Supporting Information

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SI Text

Abbreviations. SpirD, the D WH2 domain of Spire (residues 461–480); SpirCD, the Spire containing CD WH2 domains (residues 432–480); SpirBCD, the Spire containing triple WH2 domains (BCD, residues 396–480), SpirABCD, the Spire containing quadruple WH2 domains (ABCD, residues 371–480); SpirDDD, the artificial Spire WH2 construct comprising three identical D repeats.

Materials and Methods. ***Preparation of proteins.*** The vectors (pGEX-6P-1) containing *Drosophila* Spire constructs were transformed into BL21(DE3) BL21-CodonPlus(DE3)-RIL expression cells (Stratagene) and grown for approximately 4 h at 37 °C until the OD at 600 nm reached 0.9. Protein expression was carried out at 18 °C for 14–16 h after induction with 0.5 mM isopropyl-β-thiogalactopyranoside (Fermentas). The cells were harvested by centrifugation at 5,000 rpm for 15 min, and pellets were resuspended in PBS containing 1 mM EDTA, 1 mM DTT, and 0.5 mM PMSF. The lysis was performed by sonication followed by centrifugation at 20,000 rpm for 30 min. The supernatant containing GST-tagged Spire constructs was applied on Glutathione Sepharose 4 Fast Flow beads (GE Healthcare). Bound protein was washed with 50 mM Tris (pH 7.0), 150 mM NaCl, 1 mM EDTA, and 1 mM DTT and cleaved by PreScisson protease (GE Healthcare) to release the Spire protein. The cleaved Spire proteins were collected, concentrated, and further purified by Superdex 75 (SpirCD, SpirBCD, SpirDDD, and SpirABCD) or Superdex 30 (SpirC and SpirD) in PBS buffer. The His-tagged SpirNT encoded in a pET 16b vector was expressed in BL21(DE3) Codon+ RIL and purified by Ni-NTA agarose beads (Qiagen) followed by gel filtration on Superdex 200 (GE Healthcare). The purity and homogeneity of proteins were confirmed by mass spectrometry and N-terminal Edman sequencing (data not shown). The folding properties of all constructs were further analyzed by 1D NMR spectra recorded by a 650-MHz magnet. AP-actin (Drosiphila 5C A204E/P243K cytoplasmatic actin) was prepared as described by Rould et al. (1). The rabbit skeletal muscle actin was extracted according to the method of Spudich and Watt (2) and further purified on a Sephacryl S-200 gel filtration column (GE Healthcare). Latrunculin B (Sigma) was used to prevent spontaneous polymerization of rabbit actin.

Crystallization. Purified SpirABCD, SpirBCD, as well as SpirDDD, and SpirCD were mixed with AP-actin at a molar ratio of 1:4.1, 1:3.1, and 1:2:1, respectively, and separated by gel filtration on Superdex 200 (GE Healthcare) in 5 mM Tris (pH 7.0), 50 mM NaCl, 0.2 mM ATP, and 5 mM β-mercaptoethanol. Fractions from the peaks shown in Fig. S3*A* were analyzed by SDS-PAGE, pooled, and concentrated to 10–12 mg/mL. The complexes were crystallized both at 4 °C and 20 °C in several crystallization conditions, using the sitting and hanging drop vapor diffusion method. The 2- to 3-μL drops consisted of a 1:1 or 2:1 (vol/vol) mixture of protein solution and a well solution. Crystals appeared after 2 days and grew to the final size after 2–4 weeks of incubation. The best diffracting crystals of the SpirABCD/AP-actin, SpirBCD/AP-actin, and SpirDDD/AP-actin complexes grew in 0.2-M ammonium formate pH 6.6, 20% PEG 3350, and the crystals of SpirCD/AP-actin appeared in 0.2-M ammonium sulphate, 0.1-M Mes pH 6.5, 20% PEG 8000. All crystals were soaked in cryosolutions containing mother liquor supplemented with 20% 2-methyl-2,4-pentanediol or glycerol and were flash frozen in liquid nitrogen. Crystals of the SpirD/actin-latrunculin B complex appeared after one week at 4 °C in the solution containing 0.2 M magnesium formate, pH 5.9 and 20% PEG 3350. Crystals were treated as described above. To exclude the possibility of proteolytic cleavage of Spire during crystallization, we dissolved several crystals of the complexes and analyzed them by SDS-PAGE and mass spectrometry, proving that both SpirCD and SpirBCD remain intact during crystallization.

