regulates the bundling of MTs. Other factors, such as motor proteins are implicated
319 in the depolymerization of MTs, by (1) direct binding (AtKIN13-A) or by (2) modifying tubulin monomers
320 (green circle with pink center), preventing their polymerization (PHS1 Δ P, grey arrows).

321 **Figure 3.** Genetic perturbation of AFs in plant cells. When ADP-bound actin monomers are activated, the
322 ATP-bound actin monomer di- tri-merized and eventually forms actin filaments (AFs). AFs can, like MTs,
323 be bundled by the action of proteins such as fibrins or WLIM1. AFs can be depolymerized via the action
324 of the actin monomer binding protein *CYCLASE ASSOCIATED PROTEIN 1 (CAP1)*, or bacterial toxins
325 (SpvB), or by their severing, through the action of actin depolymerization factors such as cofilins. The *Actin*
326 *Interacting Protein 1 (AIP1)* enhances the actin-severing activity of the Actin Depolymerizing Factor

327 **Figure 4.** Example of the Z-lock system for reversible, light-controlled steric inhibition of AFs. In this
328 system, the LOV2 domain and Zdk are fused to each extremity of the cofilin. In the dark, the cofilin active
329 site is blocked by the dimerization of LOV2 with Zdk. Upon light induction, LOV2 changes conformation
330 and releases Zdk, uncaging the cofilin active site. The cofilin will resume its severing activity and will induce
331 AFs depolymerization upon light exposure.

332 **Figure 5.** Reversible knock-sideways of PRC1-MAP65 using an optogenetic approach. In the first part of
333 the system, PRC1 is fused to the bacterial protein SspB and localizes to MTs. The second part of the system,
334 the iLID, contains the bacterial peptide SsrA and the LOV2 domain targeted to the plasma membrane by a
335 short CAAX peptide. While the PRC1 is at the MTs in the dark, the light-induced dimerization of SsrA and
336 SspB provokes the translocation of PRC1 from MTs to the membrane.
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339 **Glossary box 462 words**

- 340
- 341 • **Actin depletion zone (ADZ):** ADZ: zone at the plasma membrane that was reported in
342 some species
 - 343 • **Actin Interacting Protein 1 (AIP1).** Conserved WD-repeat protein found in fungi, slime
344 molds, nematodes, frogs, mammals, and plants. AIP1 caps AFs weakly and enhances the actin-
345 severing activity of the Actin Depolymerizing Factor.
 - 346 • **Amiprophos-methyl (APM).** The herbicide amiprophos-methyl (APM), completely
depolymerized both cortical and mitotic MT arrays in cell culture.

