

The Arp2/3 Regulatory System and Its Deregulation in Cancer

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41 ABSTRACT

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

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82 I. INTRODUCTION

83

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

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

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110 II. THE ARP2/3 COMPLEX

Α.

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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 (Fig.1) (200). The Arp2/3 complex creates branched actin networks (16). In the active 118 conformation, the Arp2/3 complex contributes a branched junction of two actin filaments, 119 120 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

The canonical Arp2/3 complex

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 increased by Nucleation Promoting Factors (NPFs), such as N-WASP or WAVE (82, 203). 125 The conformationally activated Arp2/3 complex can then interact with a pre-existing actin 126 filament, often referred to as the mother filament, in order to initiate the elongation of a lateral 127 branch, the daughter actin filament (204). The widely used Arp2/3 inhibitory compound, CK-128 129 666, binds Arp2 and Arp3 and blocks their conformational rearrangement that is required for Arp2/3 activation (13, 95). 130

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 products (daughter) of the branching reaction, the process has been described as autocatalytic 133 and indeed generates an exponential increase of actin filaments (2). The resulting branched 134 135 actin networks, also referred to as dendritic actin networks, have been demonstrated to generate a pushing force in vitro (153, 292). In the cell, the various NPFs are anchored at the 136 surface of different membranes and the force they generate through the generation of 137 138 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 protrude in fan-like migration structures called lamellipodia (241) and to promote scission of 140 141 clathrin coated pits during endocytosis (42, 61).

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- B. Novel complexities in the Arp2/3 complex
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Further complexity has recently been added to this general scheme. In the human 145 genome, the ARPC1 subunit is encoded by two paralogous genes, ARPC1A and ARPC1B, 146 and the ARPC5 subunit by ARPC5 and ARPC5L. The complexes containing ARPC1B or 147 ARPC5L promote actin polymerization more efficiently than the ones containing the 148 149 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 NIK is required for the Arp2/3 activity (141). We are probably only starting to decipher this 151 152 complex Arp2/3 regulation through phosphorylations.

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

These hybrid complexes contain Arp2 and Arp3, which template actin filaments, but lackARPC1, 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 hybrid Arp2/3 complexes to focal adhesions (37). A previously reported interaction between 166 vinculin and the Arp2/3 complex probably reflects these hybrid complexes rather than 167 vinculin transiently interacting with the whole Arp2/3 complex (49). It is likely that these 168 169 hybrid complexes contribute to actin polymerization at focal adhesions. However, understanding the role of hybrid Arp2/3 complexes awaits specific ways to inhibit their 170 assembly in the cell, given that their constituent subunits are either subunits of the Arp2/3 171 172 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).

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C. The Arp2/3 complex in cancer

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

In breast cancer cell lines and in mammary tumors obtained in a mouse model, the 192 invasive carcinoma cells were found to overexpress genes encoding Arp2/3 subunits (261, 193 262). In physiological 3D migrations of cancer cells, either reconstituted in vitro, or through 194 intravital imaging in the grafted animal, protrusions along extracellular matrix fibers are more 195 elongated than the typical fan-shaped lamellipodia observed in 2D cultures, but they 196 197 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 overexpression of the Arp2/3 complex was more pronounced in high grade invasive colorectal 199 200 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,

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

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

215 For a stable multiprotein complex like the Arp2/3 complex, overexpression of one subunit at the mRNA level may not be sufficient to produce more complex. Levels of 216 expression of most subunits are not limiting and subunits that are not assembled are thought 217 to be degraded (51). In breast cancer, however, Arp2 protein, and thus the Arp2/3 complex, is 218 219 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 complemented by immunohistochemistry, since carcinoma cells of the tumor are not always 222 the cell type that displays the overexpression. For example, the Arp2/3 complex is also 223 224 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.

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228 III. THE NUCLEATION PROMOTING FACTORS

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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 referred to as the WCA, or sometimes VCA for historical reasons. The CA binds to the 233 234 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 initiate an actin branch (193). In NPFs, there are sometimes more than one WH2 motif. The 236 N-terminus vary considerably between NPFs. The N-terminus has a regulatory role. It 237 238 determines how the WCA, the Arp2/3 activatory region, is masked in an inactive conformation at resting state, and how, in response to activatory signals, the WCA is going to 239 240 be exposed to activate the Arp2/3 complex.