Data collection and structure determination. X-ray datasets of the SpirD/actin-latrunculin B (1.6 Å), SpirBCD/AP-actin (2.0 Å), SpirABCD/AP-actin (2.2 Å), SpirDDD/AP-actin (2.1 Å), and SpirCD/AP-actin (2.6 Å) were collected on the Swiss Light Source beamline PXII at the Paul Scherrer Institut. The datasets were integrated, scaled, and merged by XDS and XSCALE programs (3) in space group $P2_12_12_1$ for SpirD-actin-latrunculin B, $P6_5$ for SpirCD/AP-actin, SpirABCD/AP-actin, and SpirDDD/AP-actin, and $P2_1$ for SpirBCD/AP-actin (Table S1). The structures were determined by molecular replacement using the Molrep program from the CCP4 suite (4) and the structures of AP-actin and rabbit skeletal muscle actin complexed with latrunculin A as a search model. Model building and refinement were carried out with the program XtalViev/Xfit (5) and REFMAC5 (4). Data collection refinement and statistics are shown in the Table S1.

1. Rould MA, Wan Q, Joel PB, Lowey S, Trybus KM (2006) Crystal structures of expressed non-polymerizable monomeric actin in the ADP and ATP states. *J Biol Chem* 281:31909–31919.
2. Spudich JA, Watt S (1971) The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J Biol Chem* 246:4866–4871.
3. Kabsch W (1993) Automatic processing of rotation diffraction data from crystals of initially unknown symmetry and cell constants. *J Appl Crystallogr.* 26:795–800.
4. CCP4 (Collaborative Computational Project, Number 4) (1994) The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D Biol Crystallogr* 50:760–763.
5. McRee DE (1999) XtalView/Xfit. A versatile program for manipulating atomic coordinates and electron density. *J Struc Biol* 125:156–165.

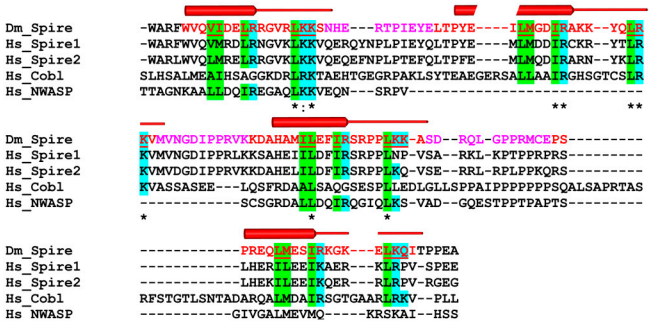


Fig. S1. Amino acid sequence alignment of the WH2-containing actin nucleators. Dm_Spire (Drosophila melanogaster; Q9U1K1); Hs_Spire1 (Homo sapiens; Q08AE8); Hs_Spire2 (Homo sapiens; Q8WWL2); Hs_Cobl (Homo sapiens; O75128); Hs_NWASP (Homo sapiens; O00401). Conserved residues are colored as follows: green, hydrophobic; cyan, basic; residues present in all WH2 domains are marked with asterisks. The residues directly interacting with actin are underlined. Sequence of the Spire protein studied in this work is shown in red (WH2 domain) and magenta (linkers). The secondary structure is depicted above the sequence.