- 347 • **Bisphenol-A (BPA).** Widespread environmental pollutant, reportedly harmful to living
348 organisms.
- 349 • **BY2-cells.** Plant *cell* line established from a callus induced on a seedling of *Nicotiana tabacum*
350 cv. BY-2
- 351 • **Carbamate herbicides.** N-Phenyl carbamate herbicides, such as protham chlorprotham, (N-(
352 3-chlorophenyl)-carbamate, CIPC) and pronamide, bind to plant tubulin and directly affect MTs
353 polymerization in cells.
- 354 • **Colchicine.** Alkaloid from the autumn crocus, binds to tubulin dimers in vitro and the formation
355 of such tubulin–colchicine complex prevents MT assembly.
- 356 • **cortical division zone (CDZ):** zone at the cortex of the cell define early during division that
357 mark the future division plane
- 358 • **CYCLASE ASSOCIATED PROTEIN 1 (CAP1).** Predicted plant protein of 476 amino acids
359 highly homologous with the yeast CAPp which binds to actin in vitro.
- 360 • **Cytochalasin-D.** Mycotoxin produced by molds such as *Helminthosporium*, a potent inhibitor
361 of F-actin polymerization.
- 362 • **Disassembly-promoting, encodable Actin tools (DeActs).** This system is based on the
363 expression of the mono(ADP-ribosyl)transferase domain of the *Salmonella enterica* SpvB
364 which induces the ADP-ribosylation of actin monomers. This process prevents the formation of
365 AFs, and results in the disassembly of all dynamic AFs.
- 366 • **iLID system.** Light-induced dimers that can be turned on and off with high spatial and temporal
367 resolution in living *systems*
- 368 • **Latrunculins.** Toxin by sponges, such as the *Latrunculia Magnifica Keller* from the red sea.
- 369 • **Light-Oxygen-Voltage-sensing (LOV) domain:** protein sensor responsible for *detecting* blue
370 *light*
- 371 • **MG132 (carbobenzoxy-Leu-Leu-leucinal).** Potent, reversible, and cell-permeable proteasome
372 inhibitor.
- 373 • **Optogenetic.** Optogenetic. Genetically-encoded system that allows light-stimulated
374 manipulation of a biological pathway
- 375 • **Oryzalin.** Herbicide of the dinitroaniline class, which has a strong binding affinity for plant
376 tubulins and acts through the depolymerization of MTs.
- 377 • **Paclitaxel/Taxol.** Toxin from the Pacific yew, Paclitaxel binds reversibly to MTs, with a
378 stoichiometry approaching 1:1, relative to the tubulin heterodimer.
- 379 • **PHS1.** mitogen-activated protein kinase phosphatase containing an atypical kinase
380 domain, with weak similarity to the slime mold Actin-Fragmin 311 kinase, adjoining the
381 phosphatase domain.
- 382
- 383 • **Propyzamide.** Benzamide herbicide which inhibited the polymerization of tobacco tubulin,
384 while no effect was observed for animal tubulin.
- 385 • **splakinolide (JAS).** Macrocyclic peptide isolated from the marine sponge *Jaspis Johnstoni*, a
386 potent inducer of actin polymerization in vitro by stimulating MFs nucleation.
- 387 • **Swinholide-A/bistheonellide (BA).** Found mostly in the marine sponge *Theonella*,.
- 388 • **Trifluralin.** Herbicide, banned in the European Union since 2008, primarily due to high toxicity
389 to aquatic life.

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- **Vinblastine (VBL).** Binds to the tubulin dimer and causes depolymerization of MTs into free tubulin subunits.

