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The Arp2/3 regulatory system and its deregulation in cancer

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41 **ABSTRACT**

42

43 The Arp2/3 complex is an evolutionary conserved molecular machine that generates branched
44 actin networks. When activated, the Arp2/3 complex contributes the actin branched junction
45 and thus cross-links the polymerizing actin filaments in a network that exerts a pushing force.

46 The different activators initiate branched actin networks at the cytosolic surface of different
47 cellular membranes in order to promote their protrusion, movement or scission in cell
48 migration and membrane traffic. Here we review the structure, function and regulation of all
49 the direct regulators of the Arp2/3 complex that induce or inhibit the initiation of a branched
50 actin network and that controls the stability of its branched junctions. Our goal is to present
51 recent findings concerning novel inhibitory proteins or the regulation of the actin branched
52 junction and place these in the context of what was previously known in order to provide a
53 global overview of how the Arp2/3 complex is regulated in human cells. We focus on the
54 human set of Arp2/3 regulators to compare normal Arp2/3 regulation in untransformed cells
55 to the deregulation of the Arp2/3 system observed in patients affected by various cancers. In
56 many cases, these deregulations promote cancer progression and have a direct impact on
57 patient survival.

58

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82 I. INTRODUCTION

83

84 The Arp2/3 complex is a major actin nucleating molecular machine, which is conserved
85 in eukaryotes. This is the only molecular machine that generates branched actin networks.
86 The Arp2/3 complex was first discovered as a multiprotein complex containing actin-related
87 proteins (160) and rediscovered as an actin nucleating machine (270). Ever since, the Arp2/3
88 complex has been associated with many functions, which we review here as we describe the
89 regulators of Arp2/3 activity. A flurry of recent papers have reported novel regulators of the
90 Arp2/3 complex, with, for example, several inhibitory proteins or regulators of the actin
91 branched junction. The major goal of this review is to integrate these new findings into
92 established knowledge of Arp2/3 regulation in order to provide a global overview of the
93 Arp2/3 regulatory system.

94 The reader can directly focus on his or her molecule of interest, which are classified
95 according to the type of regulation they provide to the Arp2/3 complex (Fig.1). The
96 monograph of each regulator is structured to describe, in this order, its molecular structure, its
97 main cellular function, and then how it is regulated in the normal mammalian cell and
98 deregulated in the cancer cell. We believe that this review organization can help the
99 newcomer to enter in this profuse field.

100 Deregulation of the Arp2/3 regulatory system in cancer has been described over the
101 years. Examination of all these examples highlights the fact that over activation of the Arp2/3
102 complex generally promotes cancer progression (Table 1). However, we will also describe
103 notable exceptions, and attempt to provide an explanation as to why these anomalies still lead
104 to cancer. The topic of the Arp2/3 complex and cancer has been less frequently covered than
105 the hijacking of the Arp2/3 complex by pathogens, which has been nicely reviewed recently
106 (271), and will not be addressed here. This topic also justifies focusing on the human genes
107 and gene products, and hence to use the consensus human nomenclature to describe their
108 activities.

109

110 II. THE ARP2/3 COMPLEX

111

112 A. The canonical Arp2/3 complex

113

114 The Arp2/3 complex is a stable multiprotein complex of 7 subunits, with a total mass of
115 about 250 kDa. Two of these subunits are Actin related proteins, Arp2 and Arp3. The crystal
116 structure of the complex has revealed an inactive conformation, where Arp2 and Arp3 are
117 maintained far apart in the architecture provided by the 5 other subunits, named ARPC1 to 5
118 (Fig.1) (200). The Arp2/3 complex creates branched actin networks (16). In the active
119 conformation, the Arp2/3 complex contributes a branched junction of two actin filaments,
120 whose structure was revealed by electron microscopy (208, 257). This active conformation
121 involves bringing together Arp2 and Arp3 within the complex, so that these two subunits

122 adopt the conformation of actin molecules within a filament (Fig.1). This conformation,
123 however, can also be detected in a population of soluble Arp2/3 complexes, in the absence of
124 any actin. The population of Arp2/3 complexes displaying the active conformation is greatly
125 increased by Nucleation Promoting Factors (NPFs), such as N-WASP or WAVE (82, 203).
126 The conformationally activated Arp2/3 complex can then interact with a pre-existing actin
127 filament, often referred to as the mother filament, in order to initiate the elongation of a lateral
128 branch, the daughter actin filament (204). The widely used Arp2/3 inhibitory compound, CK-
129 666, binds Arp2 and Arp3 and blocks their conformational rearrangement that is required for
130 Arp2/3 activation (13, 95).

131 The function of the Arp2/3 complex is to induce an explosive actin polymerization in
132 response to signaling pathways. Because actin filaments are both substrates (mother) and
133 products (daughter) of the branching reaction, the process has been described as autocatalytic
134 and indeed generates an exponential increase of actin filaments (2). The resulting branched
135 actin networks, also referred to as dendritic actin networks, have been demonstrated to
136 generate a pushing force in vitro (153, 292). In the cell, the various NPFs are anchored at the
137 surface of different membranes and the force they generate through the generation of
138 branched actin networks remodel these membranes to perform various cellular functions. For
139 example, the pushing force of branched actin networks allows the plasma membrane to
140 protrude in fan-like migration structures called lamellipodia (241) and to promote scission of
141 clathrin coated pits during endocytosis (42, 61).

142

143 B. Novel complexities in the Arp2/3 complex

144

145 Further complexity has recently been added to this general scheme. In the human
146 genome, the ARPC1 subunit is encoded by two paralogous genes, ARPC1A and ARPC1B,
147 and the ARPC5 subunit by ARPC5 and ARPC5L. The complexes containing ARPC1B or
148 ARPC5L promote actin polymerization more efficiently than the ones containing the
149 alternative paralogous subunits (1). The Arp2/3 activity is also regulated by phosphorylation
150 of its subunits (140, 169). The phosphorylation of Arp2 by the serine threonine protein kinase
151 NIK is required for the Arp2/3 activity (141). We are probably only starting to decipher this
152 complex Arp2/3 regulation through phosphorylations.

153 Strikingly, even the most fundamental property of the Arp2/3 complex, i.e. its ability to
154 generate branched actin networks, has recently been found to admit exceptions. SPIN90, a
155 protein of the WISH/Dip1 family with a conserved role in endocytosis (14, 126, 185), has
156 been shown to induce actin polymerization without the need of a pre-existing filament. The
157 SPIN90-Arp2/3 complex is in the active conformation and allows direct actin elongation from
158 the rearranged Arp2-Arp3 template (258). Such a process normally does not take place with
159 regular NPFs, since the NPF needs to dissociate from the rearranged Arp2/3 complex and the
160 activated Arp2/3 needs to bind to a preexisting mother actin filament, in order to elongate an
161 actin filament from the rearranged Arp2/3 (224). Linear actin filaments are also likely
162 nucleated by hybrid complexes containing some, but not all, subunits of the Arp2/3 complex.

163 These hybrid complexes contain Arp2 and Arp3, which template actin filaments, but lack
164 ARPC1, ARPC4 and ARPC5, which mediate many contacts with the mother filament (37).

165 The hybrid complexes contain vinculin or vinculin and α -actinin, which target these
166 hybrid Arp2/3 complexes to focal adhesions (37). A previously reported interaction between
167 vinculin and the Arp2/3 complex probably reflects these hybrid complexes rather than
168 vinculin transiently interacting with the whole Arp2/3 complex (49). It is likely that these
169 hybrid complexes contribute to actin polymerization at focal adhesions. However,
170 understanding the role of hybrid Arp2/3 complexes awaits specific ways to inhibit their
171 assembly in the cell, given that their constituent subunits are either subunits of the Arp2/3
172 complex or structural components of focal adhesions. Similarly, studying the molecular
173 mechanism, by which these hybrid complexes generate linear actin filaments, awaits their
174 purification, which is likely to be challenging given their low abundance. Moreover,
175 reconstitution of the activity of hybrid Arp2/3 complexes may require elaborate in vitro
176 systems, to reproduce the mechanosensitive behavior of vinculin in focal adhesions (38, 78).

177

178 C. The Arp2/3 complex in cancer

179

180 Arp2/3 subunits have been found by immunohistochemistry to be overexpressed in a
181 variety of cancers, including lung (216), breast (109), gliomas (152), gastric (303) and
182 colorectal cancers (110, 189). Since the Arp2/3 subunits assemble into a complex, the
183 presence of one subunit is likely indicative of the whole complex. For example, similar
184 staining were obtained with Arp2 and Arp3 antibodies in serial sections (189). However, a
185 free pool of ARPC1B with a role in centrosomal homeostasis has also been reported (177).
186 Three publications reported simultaneous overexpression of Arp2 and of one of its NPFs,
187 WAVE2, in the same discrete cells of lung carcinomas (216), of breast carcinomas (109) and
188 of colorectal carcinomas (110), indicating that, within tumors, which are often heterogeneous,
189 overexpression of both the Arp2/3 complex and of WAVE2 corresponds to a coordinated
190 program. Double positive cells were frequent at the invading front of breast and colorectal
191 carcinomas (109, 110).

192 In breast cancer cell lines and in mammary tumors obtained in a mouse model, the
193 invasive carcinoma cells were found to overexpress genes encoding Arp2/3 subunits (261,
194 262). In physiological 3D migrations of cancer cells, either reconstituted in vitro, or through
195 intravital imaging in the grafted animal, protrusions along extracellular matrix fibers are more
196 elongated than the typical fan-shaped lamellipodia observed in 2D cultures, but they
197 nevertheless require the Arp2/3 complex (80, 261, 262, 286). Arp2/3 overexpression is tightly
198 associated with cancer progression and tumor cell invasion. In patient biopsies, the
199 overexpression of the Arp2/3 complex was more pronounced in high grade invasive colorectal
200 carcinomas and was predictive of liver metastasis (110, 189). Arp2/3 mediated migration of
201 colorectal carcinoma cells is not only important to seed metastases, but also to ensure their
202 tumor growth through the co-optation of pre-existing blood vessels (67). Arp2/3
203 overexpression was associated with poor patient survival in lung (216) and breast cancers
204 (109). Arp2/3 overexpression in breast cancer is associated with HER2 overexpression, and,

205 in breast carcinoma cell lines that overexpress HER2, the therapeutic monoclonal antibody
206 trastuzumab, which inhibits HER2 signaling, blocks lamellipodia formation and tumor cell
207 invasion (294).