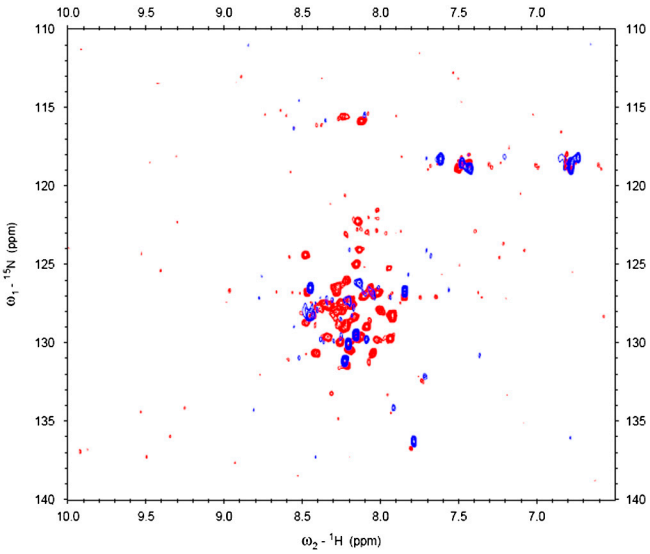


Fig. S2. Titration of the ^{15}N-labeled SpirCD with unlabeled (^{14}N) AP-actin. The ^{15}N-HSQC spectra of SpirCD before (red), and after addition of actin (blue). Only ^{1}H-^{15}N NMR signals of SpirCD are visible. NMR indicates that free SpirCD is not structured in solution giving a typical NMR spectrum of the polypeptide chain in a random conformation (1, 2) On forming the complex with actin, most of the NMR signals (blue) of SpirCD disappear. This is because upon binding the SpirCD residues participate in a well-defined structure of a large SpirCD/actin complex (1, 2). The observed $1/T_2$ transverse relaxation rate of the bound Spire in the complexes increases thus significantly and broadening of NMR resonances results in the disappearance of its signals in the spectra. No strong, sharp NMR signals are visible in the blue spectrum of the complex, indicating that there are no highly flexible residues present in the actin-bound SpirCD.

1. D'Silva L, et al. (2005). Monitoring the effects of antagonists on protein-protein interactions with NMR spectroscopy. *J Am Chem Soc* 127:13220–13226.
2. Wüthrich K (1986) *NMR of Proteins and Nucleic Acids* (Wiley, New York).

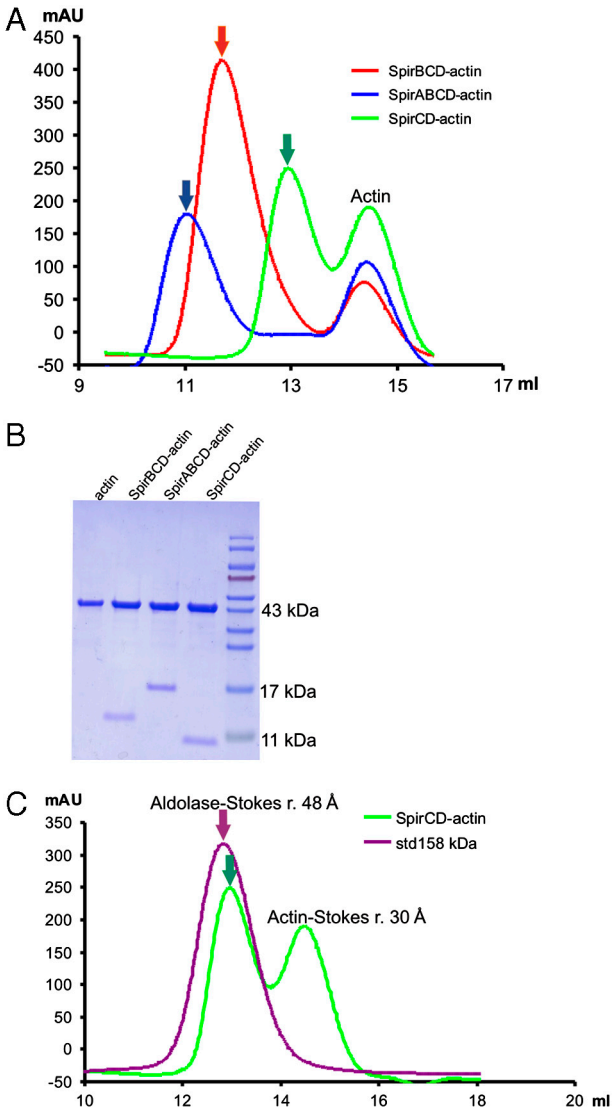


Fig. S3. Purification of Spire-actin complexes. (*A*) A Superdex 200 elution profile of SpirCD/AP-actin (green), SpirBCD/AP-actin (red), and SpirABCD/AP-actin (blue). Spire and actin form tight and stable complexes clearly separated from monomeric actin. (*B*) SDS-PAGE analysis of peak fractions eluted from the column. (*C*) Comparison of the SpirCD-actin complex (green) with the molecular weight standard—aldolase (158 kDa; Stokes radius—48 Å) (violet).