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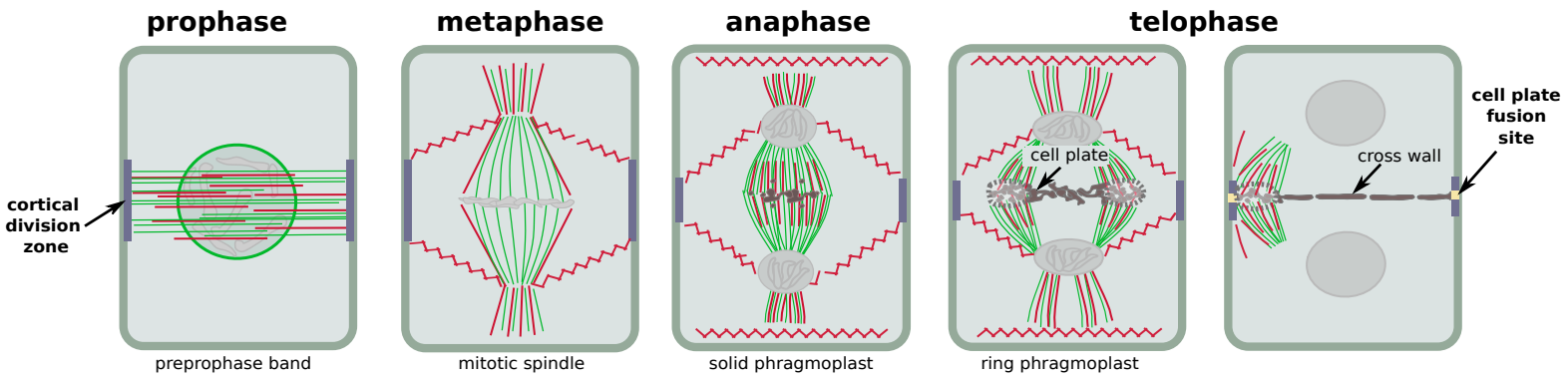
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