The domains present in the N-terminus define the different families of NPFs (Fig.2), which activate the Arp2/3 complex at various subcellular functions to perform different functions. There are 4 families of NPFs in the human genome, WAVE, N-WASP, WASH and WHAMM families. 245

A. The WAVE family

246 247

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

and releases the masked WCA (36, 51).

The WAVE complex localizes at the edge of lamellipodia, where new actin molecules 259 incorporate into dense branched actin networks beneath the plasma membrane (88, 136, 232). 260 The WAVE complex localizes to the plasma membrane through its interaction with the 261 phospholipid PIP3 or with membrane receptors (35, 186). Knock-down and knock-out 262 experiments have established that the WAVE complex is a critical Rac effector for 263 264 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).

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

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

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 example, WAVE3 is required for lamellipodium formation and cell invasion through 290 291 transwell filters (227). These in vitro results were however challenged (228). It is most of the time unclear where discrepancies come from. The extreme plasticity of tumor cell migration, 292 293 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 WAVE3 expression (248, 294). WAVE3 is stabilized within the WAVE complex and 295 296 destabilizing the complex with peptides forming interfaces between subunits results in reduced invasion of carcinoma cell lines in vitro (247, 249). WAVE3 depletion reduced the 297 298 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 were found to be overexpressed in carcinomas of various origins, of the breast (62, 109, 135), 302 of the colon (110, 302), of the liver (111, 291), of the lung (216), of the ovary (299), of the 303 prostate (63, 64). In all these cases, but one, the overexpression of WAVE proteins is 304 associated with reduced survival and prognosis markers of poor survival, such as high grade, 305 lymph node invasion and metastases. The only exception is a study, which reports that 306 WAVE3 overexpression is associated with better prognosis and better markers in colorectal 307 carcinomas (302). This is in stark contrast to the overexpression of WAVE3 in breast and 308 liver carcinomas, which is a predictor of poor outcome (111, 135). 309

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

However, two studies contradict this trend. The first one is the report of loss-of-function 319 mutations of ABI1 in prostate cancer (283). This observation is supported by the appearance 320 of prostate neoplasia in conditional ABI1 knock-out mice in the same study. The second study 321 322 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 two studies have not yet been confirmed with retrospective cohorts of patients. 327

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

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336 337 B. The N-WASP family

338 The WASP family is composed of two paralogous proteins in the human genome, the ubiquitous N-WASP and the hematopoietic WASP. N-WASP is auto-inhibited, since its 339 WCA domain is masked by its own N-terminal WH1 domain (124). Even though this 340 autoinhibition can be observed with N-WASP alone, N-WASP is normally complexed to one 341 of the WIP family proteins and this heterodimeric complex is more tightly autoinhibited than 342 343 N-WASP in isolation (51, 96, 97). The WIP family contains WICH and CR16 in addition to WIP. The small GTPase Cdc42 is the best characterized activator of N-WASP in vitro (51). 344 345 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) and a binding site for active Cdc42. N-WASP binds to both Cdc42 and these F-BAR 347 348 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, upon ß1 integrin depletion, N-WASP is destabilized and its proteasomal degradation can be 363 364 rescued by WIP overexpression. However, after an early involvement of the Cdc42-N-WASP pathway, lamellipodia seem rather to depend on the Rac—WAVE pathway. 365

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

induce and/or sense membrane curvature (108, 253). BAR domain containing proteins couple 369 370 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 invagination and to compress the collar of the invagination (42, 61). This sequence of events 372 promotes membrane scission, which eventually involves the GTPase dynamin. Actin 373 polymerization and dynamin-mediated scission are intimately coupled (61, 245). 374 Nevertheless, the role of actin polymerization in endocytosis is important, but dispensable in 375 standard conditions (15). Actin polymerization becomes critical, when high membrane 376 tension must be counteracted (18, 121). 377

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

395 In patients, N-WASP is not systematically overexpressed during cancer progression. In fact, N-WASP is overexpressed in pancreatic ductal adenocarcinomas (87), in lung cancer 396 (68) and in hepatocellular carcinomas (114). But N-WASP displays overall normal levels in 397 398 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 cell carcinomas, despite the overall normal levels, high N-WASP, within its natural 401 402 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 effect is independent from N-WASP (206). In gliomas, the N-WASP partner WIP promotes 408 409 cancer progression by stabilizing the YAP/TAZ transcription factors (74).