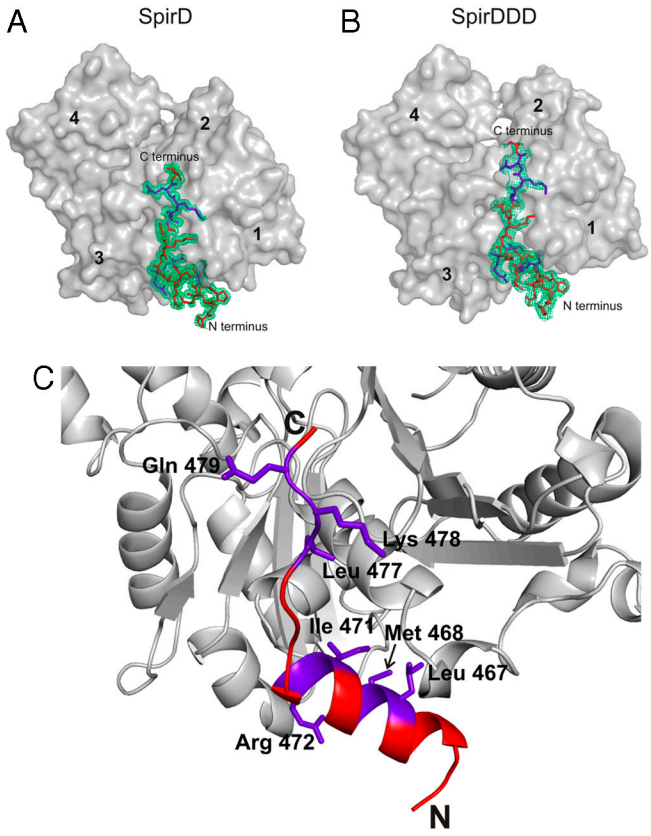


Fig. S4. Structures of WH2 domain of Spire (red and purple) and actin (gray). (*A*) Surface plot of SpirD/actin-latrunculin B complex. (*B*) Surface plot of a single WH2-D domain of Spire bound to actin in SpirDDD/AP-actin complex. (*C*) Conserved residues of WH2 domain (purple). The conserved Leu477, belonging to LKQI motif, directly interacts with the hydrophobic pocket of actin residues Ile341 and Ile345. This interaction appears to be essential for the binding of the C-terminal end of SpirD WH2 domain to actin; however, the long-range electrostatic interaction between Spire's Lys478 and Lys475 with a negatively charged surface formed by actin Asp24, Asp25, should also contribute to the binding. Additionally the entire C-terminal tail of Spire, between Spire Arg462 and Ile480, is stabilized by hydrogen bonds created by the guanidinium group of Spire Arg472, which is the last amino acid of the α-helix, with both the hydroxyl group of actin Thr148, and the carboxyl group of actin Glu167, and the ε-amino group of Spire Lys475 is hydrogen-bonded to the hydroxyl group of actin Ser348 (at the C terminus). The side chain of Spire Arg472 forms in addition a salt bridge with actin Glu167 and Spire's Glu465, which may further stabilize Spire's α-helix. The N-terminal part of the α-helix directly interacts with actin through hydrogen bonds formed between the side chain of Spire Arg464 and the carbonyl group of actin Glu167.

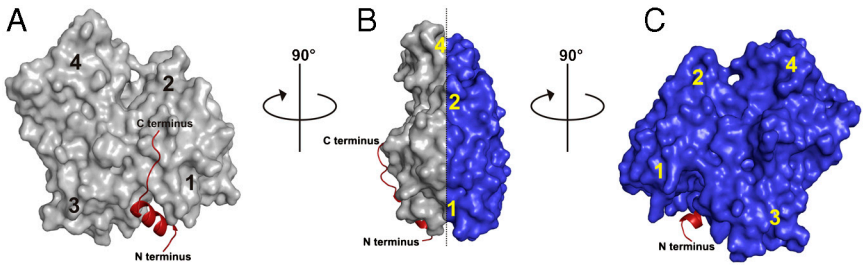


Fig. S5. The sides of the actin molecule. (*A*) The front view of the outer side of the actin molecule (gray). (*B*) The side view, the outer side is in gray and the inner one in blue. (*C*) The front view of the inner side of actin (blue). The subdomains of actin are marked with numbers. Spire is depicted as a ribbon plot and colored red. This designation should not be confused with that of Holmes (1).