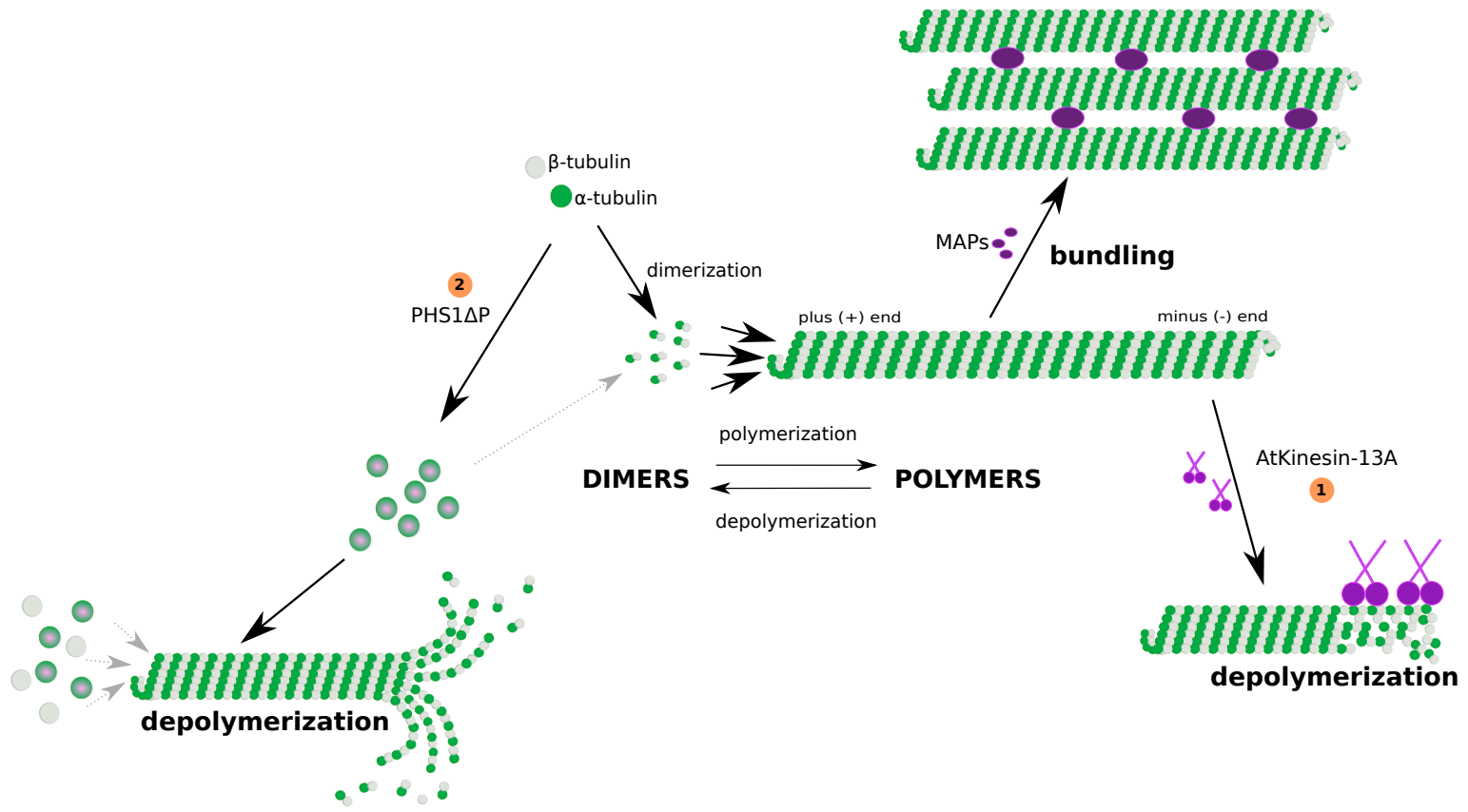
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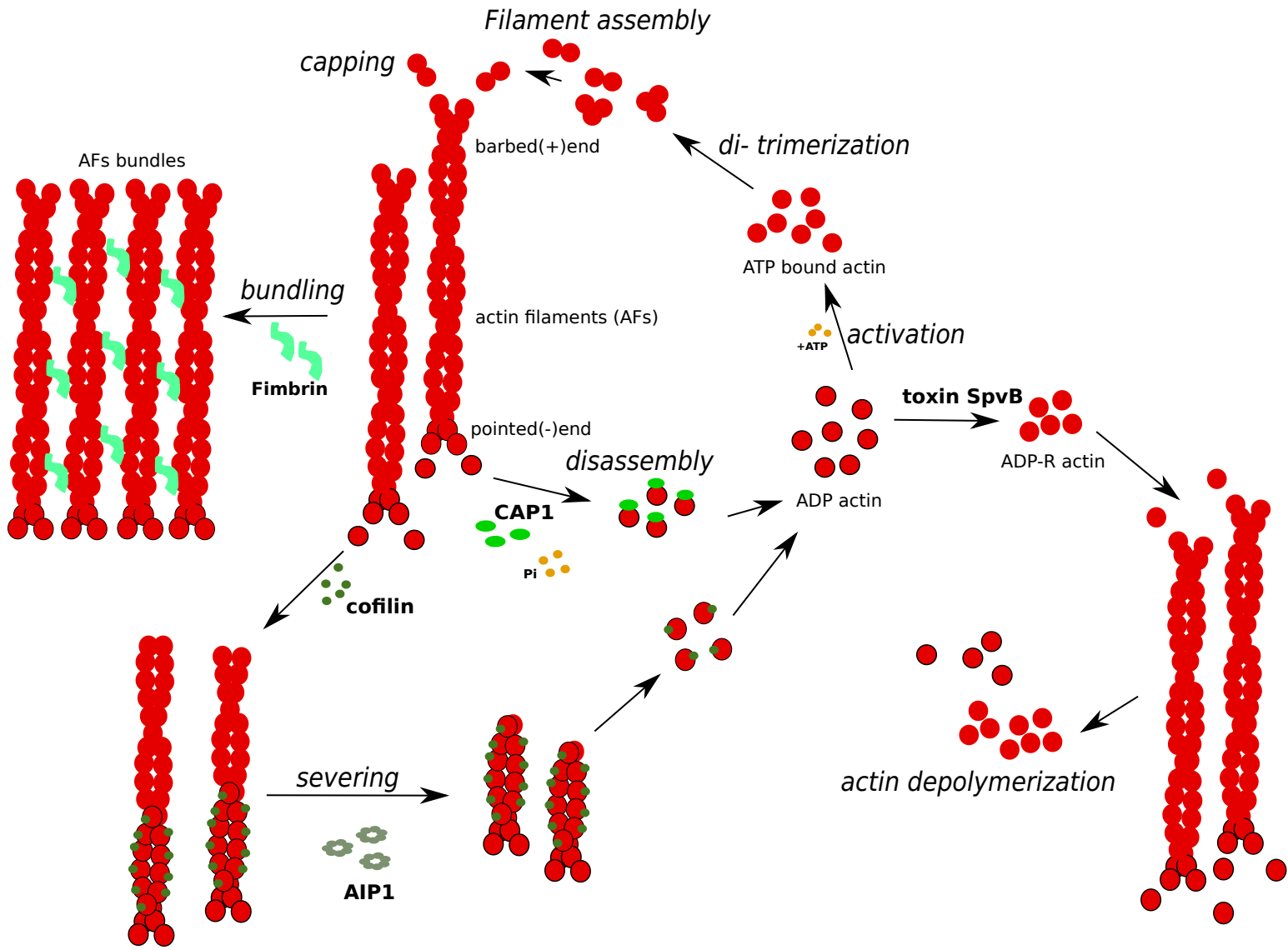
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