208 Other very invasive cancers exhibit Arp2/3 overexpression. Arp2/3 overexpression is a
209 marker, out of a set of only 5, used to distinguish malignant melanomas from benign nevi
210 (119). In pancreatic cancer, the two genes encoding the two paralogous ARPC1 subunits are
211 overexpressed (138). These two genes are localized in the 7q21-q22 region, which is
212 frequently amplified in this cancer (138). As in other cell models, it was verified in pancreatic
213 cancer cell lines that RNAi mediated depletion of the Arp2/3 complex decreases migration
214 and invasion (199).

215 For a stable multiprotein complex like the Arp2/3 complex, overexpression of one
216 subunit at the mRNA level may not be sufficient to produce more complex. Levels of
217 expression of most subunits are not limiting and subunits that are not assembled are thought
218 to be degraded (51). In breast cancer, however, Arp2 protein, and thus the Arp2/3 complex, is
219 correlated to the Arp2 mRNA level (110), indicating either that the level of Arp2 is limiting
220 or that all subunits are up-regulated in a coordinated manner in order to assemble more
221 Arp2/3 complexes. Levels of mRNA measured by quantitative PCR should systematically be
222 complemented by immunohistochemistry, since carcinoma cells of the tumor are not always
223 the cell type that displays the overexpression. For example, the Arp2/3 complex is also
224 overexpressed in cancer-associated fibroblasts in colorectal cancer (189). Interestingly,
225 overexpression in stromal cells is also associated with cancer progression toward the invasive
226 stage of the carcinoma cells, in a cross-talk between stromal and carcinoma cells.

227

228 III. THE NUCLEATION PROMOTING FACTORS

229

230 Arp2/3 activators are called Nucleation Promoting Factors, or NPFs in short. They are
231 characterized by their C-terminal domain that contains three short peptide motifs, a WH2, a
232 Connector motif, and an Acidic end, in this order (Fig.2). This characteristic C-terminus is
233 referred to as the WCA, or sometimes VCA for historical reasons. The CA binds to the
234 Arp2/3 complex and induces its conformational activation. The WH2 motif binds to one
235 globular actin molecule and delivers it to the rearranged Arp2/3. Both events are required to
236 initiate an actin branch (193). In NPFs, there are sometimes more than one WH2 motif. The
237 N-terminus vary considerably between NPFs. The N-terminus has a regulatory role. It
238 determines how the WCA, the Arp2/3 activatory region, is masked in an inactive
239 conformation at resting state, and how, in response to activatory signals, the WCA is going to
240 be exposed to activate the Arp2/3 complex.

241 The domains present in the N-terminus define the different families of NPFs (Fig.2),
242 which activate the Arp2/3 complex at various subcellular functions to perform different
243 functions. There are 4 families of NPFs in the human genome, WAVE, N-WASP, WASH and
244 WHAMM families.

245

246 A. The WAVE family

247

248 The WAVE family of NPFs consists of 3 WAVE proteins, sometimes also referred to as
249 Scar proteins. WAVE2 is the ubiquitous one, WAVE1 and 3 being more restricted in their
250 tissue expression (234). All WAVE proteins are embedded into a stable multiprotein complex,
251 also containing ABI, CYFIP, NAP1 and BRK1 for a total of 5 subunits (58, 76, 107, 230,
252 231). All of them, except BRK1, are encoded by paralogous genes yielding to a combinatorial
253 complexity in the assembly of WAVE complexes (51). Moreover, some subunits, like ABI1,
254 are alternatively spliced. Functional specializations of isoforms have been demonstrated in a
255 couple of cases (57, 236). The WAVE1 complex has been crystallized (36). The output WCA
256 domain is masked by its interaction with other subunits of the complex. The small GTPase
257 Rac, the most established activator of the WAVE complex, interacts with the CYFIP1 subunit
258 and releases the masked WCA (36, 51).

259 The WAVE complex localizes at the edge of lamellipodia, where new actin molecules
260 incorporate into dense branched actin networks beneath the plasma membrane (88, 136, 232).
261 The WAVE complex localizes to the plasma membrane through its interaction with the
262 phospholipid PIP3 or with membrane receptors (35, 186). Knock-down and knock-out
263 experiments have established that the WAVE complex is a critical Rac effector for
264 lamellipodium formation (171, 229, 230, 289, 290) (Fig.3). In addition to Rac, many
265 phosphorylations are important to control WAVE complex activity (132). The major function
266 of the WAVE complex, downstream of the small GTPase Rac, is to control cell migration,
267 and especially persistent directional migration (132).

268 Protrusion of the plasma membrane appears coordinated with intracellular traffic. The
269 clathrin heavy chain has been demonstrated to interact with the WAVE complex and to
270 promote its activation at the lamellipodium tip in an endocytosis-independent manner (75).
271 Clathrin-mediated endocytosis is indeed not detected in lamellipodia. In contrast, the exocyst
272 component Exo70 favors membrane protrusion by promoting the WAVE-Arp2/3 interaction
273 (150, 307). A shut-down in endocytosis and an activation of exocytosis contribute an excess
274 of membrane, and thus a release of membrane tension. Such a drop in membrane tension
275 rapidly induces actin polymerization, formation of a lamellipodium and restoration of
276 membrane tension (101, 146, 147, 198).

277 In fibroblasts, the Rac—WAVE—Arp2/3 pathway is critical for haptotaxis, i.e.
278 migration up a gradient of immobilized fibronectin, in line with the fact that lamellipodia are
279 adherent membrane protrusions (127, 273). Whether this pathway is also involved in
280 chemotaxis is controversial (240, 273). In epithelial cells, the Rac—WAVE—Arp2/3 pathway
281 has also been involved in the formation and maintenance of cell-cell junctions (256, 288).
282 Branched actin networks are prerequisites for the development of junctional tension through
283 myosin contractility (255). Neogenin is a transmembrane protein of the junction that directly
284 recruits the WAVE complex and promotes its activation by Rac at the junction (142).
285 Through the interplay between lamellipodia and cell-cell junctions, the WAVE complex
286 regulates collective migration of epithelial cells (181).

287 In transformed cells of human origin, lamellipodia are usually less prominent than in
288 untransformed cells (Fig.4). Nonetheless, many reports indicate an important role of the
289 WAVE complex in cell migration and tumor cell invasion. In breast invasive cell lines, for
290 example, WAVE3 is required for lamellipodium formation and cell invasion through
291 transwell filters (227). These in vitro results were however challenged (228). It is most of the
292 time unclear where discrepancies come from. The extreme plasticity of tumor cell migration,
293 where the Rac—WAVE—Arp2/3 contributes only the so-called mesenchymal migration is
294 probably part of the explanation (6, 210). HER2 overexpression induces WAVE2 and
295 WAVE3 expression (248, 294). WAVE3 is stabilized within the WAVE complex and
296 destabilizing the complex with peptides forming interfaces between subunits results in
297 reduced invasion of carcinoma cell lines in vitro (247, 249). WAVE3 depletion reduced the
298 ability of breast carcinoma cell lines to generate lung metastasis in experiments, where
299 genetically modified cell lines were grafted into immunocompromised mice (243, 248).

300 In cancer, components of the WAVE complex are overexpressed and this
301 overexpression is associated with poor patient prognosis (Table 1). All 3 WAVE proteins
302 were found to be overexpressed in carcinomas of various origins, of the breast (62, 109, 135),
303 of the colon (110, 302), of the liver (111, 291), of the lung (216), of the ovary (299), of the
304 prostate (63, 64). In all these cases, but one, the overexpression of WAVE proteins is
305 associated with reduced survival and prognosis markers of poor survival, such as high grade,
306 lymph node invasion and metastases. The only exception is a study, which reports that
307 WAVE3 overexpression is associated with better prognosis and better markers in colorectal
308 carcinomas (302). This is in stark contrast to the overexpression of WAVE3 in breast and
309 liver carcinomas, which is a predictor of poor outcome (111, 135).

310 Similarly, for the other WAVE complex subunits, the general trend is an overexpression
311 associated with decreased survival. This was reported for NAP1 in breast carcinomas (154),
312 for its hematopoietic homologue HEM1 in leukemia (115), for ABI1 in breast and ovary
313 carcinomas (259, 298). In lung carcinomas, the overexpression of the small subunit BRK1 is
314 also associated with markers of poor prognosis, such as lymph node invasion and high grade
315 (27). The BRK1 gene is located next to the tumor suppressor gene VHL. Large deletions that
316 affect BRK1 at the same time as the VHL tumor suppressor protect patients from developing
317 renal cell carcinomas, emphasizing the importance of the WAVE complex for cancer
318 progression (60).

319 However, two studies contradict this trend. The first one is the report of loss-of-function
320 mutations of ABI1 in prostate cancer (283). This observation is supported by the appearance
321 of prostate neoplasia in conditional ABI1 knock-out mice in the same study. The second study
322 at odds with the general trend is the report of CYFIP1 downregulation in multiple carcinomas
323 including breast, lung and colon cancers (221). Using mice engraftments with genetically
324 modified cell lines, this study shows that CYFIP1 suppresses tumor invasion, rather than
325 promotes it, as one could expect from the majority of cancer studies. Despite the causal
326 relationships that these mouse models provide, it must be stressed that the importance of these
327 two studies have not yet been confirmed with retrospective cohorts of patients.

328 There are 36 ways to assemble pentameric WAVE complexes with the 11 genes that
329 encode subunits. It is likely that all paralogous genes do not encode equivalent subunits in
330 terms of activity or regulation. It is also likely that functional specializations are only manifest
331 in some, but not all, tissues. What remains to be established, however, is whether the few
332 studies at odd with all the others reveal that a few specific compositions of WAVE complexes
333 inhibit, rather than activate, actin polymerization and cell migration.