1. Holmes KC (2009) Actin in a twist. *Nature* 457:389–390.

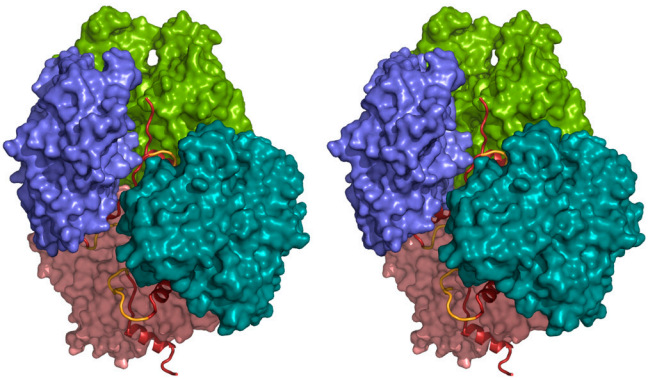


Fig. S6. Stereo representation of the SpirABCD/AP-actin complex in the "compacted side-to-side" configuration. Actin molecules colored green, blue, and cyan belong to one asymmetric unit (in the $P2_1$ space group). The fourth actin of the complex (colored in brown) belongs to the neighboring asymmetric unit. The WH2 domains are shown in red; "missing" linkers are depicted as ribbons and colored in yellow.

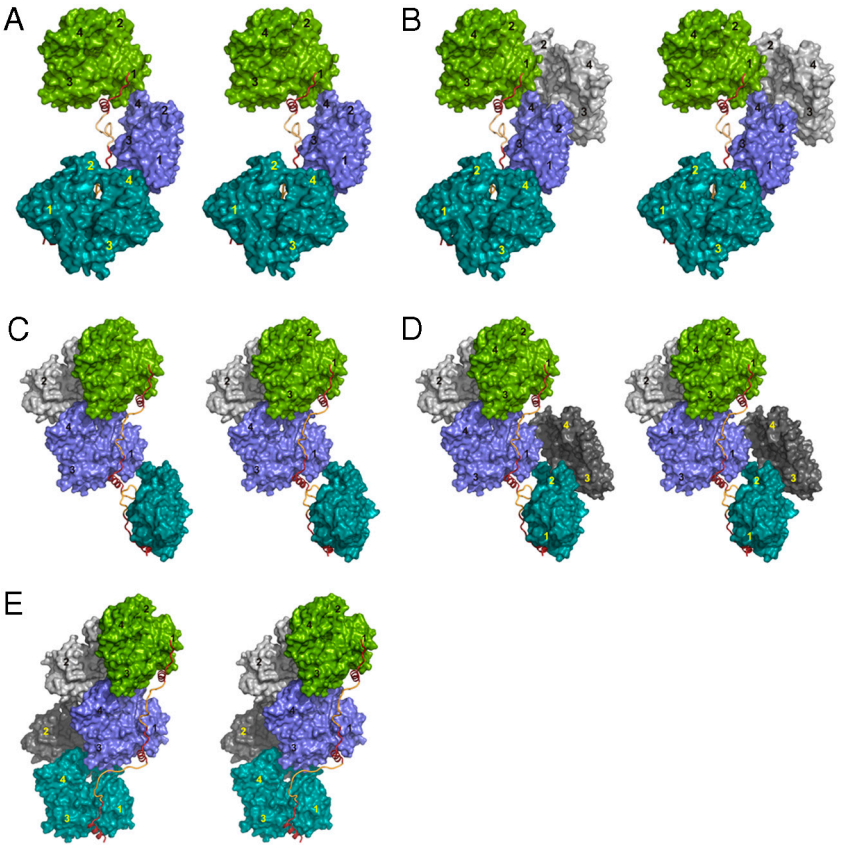
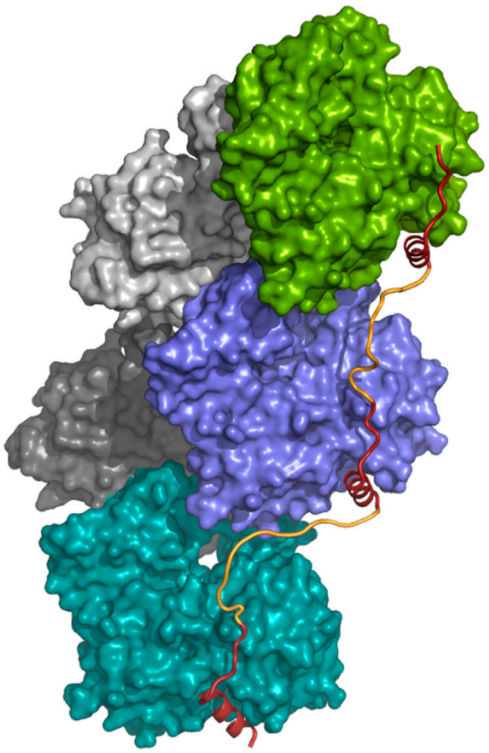