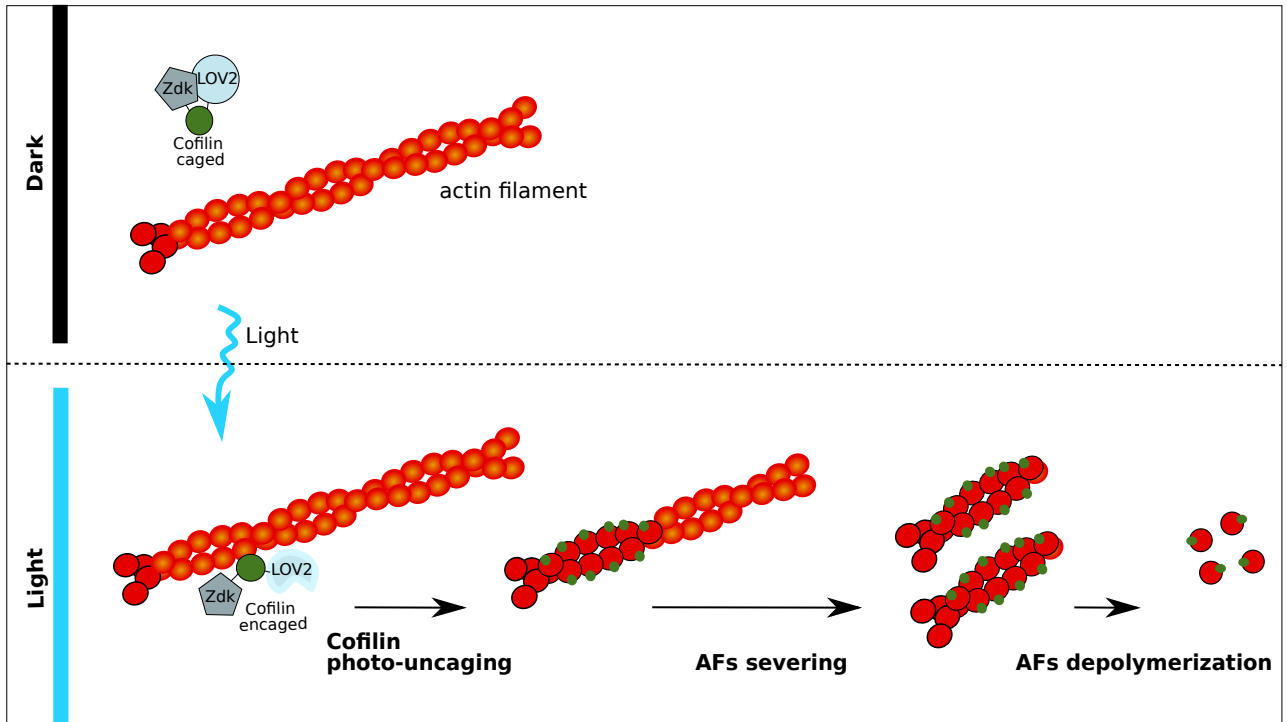
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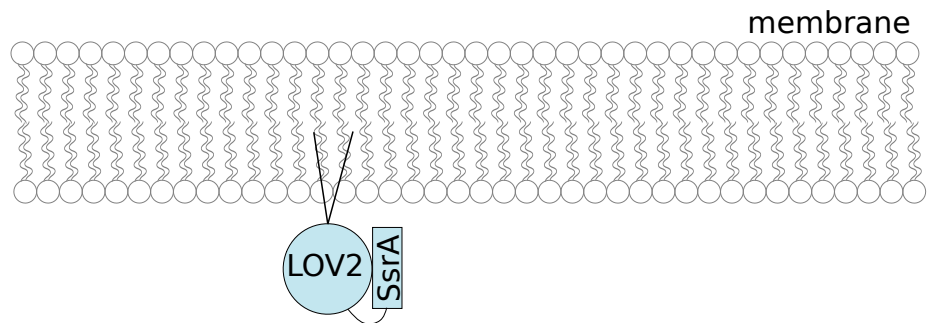