334

335

336 B. The N-WASP family

337

338 The WASP family is composed of two paralogous proteins in the human genome, the
339 ubiquitous N-WASP and the hematopoietic WASP. N-WASP is auto-inhibited, since its
340 WCA domain is masked by its own N-terminal WH1 domain (124). Even though this
341 autoinhibition can be observed with N-WASP alone, N-WASP is normally complexed to one
342 of the WIP family proteins and this heterodimeric complex is more tightly autoinhibited than
343 N-WASP in isolation (51, 96, 97). The WIP family contains WICH and CR16 in addition to
344 WIP. The small GTPase Cdc42 is the best characterized activator of N-WASP in vitro (51).
345 To activate the N-WASP/WIP complex, however, the Cdc42 signal must be transduced
346 through proteins of the (TOCA1, FBP17, CIP4) family, which contain a F-BAR domain (96)
347 and a binding site for active Cdc42. N-WASP binds to both Cdc42 and these F-BAR
348 containing proteins.

349 The major functions of N-WASP are at the plasma membrane, like the WAVE complex.
350 However, in untransformed cells, the contribution of N-WASP to membrane protrusions
351 appears limited, despite clear Cdc42 activation at the leading edge (182). The N-WASP
352 knock-out fibroblasts generate normal lamellipodia and filopodia (155, 225). When N-WASP
353 was directly compared to WAVE2, both proteins were found enriched at the leading edge
354 protrusions, but RNAi mediated depletion of WAVE2 yielded a more severe migration defect
355 than N-WASP depletion (122). In fact, the role of N-WASP might be restricted to early
356 adhesions to the extracellular matrix, as it is best revealed in cell spreading assays. N-WASP
357 is important for adhesion to fibronectin and to the development of vinculin positive focal
358 adhesions (173). Upon cell spreading, N-WASP enters in a complex with the Focal Adhesion
359 Kinase, FAK (300). FAK also directly binds to the Arp2/3 complex through its FERM
360 domain (217). In vitro, the FERM domain of FAK potentiates the actin polymerization
361 induced by the Arp2/3 complex and the WCA domain of N-WASP. N-WASP stability
362 requires both the formation of its native complex with WIP and β 1 integrin (128). Indeed,
363 upon β 1 integrin depletion, N-WASP is destabilized and its proteasomal degradation can be
364 rescued by WIP overexpression. However, after an early involvement of the Cdc42—N-
365 WASP pathway, lamellipodia seem rather to depend on the Rac—WAVE pathway.

366 The most conserved role of N-WASP at the plasma membrane is during endocytosis
367 (246, 268). N-WASP, but not WAVE, is specifically detected at the clathrin coated pit (15,
368 167). N-WASP recruitment at this location relies on BAR domain containing proteins, which

369 induce and/or sense membrane curvature (108, 253). BAR domain containing proteins couple
370 direct membrane remodeling with Arp2/3-mediated actin polymerization (233, 277). The
371 force generated by branched actin networks appears to elongate the pit into a pronounced
372 invagination and to compress the collar of the invagination (42, 61). This sequence of events
373 promotes membrane scission, which eventually involves the GTPase dynamin. Actin
374 polymerization and dynamin-mediated scission are intimately coupled (61, 245).
375 Nevertheless, the role of actin polymerization in endocytosis is important, but dispensable in
376 standard conditions (15). Actin polymerization becomes critical, when high membrane
377 tension must be counteracted (18, 121).

378 In transformed cells, in contrast, N-WASP becomes important to generate protrusions.
379 In addition to lamellipodia, transformed cells generate invadopodia that degrade the
380 extracellular matrix by localized delivery of the MT1-MMP metalloprotease (192).
381 Invadopodia are easily recognizable ventral protrusions on 2D substrates that coexist with
382 peripheral lamellipodia (Fig.4). They cannot be distinguished, however, from leading edge
383 protrusions in 3D substrates. N-WASP is present and activated in invadopodia (156).
384 Invadopodia depend on the N-WASP/WIP complex (72, 73, 176, 287). In grafted animals,
385 breast invasive cells required N-WASP to invade, intravasate into blood circulation and to
386 generate lung metastasis (81). Two N-WASP activators, Cdc42 and the F-BAR containing
387 protein CIP4, are present in invadopodia and are required for tumor cell invasion (12, 191,
388 206). This is in contrast with clathrin coated pits, which depend on F-BAR containing
389 proteins, but not on Cdc42, and with lamellipodia, which depend on Cdc42, but not on the F-
390 BAR containing proteins. Specific upstream signaling is thus responsible for N-WASP
391 recruitment and activation in different subcellular structures. In vitro, in 3D collagen gels,
392 RNAi mediated depletion of the WAVE complex induces N-WASP dependent cell invasion
393 (242), indicating a modified interplay between the two major NPFs of the plasma membrane
394 in cancer cells compared to normal cells.

395 In patients, N-WASP is not systematically overexpressed during cancer progression. In
396 fact, N-WASP is overexpressed in pancreatic ductal adenocarcinomas (87), in lung cancer
397 (68) and in hepatocellular carcinomas (114). But N-WASP displays overall normal levels in
398 esophageal squamous cell carcinomas (263) and even down-regulation in breast carcinomas
399 (165). In pancreatic ductal adenocarcinomas and in hepatocellular carcinomas, high N-WASP
400 levels are associated with risk factors and decrease overall survival. In esophageal squamous
401 cell carcinomas, despite the overall normal levels, high N-WASP, within its natural
402 fluctuations of expression, is also associated with high grade carcinomas and lymph node
403 invasion. Overall, as expected from its important role in invadopodia formation, N-WASP
404 promotes cancer progression, but breast cancer appears as an exception. In breast cancer, N-
405 WASP is down regulated, instead of up regulated, and this down-regulation is associated with
406 poor prognosis (165). Consistent with the exception of breast cancer, the N-WASP activator
407 CIP4 is overexpressed in mammary carcinomas and associated with poor prognosis, but this
408 effect is independent from N-WASP (206). In gliomas, the N-WASP partner WIP promotes
409 cancer progression by stabilizing the YAP/TAZ transcription factors (74).

410

411 C. The WASH family

412

413 The WASH family in the human genome displays a variable number of genes, from 15
414 to 20 depending on individuals (149). This is due to the fact that the WASH gene is located in
415 a subtelomeric region, which is prone to recombination. The genes were so recently
416 duplicated, however, that, in practice, one can deplete all WASH proteins with single siRNA
417 sequences, like any other gene product. In mice and rats, there is a single WASH gene. The
418 WASH protein is embedded into a stable multiprotein complex (54, 83). This stable
419 multiprotein complex is distantly related to the WAVE complex, with a one-to-one
420 correspondence of the 5 subunits (113). Most subunits are encoded by single genes, and so the
421 WASH complex is less diverse than the WAVE complex (52). Strikingly, however, the
422 WASH complex recruits a pre-existing complex, which also exists on its own in the cytosol,
423 the so-called capping protein (CP) (54). CP is in fact a heterodimer, which blocks the
424 elongation of actin filaments. CP interaction has been shown to be important for WASH
425 function in the amoeba *Dictyostelium discoideum* (190), but this is not yet understood why
426 these two activities of actin nucleation and actin capping have to be coordinated in the same
427 multiprotein complex.

428 The WASH complex activates the Arp2/3 complex at the surface of endosomes (54,
429 83). It is recruited to endosomes through its subunit FAM21, which contains multiple binding
430 sites for the retromer (90, 93, 112). Importantly, the WASH complex and the retromer do not
431 cover the whole surface area of endosomes, but rather define a microdomain, whose area is
432 controlled by actin polymerization itself (53). The polymerization of branched actin is thought
433 to promote membrane scission of transport intermediates that contain sorted receptors, in a
434 manner similar to N-WASP during clathrin mediated endocytosis (77). The retromer performs
435 sorting of endosomal cargoes that are either destined to follow the retrograde route to the
436 trans-Golgi network or which are recycled to the plasma membrane (215). The WASH
437 complex is activated through interaction with the phospholipid PI4P (56) and through
438 ubiquitination of WASH using a K63 linkage, a modification which does not target
439 conjugated proteins to the proteasome (89).

440 In transformed cells, WASH promotes tumor cell invasion function through the sorting
441 of endosomal cargoes it provides. The WASH dependent recycling of $\alpha 5 \beta 1$ integrins to the
442 plasma membrane drives invasion of ovarian carcinoma cells in fibronectin containing 3D
443 matrix (296). In breast carcinoma cells, the WASH complex interacts with the exocyst and
444 contributes to the focal delivery of the metalloprotease MT1-MMP to invadopodia (178). This
445 event requires transient tubular connexions between late endosomes and the plasma
446 membrane. In cancer patient biopsies, there has been little examination of the WASH
447 complex so far. The WASH complex subunit Strumpellin is overexpressed in high grade
448 prostatic carcinomas, due to genetic amplification (194).

449

450 D. The WHAMM family

451

452 WHAMM and JMY are two homologous proteins, which share a common modular
453 organization (131, 254). They are composed of an amino-terminal domain, a long central α -
454 helical domain and the carboxy-terminal WCA that activates the Arp2/3 complex (120). JMY
455 has the particularity to have 3 WH2 motifs, which bind actin. These 3 motifs were shown to
456 be sufficient to nucleate actin in vitro in a WH2-only mechanism (305). WHAMM seems to
457 be more expressed in epithelial tissues, such as colon, kidney and lung (29), whereas JMY is
458 enriched in the brain, testis and lymphoid organs (3, 66).

459 They are both localized at the Golgi, cis-Golgi for WHAMM (29), trans-Golgi for JMY
460 (212), and they are both involved in anterograde transport, from ER to cis-Golgi and then
461 from cis- to trans-Golgi cisternae. The role of WHAMM in trafficking involves binding of the
462 N-terminal domain to Rab1 (209) and binding of the central α -helical domain to microtubules
463 (219). Regulations by Rab1 and microtubules allow WHAMM to control the formation of
464 membrane tubules, which are thought to mediate intracellular transport of anterograde cargoes
465 (29, 209, 219).