Fig. S7. Model of actin nucleation by Spire. (*A*) The crystal structure of a SpirBCD-actin primary nucleus in the "elongated side-to-side" arrangement (side view perpendicular to the long axis). (*B* and *C*) Model of G-actin recruitment and nucleus rearrangement. (*D*) Filament formation and elongation by addition of actin monomers to the barbed end of growing filament. (*E*) The filament has been formed.



Movie S1. A proposed model of actin nucleation by Spire. Spire (red ribbons) aligns actin molecules (colored green, blue, cyan, and pink) into the elongated side-to-side arrangement and forms the primary nucleus. Binding of free actins (colored in gray) induces transition to the F-actin conformation, followed by elongation upon addition of actin monomers to the barbed end of the growing filament.
Movie S1 (WMV)

Table S1. Data collection and refinement statistics

	SpirD	SpirCD	SpirBCD	SpirABCD	SpirDDD
Data collection					
Space group	$P2_12_12_1$	$P6_5$*	$P2_1$*	$P6_5$	$P6_5$
Cell constant:					
a	52.96	125.51	125.30	126.08	125.82
b	71.81	125.51	55.95	126.08	125.82
c	100.72	56.1	$125.37/\beta = 120.036$	56.37	56.02
Resolution range (Å)	50 – 1.5	50 – 2.6	50 – 2.0	50 – 2.2	50 – 2.1
Observed reflections	380,081	84,313	370,098	228,321	266,481
Unique reflections	49,209	11,584	90,708	22,506	26,371
Whole resolution range:					
Completeness (%)	79.0	73.6	80.8	76.2	86.4
R_{merge}	4.9	8.6	5.6	6.7	3.0
I/σ (I)	21.4	12.6	19.0	15.54	32.6
Last resolution shell:					
Resolution range (Å)	1.5 – 1.6	2.6 – 2.8	2.0 – 2.1	2.2 – 2.3	2.1 – 2.2
Completeness (%)	38.1	40.5	47.6	38.5	36.9
R_{merge}	20.3	29.7	19.5	26.9	23.6
I/σ (I)	4.4	4.4	5.0	3.9	4.0
Refinement					
No. of reflections	46,688	9,765	78,737	20,832	23,718
Resolution (Å)	39 – 1.5	20 – 2.8	50 – 2.0	50 – 2.0	50 – 2.0
R factor (%)	17.2	24.1	19.0	18.4	25.6
R_{free} (%)	21.6	33.0	23.9	25.1	27.5
Average B ($Å^2$)	19.8	51.0	32.8	42.8	57.8
rms bond length (Å)	0.027	0.012	0.011	0.018	0.006
rms angles (°)	2.4	1.5	1.2	1.5	0.9
Content of asymmetric unit					
No. of complexes	1	1	3	1	1
No. of protein residues/atoms	367/3,418	381/2,974	1,132/9,613	377/2,912	378/2,967
No. of solvent atoms	489	30	781	142	63
PDB Data Bank ID code	3MN5	3MMV	3MN6	3MN9	3MN7

*Space groups $P6_5$ and $P2_1$ are equivalent (See the section: Crystal structures of the SpirCD/AP-actin, SpirBCD/AP-actin, SpirDDD/AP-actin and SpirABCD/AP-actin complexes.)