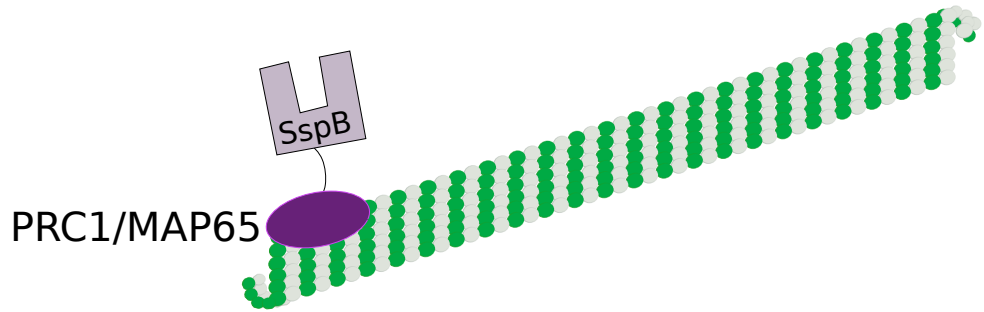




Dark

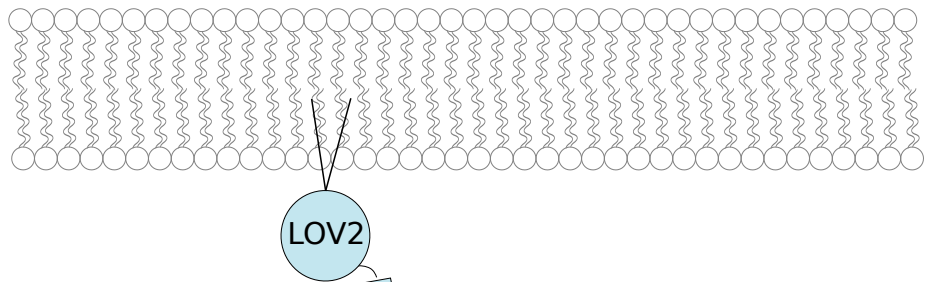


microtubule



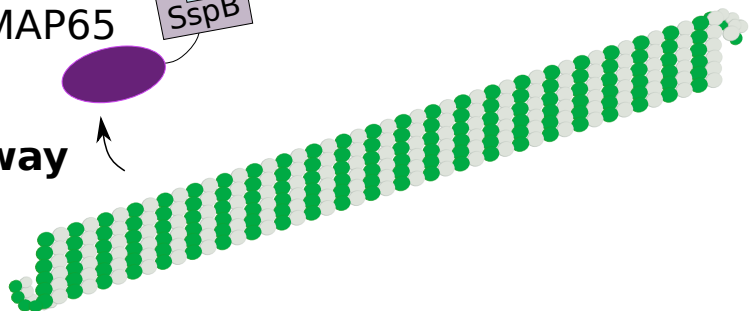
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Light



PRC1/MAP65

Knock-side way



	Name	Mode of Action	Cell cycle	PPB/CDZ establishment	metaphase/anaphase
preventing AF polymerization	Cytochalasin D	binds to AFs preventing polymerization	BY-2 cells, 100 μ M for 4 h (0.5 h after the release from propyzamide): 1/3	onion cells, 200 μ M 30 min: no effect on PPB formation	carrot suspension cells, 10 μ M : often causes the spindle axis to reorient
AF depolymerization drugs	Latrunculin B	prevents actin monomers from polymerizing	premitotic BY-2 cells, 1 μ M : arrested cytokinesis BY-2 cells, 0.2 μ M supply during		
	Swinholide A /bistheonellide (BA)	disrupts AFs, reversible			
	Jasplakinolide	stabilizes actin oligomers that can serve as extendable nuclei for polymerization	not commonly used in dicots	wheat protoplasts, 30 nM: AF bundles	BY2 cells, 30 nM: PPB not altered
induction of AFs polymerization					
MT polymerization	Oryzalin	highly dynamic MTs are more sensitive to oryzalin than stable MT arrays	Tobacco cell culture, 15-30 μ M: arrests cells at metaphase, high degree of DNA-doubling Tobacco cell	Arabidopsis root, 5 μ M: disassembly of the MTs from the PPB within 5min, no effects on the localization CDZ marker	Arabidopsis root, 100-150 nM: Spindle assembly checkpoint mutants sensitive