466 In addition, WHAMM and JMY were both recently involved in autophagy (43, 120).
467 Their N-terminal domain dictate their localization to the ER, from which autophagosomal
468 membranes originate. During autophagosome biogenesis, actin polymerization in a so-called
469 'comet tail' is required to elongate membrane tubules from the ER, independently of
470 microtubules (120). Branched actin networks also shape the autophagosome from the inside
471 (168) and are likely involved in autophagosome closure as well. Indeed, topologically,
472 autophagosome closure is a membrane scission event (130), and NPFs promote membrane
473 scission, as described above for N-WASP and WASH. Connexions between Arp2/3 and
474 autophagy appear numerous. WASH inactivation leads to massive autophagy through
475 elucidated molecular pathways implicating the major regulators Beclin1, RNF2 and
476 AMBRA1 (281, 282). However, the meaning of why WASH should suppress autophagy is
477 not yet clear.

478

479 E. An emerging function of NPFs in gene transcription

480

481 JMY was originally identified as a cofactor for the p300/CBP transcription coactivator
482 (220). JMY translocates to the nucleus, when actin concentration decreases in the cytoplasm
483 and that actin does no longer bind to its cluster of WH2 motifs (304). In the nucleus, the
484 JMY-p300 co-activator complex engages with transcription factors, such as p53 or HIF1 α to
485 induce the transcription of target genes involved in apoptosis or in cell motility (44, 45). The
486 Arp2/3 complex and the other NPFs were also described moonlighting in the nucleus to
487 regulate transcription.

488 N-WASP has a marked effect on gene transcription (279). Actin, the Arp2/3 complex
489 and N-WASP coimmunoprecipitate with the RNA polymerase II, which transcribes most
490 protein encoding genes (279, 295). Nuclear Arp2/3 generates branched actin networks like its
491 cytoplasmic counterpart (295). N-WASP might exert this transcription role through its
492 interaction with the nuclear PSF/NonO complex (279). The tyrosine phosphorylation of N-

493 WASP by Src family kinases (235) and FAK (278) favors its cytoplasmic localization at the
494 expense of its nuclear localization and can thus downregulate its transcriptional role. The
495 extent of the requirement of N-WASP for general gene transcription is, however, questioned
496 by the apparent mild phenotype associated with N-WASP knock-out (155, 225).

497 FAM21, a subunit of the WASH complex, has also been recently implicated in gene
498 transcription (50). FAM21 shuttles back and forth in the nucleus. FAM21 regulate NF- κ B
499 transcription by binding to the p65 subunit of NF- κ B and to the inhibitor of NF- κ B kinases,
500 IKK α , β and γ . This function of FAM21, however, appears relatively independent of the
501 WASH NPF.

502 The generation of nuclear branched actin can also derepress gene transcription through
503 chromatin remodeling. Toca-1, one of the BAR containing activators of N-WASP, is critical
504 to reactivate the pluripotency gene, *Oct4*, in nuclear reprogramming experiments, where
505 somatic nuclei are injected into *Xenopus* oocytes (174). The Rac—WAVE pathway is
506 similarly required (175). In T lymphocytes, the hematopoietic WASP controls the
507 lymphocytic Th1 differentiation program, because Th1 differentiation genes are occluded
508 through methylation of histone H3 in patients affected by Wiskott-Aldrich syndrome, where
509 WASP is defective (244). All together, these various experiments established that nuclear
510 Arp2/3 and NPFs may control gene transcription through both chromatin remodeling and
511 recruitment of the RNA polymerase II.

512

513

514 IV. ARP2/3 INHIBITORY PROTEINS

515

516 Three Arp2/3 inhibitory proteins have recently been reported. The general assumption is
517 that an inhibitor should be diffuse in the cytosol, in order to maintain globally silent a
518 signaling pathway that is locally activated. The surprise is that these Arp2/3 inhibitory
519 proteins are specifically localized at the surface of specific membranes, just like the
520 activators. There is mounting evidence that they counteract specific NPFs.

521

522 A. Arpin

523

524 In a bioinformatics screen for potential Arp2/3 regulators, our group identified an
525 uncharacterized protein, which contained a typical C-terminal acidic A motif, but lacked the
526 required WH2 motif for Arp2/3 activation. In vitro this protein binds to the Arp2/3 complex,
527 but cannot activate it. It thus acts as a competitive inhibitor of NPFs (47) and was called
528 Arpin as a mnemonic for Arp2/3 inhibition. There is a single Arpin gene in the human
529 genome. In vitro Arpin constitutively exposes its C-terminal acidic tail, which inhibits the
530 Arp2/3 complex (65). When bound to Arpin, the Arp2/3 complex is in the inactive
531 conformation, where Arp2 and Arp3 are far apart (226).

532 In the cell, Arpin localizes at the lamellipodial edge, i.e. where branched actin is
533 nucleated by the WAVE complex. Arpin's ability to interact with the Arp2/3 complex depends
534 on Rac activity, which induces lamellipodia (47). Indeed, by single molecule imaging, Arp2/3
535 complexes from the lamellipodial edge were not all activated and incorporated into the
536 branched actin network that undergoes retrograde flow. Some Arp2/3 complexes were seen to
537 move laterally in the plane of the plasma membrane (172) (Fig.3). The Arp2/3 complexes are
538 likely maintained inactive by Arpin at this location. Arpin is an inhibitor of cell migration
539 (84). Without Arpin, lamellipodia protrude for longer, and, as a consequence, sustain active
540 directional migration.

541 Consistent with an inhibitory role on cell migration, Arpin was found to be down-
542 regulated during breast cancer progression. In two independent retrospective cohorts of breast
543 cancer patients, Arpin down-regulation, documented at both mRNA and protein levels, was
544 associated with lymph node invasion and decreased survival (151, 154). The most powerful
545 prognosis factor, however, is obtained when patients having tumors displaying Arpin down-
546 regulation are combined with patients having tumors displaying up-regulation of the WAVE
547 complex (154). Patient analyses thus confirm the specific antagonism between Arpin and the
548 WAVE complex deduced from the analyses of cell migration in vitro.

549

550 B. Gadkin

551

552 Gadkin, also known as γ -BAR, is another protein that interacts with the Arp2/3 complex
553 through an acidic motif (163). The AP1AR gene, which encodes Gadkin, has no paralog in
554 the human genome. Gadkin localizes at steady state at the surface of endosomes (213).
555 Gadkin regulates the trans Golgi network—endosomal traffic by entering in a complex with
556 the kinesin KIF5 and the clathrin adaptor AP-1 (213). Its function and localization are thus
557 most similar to the ones of the WASH complex among the different NPFs. In Gadkin knock-
558 out cells, the Arp2/3 complex associated with endosomes appear to polymerize more F-actin
559 (211). It is thus logical to assume that Gadkin maintains the Arp2/3 complex in an inhibited
560 conformation, like Arpin. However, in in vitro assays of Arp2/3 activity, purified Gadkin does
561 not inhibit actin nucleation (163), suggesting that Gadkin's activity is not yet properly
562 reconstituted in such in vitro assays. It is tempting to speculate that Gadkin might antagonize
563 the WASH complex at the surface of endosomes, but this hypothesis awaits the demonstration
564 that they both regulate the endosomal sorting of the same receptors.

565 Gadkin has been shown to be overexpressed in breast cancer, but this overexpression is
566 not associated with patient prognosis (154).

567

568 C. PICK1

569

570 PICK1 is a protein containing a PDZ and a BAR domain. PICK1 is encoded by a single
571 gene, which is ubiquitously expressed with an enrichment in the brain (144). The PDZ

572 domain of PICK1 connects it to numerous membrane receptors and transporters, the most
573 studied of which is the AMPA receptor of the glutamate neurotransmitter. The BAR domain
574 dimerizes and induces or senses curved membranes through its banana-shaped membrane
575 binding interface (161, 233). The C-terminal part of the molecule contains an acidic motif that
576 binds and inhibits the Arp2/3 complex (202). This inhibitory activity was reported to be
577 regulated by the GTPase Arf1 (201).

578 PICK1 regulates AMPA receptor trafficking, and in particular its clathrin-dependent
579 endocytosis (9, 202). The role of PICK1 is thus most consistent with an antagonism with N-
580 WASP at the clathrin coated pits. However, the situation is more complex than previously
581 thought, since the BAR domain of PICK1 recognize mostly vesicles derived from the trans
582 Golgi network and inhibition of the Arp2/3 complex *in vitro* was not reproduced in a recent
583 study (161). PICK1 regulates long-term depression and long-term potentiation of synapses
584 (125, 201, 250). These effects are mediated by expansion or contraction of post-synaptic
585 structures called dendritic spines. Dendritic spines are actin-rich membrane structures,
586 perhaps equivalent to a mini-lamellipodium except that filaments emanating from a central
587 branched actin network are laterally elongated, thus allowing the expansion of the spine (33).
588 Like in the lamellipodium, the central branched actin network is nucleated by the Rac—
589 WAVE—Arp2/3 pathway (34). PICK1 controls the activity-regulated size of dendritic spines
590 (8). Consistent with an inhibitory role, PICK1 depletion expand spines, whereas its
591 overexpression induce spine shrinkage (180). In glial cells, PICK1 might antagonize WAVE
592 rather than N-WASP, because PICK1 depletion increases branching complexity of astrocytes,
593 whereas WAVE2 depletion decreases it, unlike N-WASP depletion (179).

594 In cancer as well, the role of PICK1 is complex. In gliomas, PICK1 expression is down-
595 regulated in aggressive astrocytic tumors (41). In contrast, in the breast, PICK1 was reported
596 to be overexpressed in tumors of poor prognosis (297). PICK1 overexpression in breast
597 cancer was, however, not observed in a second independent cohort (154).