411 C. The WASH family

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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 a subtelomeric region, which is prone to recombination. The genes were so recently 415 duplicated, however, that, in practice, one can deplete all WASH proteins with single siRNA 416 417 sequences, like any other gene product. In mice and rats, there is a single WASH gene. The WASH protein is embedded into a stable multiprotein complex (54, 83). This stable 418 multiprotein complex is distantly related to the WAVE complex, with a one-to-one 419 420 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 elongation of actin filaments. CP interaction has been shown to be important for WASH 424 425 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 sites for the retromer (90, 93, 112). Importantly, the WASH complex and the retromer do not 430 431 cover the whole surface area of endosomes, but rather define a microdomain, whose area is controlled by actin polymerization itself (53). The polymerization of branched actin is thought 432 to promote membrane scission of transport intermediates that contain sorted receptors, in a 433 manner similar to N-WASP during clathrin mediated endocytosis (77). The retromer performs 434 435 sorting of endosomal cargoes that are either destined to follow the retrograde route to the trans-Golgi network or which are recycled to the plasma membrane (215). The WASH 436 complex is activated through interaction with the phospholipid PI4P (56) and through 437 438 ubiquitination of WASH using a K63 linkage, a modification which does not target conjugated proteins to the proteasome (89). 439

440 In transformed cells, WASH promotes tumor cell invasion function through the sorting of endosomal cargoes it provides. The WASH dependent recycling of $\alpha 5\beta 1$ integrins to the 441 442 plasma membrane drives invasion of ovarian carcinoma cells in fibronectin containing 3D matrix (296). In breast carcinoma cells, the WASH complex interacts with the exocyst and 443 contributes to the focal delivery of the metalloprotease MT1-MMP to invadopodia (178). This 444 445 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).

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

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

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

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E. An emerging function of NPFs in gene transcription

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

N-WASP has a marked effect on gene transcription (279). Actin, the Arp2/3 complex and N-WASP coimmunoprecipitate with the RNA polymerase II, which transcribes most protein encoding genes (279, 295). Nuclar Arp2/3 generates branched actin networks like its cytoplasmic counterpart (295). N-WASP might exert this transcription role through its interaction with the nuclear PSF/NonO complex (279). The tyrosine phosphorylation of N- WASP by Src family kinases (235) and FAK (278) favors its cytoplasmic localization at the
expense of its nuclear localization and can thus downregulate its transcriptional role. The
extent of the requirement of N-WASP for general gene transcription is, however, questioned
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-κB 499 transcription by binding to the p65 subunit of NF-κB and to the inhibitor of NF-κB 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 reprograming experiments, where somatic nuclei are injected into Xenopus oocytes (174). The Rac-WAVE pathway is 505 similarly required (175). In T lymphocytes, the hematopoietic WASP controls the 506 lymphocytic Th1 differentiation program, because Th1 differentiation genes are occluded 507 through methylation of histone H3 in patients affected by Wiskott-Aldrich syndrome, where 508 WASP is defective (244). All together, these various experiments established that nuclear 509 510 Arp2/3 and NPFs may control gene transcription through both chromatin remodeling and 511 recruitment of the RNA polymerase II.