preventing N	Colchicine	sequestration via tubulin–colchicine complex formation	Tobacco cell culture, 0.5 mM : induces a 20 % arrest in mitosis Tobacco cell culture, 5 mM : 10	mM concentrations : block plant mitosis	
MT depolymerization drugs	Amiprophosmethy I (APM)	depolymerizes MTs, partial recovery after washout	Potatoes, 15-32 µM: arrests the cells in metaphase, micro-nucleated cells		
	Propyzamide	inhibits the polymerization of tobacco tubulin in vitro, no effect for animal tubulin	BY-2 cells, 2µM : arrests the cell cycle at metaphase	effect reversible (within 2h) and can be used to synchronized plant cells. treatment short (less than 14h), otherwise leading to abnormal cell division	tobacco cell culture, 50 µM in prometaphase : prevents the formation of microtubule-kinetochore attachments
	Chlorpropham	Triticum roots, 50µM = disappearance of all MTs of dividing cells	wheat roots, 50 µM: polyploid nuclei and incomplete walls 8h after washout		in onion root tip cells and in carrot suspension cells, 100 µM for 23h: formation of tripolar spindles
	Trifluralin	induces a minus-end specific MT depolymerization			<i>Allium cepa</i> meristematic cells, 0.42-1.67 ppm: multipolar anaphases
MT stabilizing drug	Paclitaxel (Taxol)	stabilizes MTs and promotes their assembly	low sensitivity of <i>P. patens</i> , mitotic arrest after taxol treatment		tobacco cells arrested in metaphase, 50µM for 10min:recruitment of BUBR1 to the MTs kinetochore 10 µM

Targets of both MTs and AFs	Bisphenol A (BPA)	estrogen-like chemical		Zea mays, 20 mg/L: no effects Zea mays: 100 mg/L for 3h: asymmetric unilateral PPB without perinuclear MTs	
	SMIFH2	Inhibitor of Formin FH2			
General disrupter of cytokinesis	Methylxanthines (Caffeine)	antagonist of adenosine A2A receptors			

cytokinesis	References
	[17, 38, 98]
maize root apex, 10 μ M, 12h = Increased oblique cell plates	[20, 21, 99-102]
BY-2 cells : 10% decrease in the cell plate expansion rate at the early phase and a 25% at the late phase synchronized BY2-cells, 1–10h : division planes appeared disordered (60%).	[103, 104]
BY2 cells, 30 nM: formation of triple peaks in approximately 32% of cells Microasterias, 1.5 μ M : development resumed, but the cell symmetry is disturbed	[24, 105]
	[32, 101-104]

destruction of the phragmoplast MT, arrest of vesicle trafficking toward the cell plate	[110, 111]
	[112]
	[29, 34]
in carrot suspension cells, 100 μ M for 23h: Y-shaped AF-containing phragmoplasts Allium root tips, 1 mM for 2 h: formation of highly branched and contorted cell plates	[35, 37]
Toxoplasma, micromolar concentrations: totally blocked cytokinesis	[113, 114]
BY-2 cells, 20 μ M : increases the accumulation of vesicles at the equatorial plane of the phragmoplast, inhibition of the phragmoplast expansion Arabidopsis seedlings, 0.5 and 1 μ M: sensitivity for tangled mutant	[29, 34, 115, 116]

<p>In BY2 cells: 50-100 mg, 3h : anomalous phragmoplasts (10% decrease in the cell plate expansion rate at the early phase of cytokinesis, 25% at the late phase)</p> <p>In BY2 cells, pretreatment for 1h with 1 μM: malformation of the emerging cell plate, which appears discontinuous</p>	<p>[117]</p>
<p>In BY2 cells, 5 μM for five days: slows down the phragmoplast expansion and caused cell plate swelling</p>	<p>[41, 42, 44]</p>
<p>In Arabidopsis, Low-dose caffeine abolishes MAP65-3 from the midzone in the hypersensitive bub3 mutants</p> <p>Tradescantia stamen hair system, 5 mM: no consolidation of the plate</p> <p>synchronized BY2 cells, 10 mM for 1 h : no stabilizing callose layer and the thin tubule membrane network fragments into vesicles</p>	<p>[45, 118]</p>