598

599 V. BRANCH REGULATORS

600

601 A. Cortactin

602

603 The cortactin family is composed of two members, the ubiquitous cortactin protein and
604 HS1, which is hematopoietic-specific and thus less studied. Cortactin interacts with actin
605 filaments through its specific cortactin repeats and with the Arp2/3 complex through an acidic
606 motif, which has the particularity to be located in the N-terminus of the protein (Fig.5).
607 Cortactin was previously proposed to be a NPF, because it does activate the Arp2/3 complex
608 *in vitro* at high concentration. However, cortactin strongly synergizes with NPFs, because it
609 acts after WCA induced branching nucleation by inhibiting debranching (265, 266). Cortactin
610 thus competes for Arp2/3 binding with the NPF and by doing so favors NPF detachment
611 (223). NPF detachment is a prerequisite for the elongation of a daughter filament from an

612 Arp2/3 complex that has landed onto a mother filament (59, 94, 224). Cortactin then remains
613 bound to the Arp2/3 at the actin branch and prevents it from debranching (25) (Fig.6).

614 In cells, cortactin is a good marker of branched actin networks, because it labels them
615 all along their length and independently of their subcellular location (116, 267). In contrast,
616 NPFs are specific for each organelle and are only localized at the interface between the
617 organelle and the branched actin structure. By FRAP, cortactin was seen to undergo
618 continuous exchange along the length of lamellipodial actin network (136). Further in vivo
619 evidence that cortactin associates with Arp2/3 at the branch include double immunogold
620 labeling of lamellipodial actin networks by electron microscopy (25). Elongation of actin
621 filaments induces the retrograde movement of Arp2/3 complexes that have landed on a
622 mother filament. This movement extracts the NPF from the membrane displaying it.
623 Inactivating the Arp2/3 complex or blocking actin dynamics indeed freezes the turnover of
624 NPFs and induces a striking accumulation of WAVE, WASP or WASH at the surface of their
625 respective membrane (53, 172, 269). It remains to be tested, however, if cortactin
626 overexpression prevents NPF extraction that ensures the turnover measured by FRAP.

627 Cortactin has been discovered as a tyrosine-phosphorylated protein in cells transformed
628 by the Src oncogene (274, 275). It has since been demonstrated to be a substrate of numerous
629 tyrosine kinases, of both receptor and non-receptor types (19, 46, 71, 102, 118). Cortactin is
630 also phosphorylated on serine residues by MAP kinases (28) and PAK1, the well-known
631 effector of Rac and Cdc42 pathway (86, 92). It is not known if these modifications
632 specifically regulate the branch regulatory function of cortactin, but it does signal to
633 cytoskeleton regulatory proteins. Tyrosine phosphorylation controls cortactin association with
634 cofilin and its activation (162, 188). Cortactin phosphorylation also regulates its ability to
635 bind and activate N-WASP, through its C-terminal SH3 domain (166). Cortactin is subjected
636 to other types of post-translational modifications, such as acetylation, which prevents its
637 binding to actin filaments and decreases cell migration (301).

638 Cortactin was found to promote lamellipodial persistence and cell motility in
639 transformed cells (21, 123). However, this is likely not a direct effect of its branch stabilizing
640 activity, but rather a signaling role of cortactin, since cortactin knock-out fibroblasts have no
641 visible defects in lamellipodial ultrastructure and only modest alterations of migration
642 parameters (137). Strikingly, the defects of cortactin depleted cells can be rescued by
643 adhesion to the matrix deposited by control cells, suggesting that cortactin have defects in
644 depositing extracellular matrix (239). Invadopodia, the major protrusions of cancer invasive
645 cells, depend on cortactin, in addition to their specific NPF, N-WASP. Cortactin is a structural
646 component of the branched actin networks that induce invadopodial protrusions and its
647 activation by phosphorylation is critical for this process (10, 11, 40, 55). Intracellular traffic
648 controls the focal secretion of the metalloproteases of the invadopodia and cortactin favors
649 secretion, in addition to its role in creating the protrusion (10, 40, 99, 129). Exosome
650 secretion, which is important for communication between cancer cells and between cancer
651 cells and cancer-associated cells from their microenvironment, critically depends on
652 cortactin (222).

653 Cortactin is overexpressed in many cancers (Table 2). The CTTN gene encoding
654 cortactin is located on the 11q13 genomic region, which is frequently amplified during cancer
655 progression, especially in head and neck squamous cell carcinoma (HNSCC) and in breast
656 cancer (214). In HSNCC, cortactin overexpression, due to CTTN amplification, is associated
657 with cancer progression, invasion of lymph nodes, and recurrence (98, 205, 207). In HNSCC
658 cell lines, cortactin overexpression promotes cell growth in vitro and tumor growth in
659 xenografts (39, 252). Cortactin overexpression favors cell growth independent of growth
660 factors, of anchorage and mediates resistance to contact inhibition, which are three major
661 hallmarks of transformation. In patients, cortactin overexpression is associated with high
662 grade tumors, metastases and poor survival in squamous cell carcinomas from the larynx,
663 from the esophagus and from the oral cavity (7, 79, 157, 237, 285). In breast cancers,
664 cortactin is also overexpressed, but this overexpression was consistently found not to be
665 associated with patient prognosis (48, 104, 218). Nevertheless, in xenografts, cortactin
666 expression enhances the ability of a breast carcinoma cell line to metastasize to the bones
667 (143). These results are not as paradoxical as it might seem: Cortactin can be involved in the
668 transformation process and in cancer progression, and not be associated with a poor
669 prognosis, if the overexpression occurs most frequently in breast cancer subtypes, which are
670 not as aggressive, as the one associated with the poorest patient survival. Cortactin
671 overexpression has been associated with high grade tumors, but not yet with poor survival, in
672 ovarian carcinomas (148), in non small cell carcinomas of the lung (184), in gliomas (260).
673 Cortactin has been associated with both high grade tumors and poor survival in colorectal
674 cancer (22, 183), in gastric cancer (264), in prostate cancer (100), in melanoma (284) and in
675 hepatocellular carcinoma (103).

676

677 B. Coronin

678

679 There are 7 coronins in human, which are characterized by a so-called β -propeller
680 structure made of WD40 repeats (31). Here we will discuss only the type I coronins, among
681 which Coro1B and Coro1C are ubiquitous and Coro1A hematopoietic-specific. At the C-
682 terminus, a coiled-coil domain mediates homo and hetero-trimerization of coronins (117).
683 Coronins are binding to both the Arp2/3 complex and actin filaments. In all type I coronins, a
684 binding site for actin filaments is in the β -propeller, but Coro1C possesses a second filament
685 binding site in a unique region (31, 32). The site binding to the Arp2/3 complex is in the N-
686 terminus of coronins. Arp2/3 binding is regulated by the phosphorylation of two serines in the
687 N-terminus and C-terminus. Phosphomimetic mutations of these sites strongly decrease
688 Arp2/3 binding (23, 280).

689 In vitro, coronin enhances Arp2/3 debranching and both binding sites are required for
690 this effect (24, 25). An antagonism between cortactin and coronin has thus been proposed to
691 control the debranching rate of branched actin networks (25). Coronin does not stain the
692 branched actin structures to their top where new branches are formed, indicating that coronin
693 acts at a later stage than cortactin (25). However, one has to understand why in vivo coronin
694 is a good marker of branched actin networks at lamellipodia, as well as at the surface of

695 endosomes (26, 197). If the only role of coronin was to debranch, coronin should not stay in
696 the branched actin network. Recent data explain the conundrum that coronin remains
697 associated with the branched actin structure that it remodels: Coronin would not have the
698 same effect on the Arp2/3 complexes depending on their precise composition in paralogous
699 subunits (1). The Arp2/3 complexes containing ARPC1A and/or ARPC5 would indeed be
700 debranched by coronin, whereas the most efficient Arp2/3 complexes containing ARPC1B
701 and/or ARPC5L would be immune to coronin-induced debranching (Fig.6). Coronin could
702 thus mediate partial debranching of the branched actin networks and at the same time be a
703 good marker of the branched actin structures.

704 The serine 2 of Coronin 1B has been shown to be phosphorylated by PKCs in response
705 to factors that induce actin polymerization and cell motility, such as platelet-derived growth
706 factor (PDGF) or phorbol myristate acetate (PMA) (23, 272). These observations are
707 consistent with a shut-down of Arp2/3 debranching, when branched actin networks are
708 triggered. Depletion of coronin, however, alters lamellipodial dynamics and impairs cell
709 migration as a consequence (26). These effects might only be partially due to Arp2/3
710 debranching. Indeed, coronin recruits to branched actin networks the phosphatase slingshot
711 that activates cofilin by dephosphorylation (26). Coronin, together with cofilin, promotes the
712 disassembly of branched actin structures, when the actin filaments age (20, 69, 134). Through
713 its activities of Arp2/3 debranching and actin disassembly, coronin appears to down-regulate,
714 rather than promote, branched actin networks.

715 Coronin 1C is overexpressed in gliomas (251), in hepatocellular carcinomas (276),
716 gastric cancers (238), lymphomas (159). When it has been examined, coronin overexpression
717 is associated with high grade tumors and poor survival for patients. It might seem at odds that
718 a type I coronin, which globally down-regulates branched actin networks, might be
719 overexpressed in cancers, while the Arp2/3 complex is also overexpressed. However, coronin
720 activities in favoring debranching and actin depolymerization might be important to replenish
721 available pools of actin molecules and Arp2/3 complexes for continuous actin polymerization
722 and sustained cell movements (26, 272).