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514 IV. ARP2/3 INHIBITORY PROTEINS

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Three Arp2/3 inhibitory proteins have recently been reported. The general assumption is that an inhibitor should be diffuse in the cytosol, in order to maintain globally silent a signaling pathway that is locally activated. The surprise is that these Arp2/3 inhibitory proteins are specifically localized at the surface of specific membranes, just like the 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 uncharacterized protein, which contained a typical C-terminal acidic A motif, but lacked the 525 required WH2 motif for Arp2/3 activation. In vitro this protein binds to the Arp2/3 complex, 526 527 but cannot activate it. It thus acts as a competitive inhibitor of NPFs (47) and was called Arpin as a mnemonic for Arp2/3 inhibition. There is a single Arpin gene in the human 528 529 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 conformation, where Arp2 and Arp3 are far apart (226). 531

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

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 associated with lymph node invasion and decreased survival (151, 154). The most powerful 544 545 prognosis factor, however, is obtained when patients having tumors displaying Arpin downregulation are combined with patients having tumors displaying up-regulation of the WAVE 546 complex (154). Patient analyses thus confirm the specific antagonism between Arpin and the 547 548 WAVE complex deduced from the analyses of cell migration in vitro.

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Β.

Gadkin

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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 (211). It is thus logical to assume that Gadkin maintains the Arp2/3 complex in an inhibited 559 conformation, like Arpin. However, in in vitro assays of Arp2/3 activity, purified Gadkin does 560 561 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.

565 566 Gadkin has been shown to be overexpressed in breast cancer, but this overexpression is 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 domain of PICK1 connects it to numerous membrane receptors and transporters, the most studied of which is the AMPA receptor of the glutamate neurotransmitter. The BAR domain dimerizes and induces or senses curved membranes through its banana-shaped membrane binding interface (161, 233). The C-terminal part of the molecule contains an acidic motif that binds and inhibits the Arp2/3 complex (202). This inhibitory activity was reported to be regulated by the GTPase Arf1 (201).

PICK1 regulates AMPA receptor trafficking, and in particular its clathrin-dependent 578 579 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 (125, 201, 250). These effects are mediated by expansion or contraction of post-synaptic 584 585 structures called dendritic spines. Dendritic spines are actin-rich membrane structures, perhaps equivalent to a mini-lamellipodium except that filaments emanating from a central 586 branched actin network are laterally elongated, thus allowing the expansion of the spine (33). 587 588 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 (8). Consistent with an inhibitory role, PICK1 depletion expand spines, whereas its 590 overexpression induce spine shrinkage (180). In glial cells, PICK1 might antagonize WAVE 591 rather than N-WASP, because PICK1 depletion increases branching complexity of astrocytes, 592 593 whereas WAVE2 depletion decreases it, unlike N-WASP depletion (179).

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

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- 599 V. BRANCH REGULATORS
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- 601 A. Cortactin
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603 The cortactin family is composed of two members, the ubiquitous cortactin protein and HS1, which is hematopoietic-specific and thus less studied. Cortactin interacts with actin 604 filaments through its specific cortactin repeats and with the Arp2/3 complex through an acidic 605 606 motif, which has the particularity to be located in the N-terminus of the protein (Fig.5). Cortactin was previously proposed to be a NPF, because it does activate the Arp2/3 complex 607 in vitro at high concentration. However, cortactin strongly synergizes with NPFs, because it 608 609 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 Arp2/3 complex that has landed onto a mother filament (59, 94, 224). Cortactin then remains
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 all along their length and independently of their subcellular location (116, 267). In contrast, 615 616 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 continuous exchange along the length of lamellipodial actin network (136). Further in vivo 618 619 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 overexpression prevents NPF extraction that ensures the turnover measured by FRAP. 626

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

Cortactin was found to promote lamellipodial persistence and cell motility in 638 639 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 visible defects in lamellipodial ultrastructure and only modest alterations of migration 641 parameters (137). Strikingly, the defects of cortactin depleted cells can be rescued by 642 adhesion to the matrix deposited by control cells, suggesting that cortactin have defects in 643 depositing extracellular matrix (239). Invadopodia, the major protrusions of cancer invasive 644 cells, depend on cortactin, in addition to their specific NPF, N-WASP. Cortactin is a structural 645 component of the branched actin networks that induce invadopodial protrusions and its 646 647 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 secretion, in addition to its role in creating the protrusion (10, 40, 99, 129). Exosome 649 650 secretion, which is important for communication between cancer cells and between cancer 651 cells and cancer-associated cells from their microenvironnement, critically depends on cortactin (222). 652