723

724 C. GMF

725

726 There are two Glia-Maturation Factors (GMF) proteins encoded in humans, GMF β
727 encoded by the GMFB gene and GMF γ encoded by GMFG. GMFB is expressed ubiquitously
728 with an enrichment in the brain (91), whereas GMFG is mostly expressed in hematopoietic
729 cells and endothelial cells (105). These two homologous proteins belong to the same
730 superfamily as cofilin, but define a subfamily of their own (85, 196). GMFs are cofilins,
731 which have evolved to bind to Arp2, instead of actin. GMF uses this Arp2 binding site to
732 insert itself in between Arp2 and the actin molecule and thus severs the branch in a way
733 similar to cofilin severing actin filaments (70, 158, 293). GMF preferentially recognizes Arp2,
734 when it is bound to ADP (17). ATP hydrolysis by Arp2 is not required for branched actin
735 nucleation, since it occurs after branched actin nucleation (106, 139, 164). As a consequence
736 ATP hydrolysis by Arp2 acts as a timer, indicating that the actin branch has aged, the same

737 way ATP hydrolysis by the actin molecules in a filament indicates the older portion of the
738 filament. Cofilin also severs ADP-actin filaments more efficiently than younger ATP-actin
739 filaments (30, 170, 187). GMF binding to Arp2 is regulated by phosphorylation of its serine at
740 position 2 (105), just like cofilin (5).

741 It is not yet established whether coronin and GMF provide two independent means of
742 debranching branched actin networks for increased robustness or whether they collaborate to
743 perform efficient debranching together. GMF and coronin may play sequential roles in the
744 molecular reaction of Arp2/3 debranching. Indeed, once GMF has severed the connexion
745 between the Arp2/3 complex and the daughter filament, the Arp2/3 complex is still interacting
746 with the mother actin filament and coronin has been shown to detach such landed Arp2/3
747 complexes from filaments (25).

748 In the cell, GMF depletion alters lamellipodial dynamics by decreasing disassembly of
749 branched actin networks (91, 195). The consequence on cell migration is not dramatic, but
750 can be seen in instances, where migration is finely regulated. Depletion of GMF γ from
751 neutrophils impairs their chemotaxis towards inflammatory cytokines (4), whereas depletion
752 of GMF β from fibroblasts impairs their haptotaxis towards immobilized extracellular matrix
753 (91).

754 GMF β is overexpressed in gliomas, because it is expressed by tumor cells and
755 endothelial cells of new capillaries feeding the tumor (133). Endothelial cells within the
756 glioma thus express GMF β , instead of GMF γ for normal vessels. GMF β overexpression in
757 glioma-associated endothelial cells has been reported to be a better prognosis factor than
758 GMF β overexpression in tumor cells (133). Both GMF β and GMF γ are overexpressed in
759 ovarian cancers (145, 306). As we have seen for coronin, the overexpression of a branch
760 destabilizer might promote the turnover of branched actin networks.

761

762 VI. CONCLUSION

763

764 In about 20 years, the Arp2/3 complex has been implicated in a variety of processes. It
765 is now clear that the branched actin networks and the pushing force that they generate are
766 major means that cells use to remodel their plasma membrane in cell migration as well as
767 their internal membranes in membrane traffic. In many instances, the *in vivo* role of Arp2/3
768 regulators has been reconstituted *in vitro* and these endeavors gave rise to in depth
769 understanding of molecular and cellular functions of Arp2/3 regulation. To go back and forth
770 from the molecular level in appropriate reconstitutions to the cellular level, where molecular
771 dynamics can also be addressed using techniques such as FRAP, has allowed to identify and
772 rule out artifactual molecular behaviors sometimes observed *in vitro* in conditions far from
773 the cell physiology.

774 Here we have attempted to organize the various Arp2/3 regulators in systems. The NPFs
775 divide the labor of generating the diverse branched actin structures of the cell. The
776 combinatorial complexity in assembling NPF complexes imposes to study the repertoire of

777 paralogous subunits expressed in each cell system. Similar complexity emerged at the level of
778 the different Arp2/3 complexes, which might themselves be differentially regulated by the
779 different NPF complexes. Similarly, the identification of different Arp2/3 inhibitors revealed
780 that Arp2/3 inhibition is not global, as a means to maintain the system silent in the cytosol,
781 but the inhibitory proteins rather balance Arp2/3 activation at the surface of different cell
782 membranes. Similarly, regulators of the Arp2/3 branched junction fine-tune the ultrastructure
783 of branched actin networks and their role appear mostly in highly regulated physiological
784 processes. We begin to unravel the exquisite regulations of the Arp2/3 complex in these
785 various situations.

786 Deregulation of the Arp2/3 regulatory system in cancer offers an opportunity to
787 understand its logic. If one sets aside a couple of reports at odds with the majority of
788 publications, one can see that the Arp2/3, the WAVE and the N-WASP complexes are overall
789 overexpressed in many cancers. These overexpressions are usually associated with poor
790 prognosis for patients. The other NPFs more specifically involved in intracellular trafficking
791 like WASH, WHAMM or JMY, are not, or not yet, involved in cancer progression. Down-
792 regulation of Arpin is consistent with its inhibitory role. The situation is not yet clear for
793 Gadkin and PICK1. As for the branch regulators, the stabilizer cortactin and destabilizers,
794 coronin and GMF, are all clearly overexpressed with an overall association with high grade of
795 tumors and poor prognosis for the patients. These simple observations emphasize the notion
796 that branched actin networks have to turnover fast to perform their function. The deregulation
797 of the Arp2/3 regulatory system in cancer suggests that small molecule inhibitors of branched
798 actin dynamics might provide a therapeutic benefit to control cancer progression.

799

800

801

802

803 **Figure legends**

804

805 **Figure 1:** Conformations of the Arp2/3 complex and regulators of the equilibria between
806 them. On the left, the fully inactive Arp2/3 complex displays a conformation, where Arp2 and
807 Arp3 are far apart. On the right, the fully activated Arp2/3 complex has nucleated an actin
808 filament from the side of a pre-existing filament. The middle conformation corresponds to a
809 rearranged Arp2/3 complex, where Arp2 and Arp3 have been brought into proximity and
810 mimics the end of an actin filament. This conformation allows side binding to an actin
811 filament. Regulatory factors are represented above and below the equilibrium arrows. CK-666
812 and CK-869 are two small molecule inhibitors of Arp2/3 activation (95), which are widely
813 used in cell biology experiments.

814

815 **Figure 2:** Modular organization of Arp2/3 activatory and inhibitory proteins. Their division
816 of labor at different subcellular localizations is indicated. All activators contain a C-terminal
817 WCA domain, which binds and activates the Arp2/3 complex, whereas all inhibitors contain
818 an acidic motif (A), which binds to the Arp2/3 complex and competes with WCA-containing
819 activators. When the activators were described to form a stable complex, partner subunits are
820 depicted. An inhibitory protein that would antagonize WHAMM activatory proteins remains
821 to be identified. SHD: Scar/WAVE homology domain; B: Basic domain; P : Proline-rich
822 region; WH1: WASP homology 1; CRIB: CDC42 and Rac1 Interactive Binding region; AI:
823 Auto-Inhibition domain; WAHD1: WASH homology Domain 1; WMD: WHAMM
824 Membrane-interacting Domain; CC : Coiled coiled; PDZ : PSD95-Dlg1-ZO1 domain; BAR:
825 Bin-Amphiphysin-Rvs domain.

826

827 **Figure 3:** WAVE and Arpin control the duration of branched actin polymerization at the
828 cortex. Polymerization is triggered by Rac activation, i.e. its exchange of the bound GDP
829 nucleotide for a GTP nucleotide. GTP-bound Rac then directly recruits the WAVE complex at
830 the plasma membrane. Activated WAVE binds and activates several Arp2/3 complexes,
831 which are represented with different blue intensities. Each activated Arp2/3 complex
832 nucleates an actin branch that undergoes a retrograde movement due to actin filament
833 elongation (white arrows). This scheme represents molecular processes in the referential of
834 the cell membrane and not of the substratum: either the membrane is fixed and the branched
835 actin undergoes a retrograde flow, or the branched actin structure is fixed, due to a coupling
836 with cell adhesion structures, and the plasma membrane protrudes. Rac controls the
837 recruitment of Arpin with a delay, after the recruitment of the WAVE complex. Arpin
838 molecules locally bind Arp2/3 complexes and block their activation of Arp2/3 complexes.
839 The Arp2/3 complexes, which move laterally in the plane of the plasma membrane (white
840 arrows), are probably the ones bound to Arpin molecules.

841

842 **Figure 4:** Deregulation of the Arp2/3 regulatory system in cell transformation. In normal
843 cells, N-WASP, WASH, WHAMM and WAVE nucleate branched actin network at the
844 clathrin-coated pit, at the surface of endosomes, of the ER/Golgi and at the lamellipodial
845 edge, respectively. The Arp2/3 inhibitory proteins, Arpin, Gadkin and PICK1, locally
846 antagonize WAVE, WASH and N-WASP at their respective locations. During cell
847 transformation, N-WASP is overexpressed and forms invadopodia. In invadopodia, WASH
848 promotes focalized delivery of metalloproteases that degrade the extracellular matrix. In
849 cancer cells, WAVE is overexpressed and Arpin down-regulated, despite frequent abnormal
850 lamellipodia. Font size indicate the level of expression.

851

852 **Figure 5:** Modular organization of proteins that regulate Arp2/3 branches. Cortactin stabilizes
853 the Arp2/3 branches, whereas GMF and coronin destabilize them. R: cortactin Repeats; P =
854 Proline-rich region; SH3: Src Homology 3 domain; CC = Coiled Coil ; ADF-H = ADF/cofilin
855 Homology domain.

856

857 **Figure 6:** Regulation of the actin junctions branched by the Arp2/3 complex. Cortactin binds
858 the Arp2/3 complex at the branch and blocks actin debranching. GMF recognizes the Arp2/3
859 complex in the context of the branch and severs the branch. Coronin destabilizes the actin
860 branches formed by ArpC1A/ArpC5 containing Arp2/3 complexes. In contrast, the actin
861 branches formed by ArpC1B/ArpC5L containing Arp2/3 complexes resist coronin's effect,
862 thus explaining why coronin is a good marker of branched actin networks, like cortactin.

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878 **REFERENCES**

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Figure 1

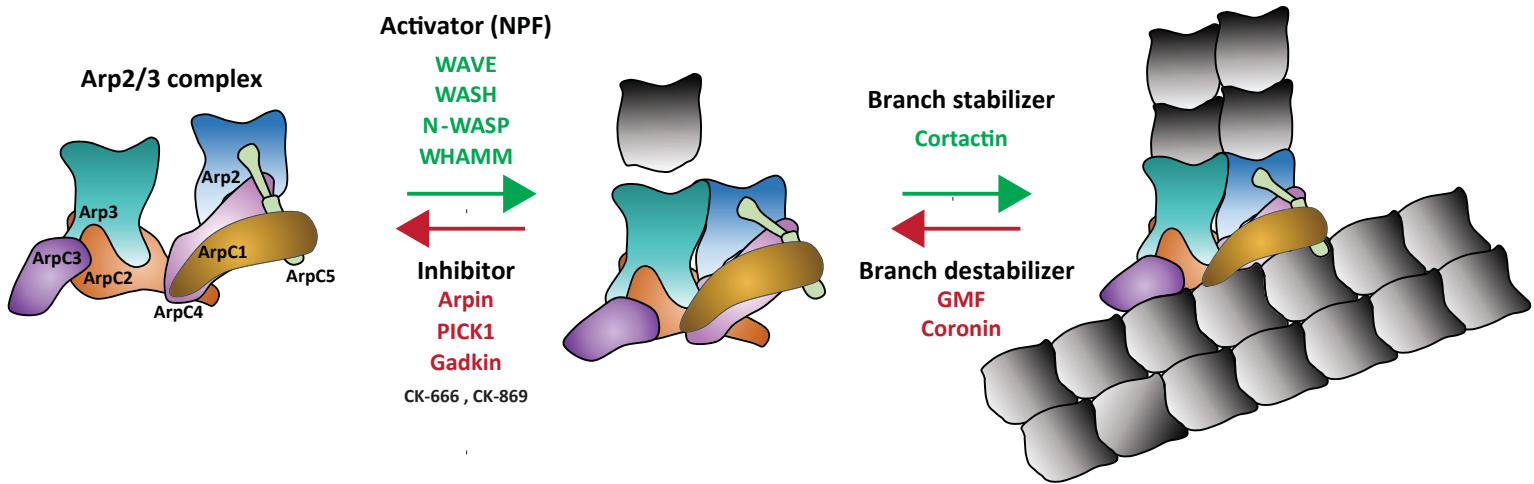


Figure 2

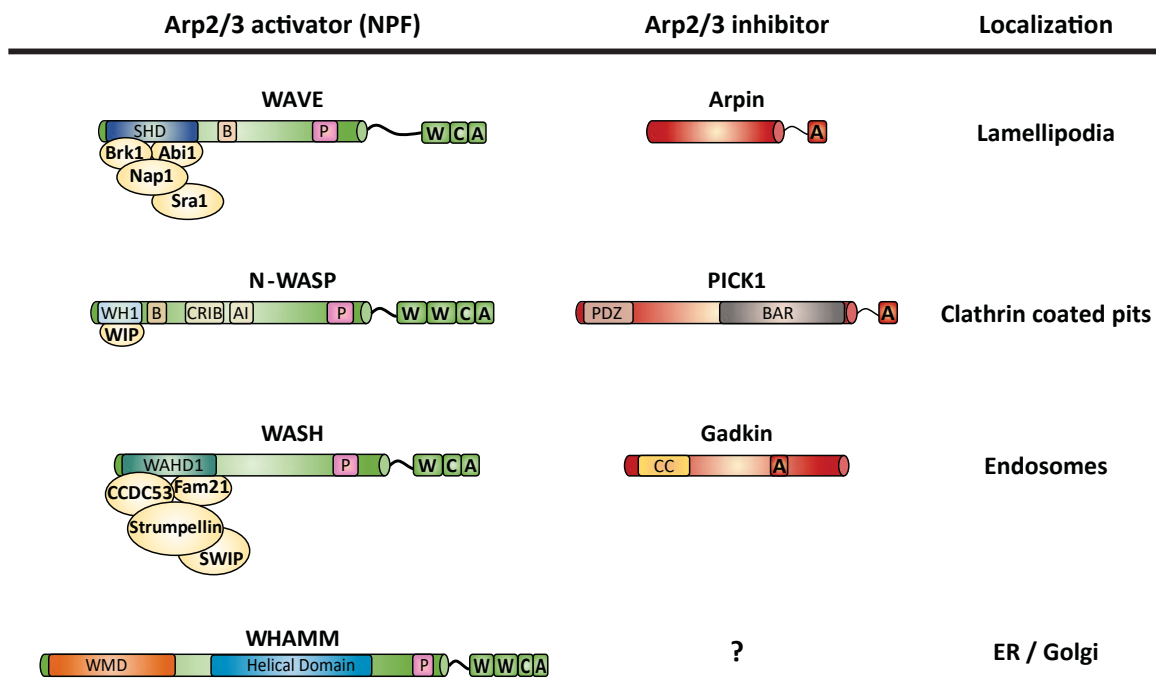


Figure 3

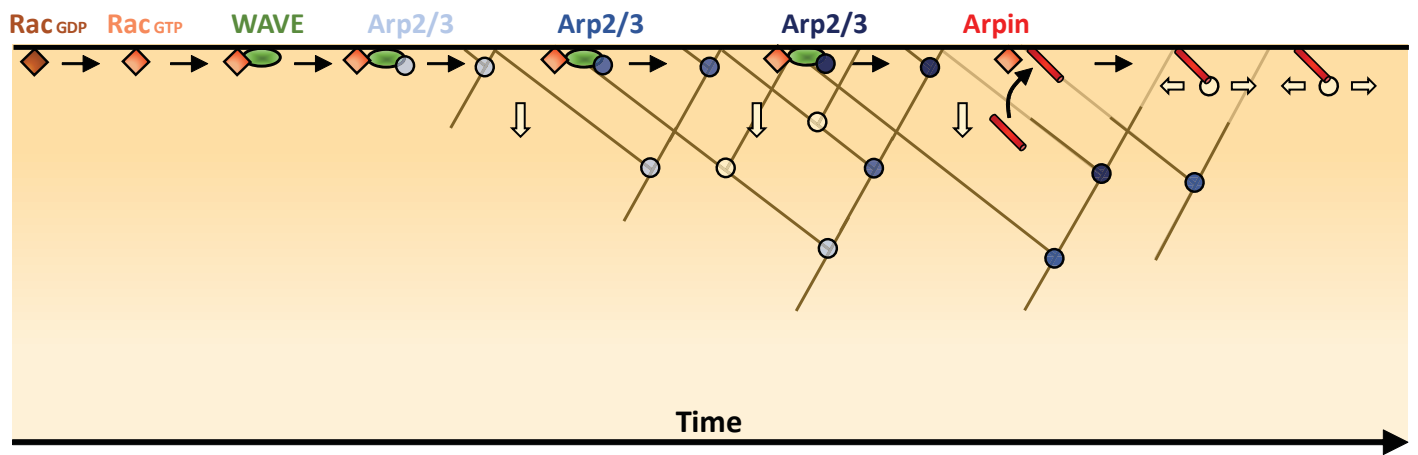


Figure 4

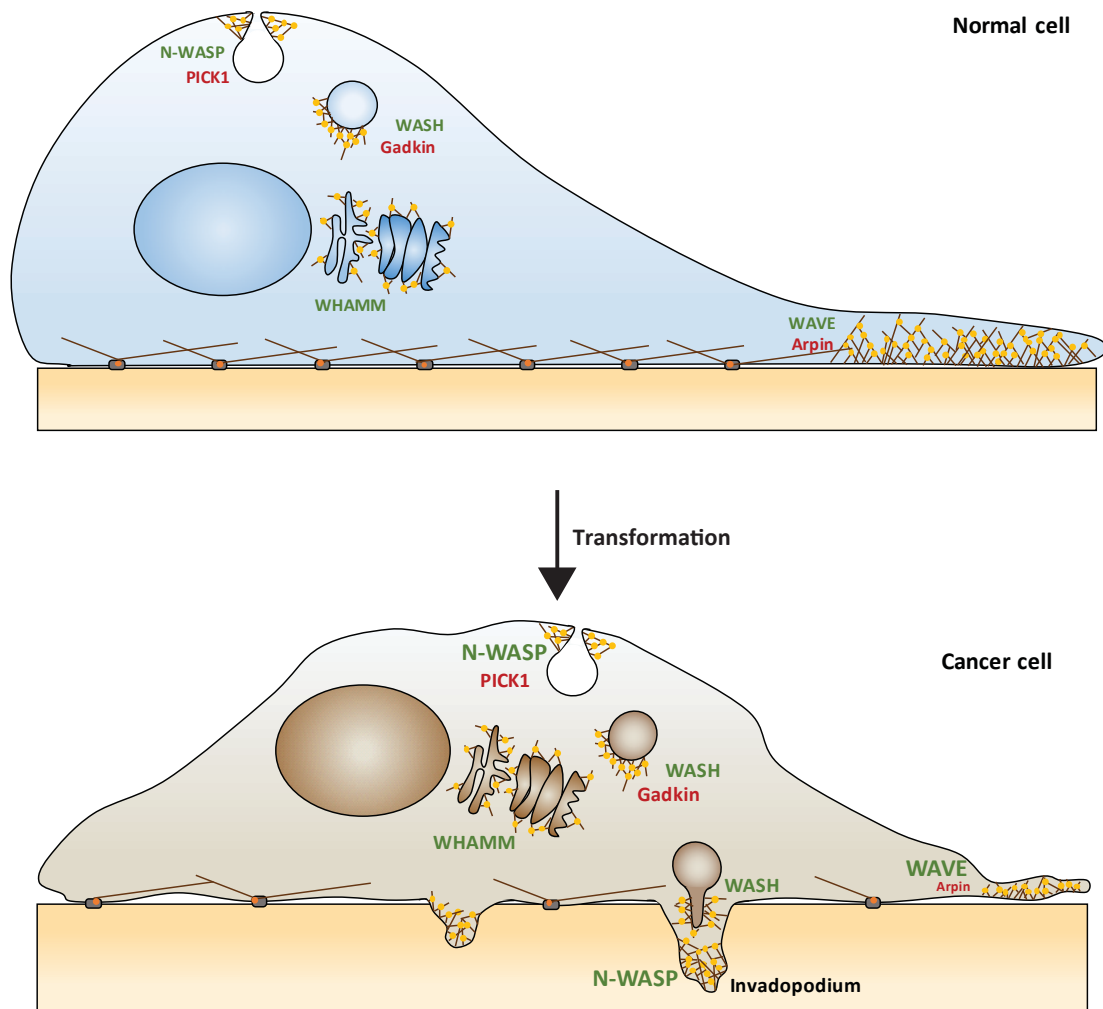


Figure 5

Branch regulators

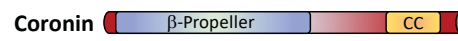
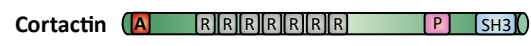