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

There are 7 coronins in human, which are characterized by a so-called β -propeller 679 structure made of WD40 repeats (31). Here we will discuss only the type I coronins, among 680 681 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 binding site for actin filaments is in the β -propeller, but Coro1C possesses a second filament 684 685 binding site in a unique region (31, 32). The site binding to the Arp2/3 complex is in the Nterminus of coronins. Arp2/3 binding is regulated by the phosphorylation of two serines in the 686 N-terminus and C-terminus. Phosphomimetic mutations of these sites strongly decrease 687 Arp2/3 binding (23, 280). 688

In vitro, coronin enhances Arp2/3 debranching and both binding sites are required for this effect (24, 25). An antagonism between cortactin and coronin has thus been proposed to control the debranching rate of branched actin networks (25). Coronin does not stain the branched actin structures to their top where new branches are formed, indicating that coronin acts at a later stage than cortactin (25). However, one has to understand why in vivo coronin 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 the branched actin network. Recent data explain the conundrum that coronin remains 696 697 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 subunits (1). The Arp2/3 complexes containing ARPC1A and/or ARPC5 would indeed be 699 700 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 good marker of the branched actin structures. 703

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 consistent with a shut-down of Arp2/3 debranching, when branched actin networks are 707 708 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 Arp2/3710 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 its activities of Arp2/3 debranching and actin disassembly, coronin appears to down-regulate, 713 714 rather than promote, branched actin networks.

715 Coronin 1C is overexpressed in gliomas (251), in hepatocellular carcinomas (276), gastric cancers (238), lymphomas (159). When it has been examined, coronin overexpression 716 is associated with high grade tumors and poor survival for patients. It might seem at odds that 717 718 a type I coronin, which globally down-regulates branched actin networks, might be overexpressed in cancers, while the Arp2/3 complex is also overexpressed. However, coronin 719 720 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

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726 There are two Glia-Maturation Factors (GMF) proteins encoded in humans, GMFB 727 encoded by the GMFB gene and GMF γ encoded by GMFG. GMFB is expressed ubiquitously with an enrichment in the brain (91), whereas GMFG is mostly expressed in hematopoietic 728 cells and endothelial cells (105). These two homologous proteins belong to the same 729 730 superfamily as cofilin, but define a subfamily of their own (85, 196). GMFs are cofilins, which have evolved to bind to Arp2, instead of actin. GMF uses this Arp2 binding site to 731 insert intself in between Arp2 and the actin molecule and thus severs the branch in a way 732 similar to cofilin severing actin filaments (70, 158, 293). GMF preferentially recognizes Arp2, 733 734 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 way ATP hydrolysis by the actin molecules in a filament indicates the older portion of the
filament. Cofilin also severs ADP-actin filaments more efficiently than younger ATP-actin
filaments (30, 170, 187). GMF binding to Arp2 is regulated by phosphorylation of its serine at
position 2 (105), just like cofilin (5).

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

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

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

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762 VI. CONCLUSION

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

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

paralogous subunits expressed in each cell system. Similar complexity emerged at the level of 777 778 the different Arp2/3 complexes, which might themselves be differentially regulated by the different NPF complexes. Similarly, the identification of different Arp2/3 inhibitors revealed 779 that Arp2/3 inhibition is not global, as a means to maintain the system silent in the cytosol, 780 but the inhibitory proteins rather balance Arp2/3 activation at the surface of different cell 781 membranes. Similarly, regulators of the Arp2/3 branched junction fine-tune the ultrastructure 782 783 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 understand its logic. If one sets aside a couple of reports at odds with the majority of 787 publications, one can see that the Arp2/3, the WAVE and the N-WASP complexes are overall 788 overexpressed in many cancers. These overexpressions are usually associated with poor 789 790 prognosis for patients. The other NPFs more specifically involved in intracellular trafficking like WASH, WHAMM or JMY, are not, or not yet, involved in cancer progression. Down-791 regulation of Arpin is consistent with its inhibitory role. The situation is not yet clear for 792 793 Gadkin and PICK1. As for the branch regulators, the stabilizer cortactin and destabilizers, coronin and GMF, are all clearly overexpressed with an overall association with high grade of 794 tumors and poor prognosis for the patients. These simple observations emphasize the notion 795 that branched actin networks have to turnover fast to perform their function. The deregulation 796 of the Arp2/3 regulatory system in cancer suggests that small molecule inhibitors of branched 797 actin dynamics might provide a therapeutic benefit to control cancer progression. 798

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803 Figure legends

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805 Figure 1: Conformations of the Arp2/3 complex and regulators of the equilibria between them. On the left, the fully inactive Arp2/3 complex displays a conformation, where Arp2 and 806 807 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 rearranged Arp2/3 complex, where Arp2 and Arp3 have been brought into proximity and 809 mimics the end of an actin filament. This conformation allows side binding to an actin 810 811 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.

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Figure 2: Modular organization of Arp2/3 activatory and inhibitory proteins. Their division 815 816 of labor at different subcellular localizations is indicated. All activators contain a C-terminal WCA domain, which binds and activates the Arp2/3 complex, whereas all inhibitors contain 817 818 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 depicted. An inhibitory protein that would antagonize WHAMM activatory proteins remains 820 821 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 elongation (white arrows). This scheme represents molecular processes in the referential of 833 834 the cell membrane and not of the substratum: either the membrane is fixed and the branched actin undergoes a retrograde flow, or the branched actin structure is fixed, due to a coupling 835 with cell adhesion structures, and the plasma membrane protrudes. Rac controls the 836 837 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.

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 edge, respectively. The Arp2/3 inhibitory proteins, Arpin, Gadkin and PICK1, locally 845 antagonize WAVE, WASH and N-WASP at their respective locations. During cell 846 847 transformation, N-WASP is overexpressed and forms invadopodia. In invadopodia, WASH promotes focalized delivery of metalloproteases that degrade the extracellular matrix. In 848 849 cancer cells, WAVE is overexpressed and Arpin down-regulated, despite frequent abnormal 850 lamellipodia. Font size indicate the level of expression.