Figure 6

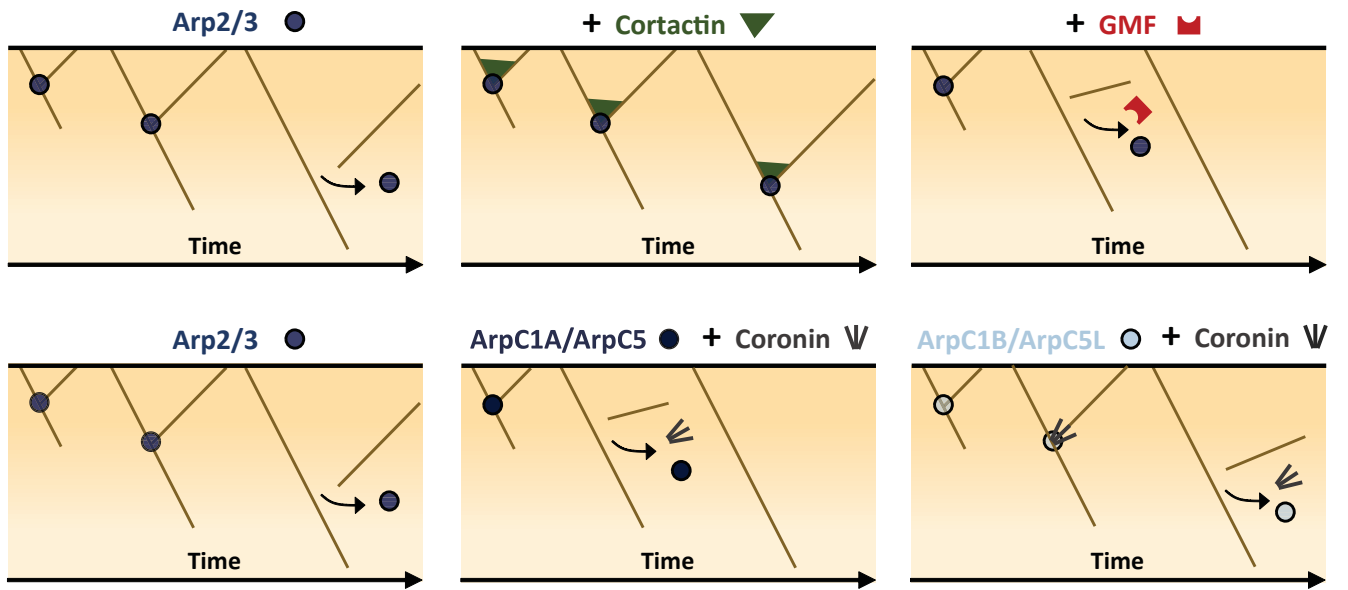


Table 1: Deregulation of the Arp2/3 system in association with cancer stage and patient prognosis.

	Protein name	Gene name	DNA	mRNA	Protein	Cancer	Overall deregulation	Association with stage	Survival prognosis	Ref.
Arp2/3 complex	ARP2	ACTR2			✓	Breast	↗	lymph node, grade	↘	Iwaya et al., 2007
					✓	Lung	↗	lymph node	↘	Semba et al., 2006
					✓	Colorectal	↗	Liver metastases	nd	Iwaya et al., 2007
					✓	Colorectal	↗	invasive stage, stromal expression	nd	Otsubo et al., 2004
					✓	Stomach	↗	stages, depth of invasion	nd	Zheng et al., 2008
					✓	Stomach	↗	stages	↘	Sun et al., 2014
	ARP3	ACTR3			✓	Colorectal	↗	invasive stage, stromal expression	nd	Otsubo et al., 2004
					✓	Gastric	↗	stages, depth of invasion	-	Zheng et al., 2008
ARPC1A	ARPC1A		✓		Pancreas	↗	nd	nd	Laurila et al., 2009	
ARPC1B	ARPC1B		✓		Pancreas	↗	nd	nd	Laurila et al., 2009	
ARPC2	ARPC2			✓	Glioma	↗	high grade	nd	nd	Liu et al., 2013
				✓	Melanoma	↗	nd	nd	nd	Kashani-Sabet et al., 2009
N-WASP complex	N-WASP	WASL			✓	Pancreas	↗	perineural invasion	↘	Guo et al., 2014
				✓		Lung	↗	lymph node	↘	Frugtniet et al., 2017
					✓	Liver	↗		↘	Jin et al., 2012
				✓	✓	Esophagus	-	lymph node invasion	-	Wang et al., 2010
				✓	✓	Breast	↘		↘	Martin et al., 2008
WAVE complex	WAVE1	WASF1			✓	Ovary	↗	high grade	↘	Zhang et al., 2012
					✓	Prostate	↗		nd	Fernando et al., 2008
	WAVE2	WASF2		✓		Breast	↗	high grade	↘	Fernando et al., 2007
					✓	Breast	↗	lymph node, grade	↘	Iwaya et al., 2007
					✓	Lung	↗	lymph node	↘	Semba et al., 2006
					✓	Colorectal	↗	Liver metastases	nd	Iwaya et al., 2007
			✓	✓	✓	Liver	↗	high grade, multiple nodules	↘	Yang et al., 2006
	WAVE3	WASF3		✓	✓	Colorectal	↗	low grade, no lymph node inv.	↗	Zhang et al., 2012
					✓	Breast	↗	tumor size, lymph node	↘	Kulkarni et al., 2012
					✓	Prostate	↗		nd	Fernando et al., 2010
			✓	✓	✓	Liver	↗	high grade, metastases	↘	Ji et al., 2015
	ABI1	ABI1		✓	✓	Ovary	↗	high grade	↘	Zhang et al., 2015
					✓	Breast	↗		↘	Wang et al., 2011
					Prostate			nd	Xiong et al., 2012	
LOF mutations					Breast	↗		↘	Lomakina et al., 2016	
HEM1	NCKAP1L		✓		Leukemia	↗		↘	Joshi et al., 2007	
SRA1	CYFIP1	deletion	✓	✓	Lung, Colon, Breast	↘	high grade	nd	Silva et al., 2009	
BRK1	BRK1			✓	Lung	↗	lymph node, grade	nd	Cai et al., 2009	
Inhibitory proteins	ARPIN	ARPIN		✓	✓	Breast	↘		↘	Lomakina et al., 2016
				✓	✓	Breast	↘		↘	Liu et al., 2016
	GADKIN	AP1AR		✓		Breast	↗		-	Lomakina et al., 2016
	PICK1	PICK1		✓	✓	Breast	↗	lymph node, grade	↘	Zhang et al., 2010
				✓	✓	Breast	-		-	-
		✓			Glioma	↘	high grade	nd	Cockbill et al., 2015	

nd: not determined; LOF: loss of function

Table II: Deregulation of the Arp2/3 branch regulators in association with cancer stage and patient prognosis.

	Protein name	Gene name	DNA	mRNA	Protein	Cancer	Overall deregulation	Association with stage	Survival prognosis	Ref.	
Branch regulators	Cortactin	CTTN	amplification			Head and Neck	↗	lymph nodes, recurrence	↘	Rodrigo et al., 2000	
			amplification		✓	Head and Neck	↗	lymph nodes	nd	Rothschild et al., 2006	
					✓	Head and Neck	↗	high grade	↘	Hofman et al., 2008	
					✓	Larynx	↗		↘	Gibcuset et al., 2008	
				✓	✓	Larynx	↗	high grade	nd	Ambrosio et al., 2011	
					✓	Esophagus	↗	high grade, metastases	↘	Lu et al., 2014	
					✓	Oral	↗	high grade	nd	Yamada et al., 2010	
			amplification		✓	Breast	↗		-	Hui et al., 1998	
			amplification		✓	Breast	↗		-	Dedes et al., 2010	
					✓	Breast	↗		-	Sheen-Chen et al., 2011	
					✓	Colon	↗		high grade	↘	Cai et al., 2010
				✓	✓	Colon	↗		high grade	↘	Ni et al., 2015
					✓	Stomach	↗		high grade, lymph nodes	↘	Wang et al., 2010
					✓	Liver	↗		high grade, metastases	↘	Huang et al., 2012
				✓	✓	Brain	↗		high grade	nd	Wang et al., 2015
					✓	Lung	↗		high grade	nd	Noh et al., 2013
					✓	Ovary	↗		high grade	nd	Linet et al., 2008
		✓	✓	Melanome	↗		high grade	nd	Xu et al., 2009		
			✓	Prostate	↗		high grade	↘	Hou et al., 2012		
	Coronin 1C	CORO1C			✓	Gliomas	↗	high grade	nd	Thal et al., 2008	
					✓	Liver	↗	high grade	nd	Wu et al., 2010	
				✓	✓	Lymphoma	↗		nd	Luan et al., 2010	
	GMFβ	GMFB			✓	Stomach	↗	high grade	↘	Sun et al., 2014	
				✓	Glioma	↗	high grade, neovascularization	↘	Kuang et al., 2016		
GMFγ	GMFG			✓	Ovary	↗	high grade	↘	Li et al., 2010		
				✓	Ovary	↗		↘	Zuo et al., 2014		

nd: not determined