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

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

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875 **DISCLOSURES**

876 The authors have no conflict of interest to declare.

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- 1824

Figure 1



Figure 2









Figure 5

Cortactin (A R R R R R R R P SH3) Coronin β-Propeller CC

Branch regulators





 Table I: 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.
					~	Breast	7	lymph node, grade	И	Iwaya et al., 2007
					~	Lung	Z	lymph node	К	Semba et al., 2006
	A P D 2	ACTRO			~	Colorectal	7	Liver metastases	nd	Iwaya et al., 2007
	ANF 2	ACTN2			~	Colorectal	7	invasive stage, stromal expression	nd	Otsubo et al., 2004
					~	Stomach	7	stages, depth of invasion	nd	Zheng et al, 2008
Arp2/3					~	Stomach	7	stages	ע	Sun et al, 2014
complex	A D D 2	ACTR2			~	Colorectal	7	invasive stage, stromal expression	nd	Otsubo et al., 2004
	AILE 2	ACINS			~	Gastric	7	stages, depth of invasion	-	Zheng et al, 2008
	ARPC1A	ARPC1A		~		Pancreas	7	nd	nd	Laurila et al., 2009
	ARPC1B	ARPC1B		~		Pancreas	7	nd	nd	Laurila et al., 2009
	ARPC2	ARPC2			~	Glioma	7	high grade	nd	Liu et al., 2013
	ANI C2	ANTEZ			~	Melanoma	7	nd	nd	Kashani-Sabet et al., 2009
					~	Pancreas	7	perineural invasion	R	Guo et al., 2014
N-WASP				~		Lung	7	lymph node	R	Frugtniet et al., 2017
complex	N-WASP	WASL			~	Liver	7		ע	Jin et al., 2012
complex				~	~	Esophagus	-	lymph node invasion	-	Wang et al., 2010
				~	~	Breast	К		Ц	Martin et al., 2008
	W/A//F1	W/ASE1			~	Ovary	7	high grade	R	Zhang et al., 2012
	WAVEI	WASFI			~	Prostate	7		nd	Fernando et al., 2008
1		WASF2		~		Breast	7	high grade	ע	Fernando et al., 2007
	WAVE2				~	Breast	7	lymph node, grade	К	Iwaya et al., 2007
					~	Lung	7	lymph node	ע	Semba et al., 2006
					~	Colorectal	7	Liver metastases	nd	Iwaya et al., 2007
				~	~	Liver	7	high grade, multiple nodules	R	Yang et al., 2006
	WAVE3	WASF3		~	~	Colorectal	7	low grade, no lymph node inv.	7	Zhang et al., 2012
WAVE				~	~	Breast	7	tumor size, lymph node	R	Kulkarni et al., 2012
complex					~	Prostate	7		nd	Fernando et al., 2010
					~	Liver	7	high grade, metastases	R	Ji et al., 2015
				~	~	Ovary	7	high grade	Ц	Zhang et al., 2015
	ABI1	ABI1			~	Breast	7		R	Wang et al., 2011
			LOF mutations			Prostate			nd	Xiong et al., 2012
	NAP1	NCKAP1		~		Breast	7		R	Lomakina et al., 2016
	HEM1	NCKAP1L		~		Leukemia	7		R	Joshi et al., 2007
	SRA1	CYFIP1	deletion	~	~	Lung, Colon, Breast	لا	high grade	nd	Silva et al., 2009
	BRK1	BRK1			~	Lung	7	lymph node, grade	nd	Cai et al., 2009
	ARDIN	ARPIN		V	~	Breast	К		И	Lomakina et al., 2016
Inhibitory				V	~	Breast	И		И	Liu et al., 2016
	GADKIN	AP1AR		~		Breast	7		-	Lomakina et al., 2016
proteins				~	~	Breast	7	lymph node, grade	R	Zhang et al., 2010
	PICK1	PICK1		 ✓ 		Breast	-		-	Lomakina et al., 2010
				~		Glioma	К	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.
			amplification			Head and Neck	7	lymph nodes, recurrence	R	Rodrigo et al., 2000
			amplification		√	Head and Neck	7	lymph nodes	nd	Rothschild et al., 2006
					~	Head and Neck	7	high grade	К	Hofman et al., 2008
					√	Larynx	7		R	Gibcuset al., 2008
				√	✓	Larynx	7	high grade	nd	Ambrosio et al., 2011
					~	Esophagus	7	high grade, metastases	К	Lu et al., 2014
					√	Oral	7	high grade	nd	Yamada et al., 2010
			amplification		√	Breast	7		-	Hui et al., 1998
			amplification		✓	Breast	7		-	Dedes et al., 2010
	Cortactin	CTTN			~	Breast	7		-	Sheen-Chen et al., 2011
					√	Colon	7	high grade	R	Cai et al., 2010
				√	✓	Colon	7	high grade	Ы	Ni et al., 2015
Branch regulators					~	Stomach	7	high grade, lymph nodes	К	Wang et al., 2010
					√	Liver	7	high grade, metastases	R	Huang et al., 2012
				✓	✓	Brain	7	high grade	nd	Wang et al., 2015
					✓	Lung	7	high grade	nd	Noh et al., 2013
					√	Ovary	7	high grade	nd	Linet al., 2008
			√		✓	Melanome	7	high grade	nd	Xu et al., 2009
					✓	Prostate	7	high grade	R	Hou et al., 2012
		CORO1C			\checkmark	Gliomas	7	high grade	nd	Thal et al., 2008
	Coronin 1C				\checkmark	Liver	7	high grade	nd	Wu et al., 2010
				✓		Lymphoma	7		nd	Luan et al., 2010
					\checkmark	Stomach	7	high grade	R	Sun et al., 2014
	GMEB	CMER			~	Glioma	7	high grade, neovascularization	R	Kuang et al., 2016
	Givin p	GIVIT			1	Ovary	7	high grade	R	Li et al., 2010
	GMFγ	GMFG			\checkmark	Ovary	7		R	Zuo et al., 2014

nd: not determined