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Phosphorylation of VASP by AMPK alters actin binding and occurs at a novel site

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ABSTRACT

Vasodilator-stimulated phosphoprotein (VASP) is an actin regulatory protein that functions in adhesion and migration. In epithelial cells, VASP participates in cell–cell adhesion. At the molecular level, VASP drives actin bundling and polymerization. VASP activity is primarily regulated by phosphorylation. Three physiologically relevant phosphorylation sites significantly reduce actin regulatory activity and are targeted by several kinases, most notable Abl and protein kinases A and G (PKA and PKG). AMP-dependent kinase (AMPK) is best characterized as a cellular sensor of ATP depletion, but also alters actin dynamics in epithelial cells and participates in cell polarity pathways downstream of LKB1. While little is known about how AMPK direct changes in actin dynamics, AMPK has been shown to phosphorylate VASP at one of these three well-characterized PKA/PKG phosphorylation sites. Here we show that phosphorylation of VASP by AMPK occurs at a novel site, serine 322, and that phosphorylation at this site alters actin filament binding. We also show that inhibition of AMPK activity results in the accumulation of VASP at cell–cell adhesions and a concomitant increase in cell–cell adhesion.

1. Introduction

Actin plays a fundamental role in cell migration and adhesion, including cell–cell adhesion in epithelial cells [1]. Reorganization of actin structures to drive cell migration and adhesion requires a large number of actin regulatory proteins, whose relative contribution to actin dynamics must be carefully coordinated. A major emphasis in cell biology is determining the biochemical mechanism and regulation of actin regulatory systems that contributed to larger cellular processes like adhesion and migration. VASP is an actin regulatory protein that functions in cell migration [2,3] and adhesion [4], including cell–cell adhesion of epithelial cells [5–8]. VASP localizes to the extreme edge of lamellipodia [9], focal adhesions [10], and cadherin-based cell–cell contacts [7,9,11]. When VASP function is perturbed, alterations in the rate of migration [2] and strength of cell–cell adhesion are observed [6,7,12].

The biochemical mechanisms underlying VASP function have been carefully studied. VASP contains G-actin, profilin, and F-actin binding sites that are coordinated to allow VASP to act as an actin barbed end binding protein [13] that facilitates actin filament elongation [14–16] and protects barbed ends from the activity of capping protein [17,18]. These actin regulatory functions require tetramerization via a tetramerization domain at the extreme COOH terminus of the VASP protein [19]. As a homotetrameric protein complex, VASP also facilitates bundling of actin filaments [20] and may antagonize formation of Arp2/3 complex in forming branched actin networks [21]. The actin regulatory functions of VASP are highly sensitive to salt concentrations, suggesting that phosphorylation of this protein can have dramatic effects on activity. This idea has been borne out by observations that serine/threonine phosphorylation at three highly conserved sites is associated with decreased VASP activity at both the molecular and cellular level [22–25]. Though VASP phosphorylation sites are highly conserved PKA/PKG targets, several other kinases, including AMP-dependent protein kinase (AMPK) [26], have been shown to phosphorylate these sites also.

AMP-activated protein kinase (AMPK) is a serine/threonine kinase that acts as an important sensor of cellular energy charge. When ATP consumption is accelerated, replenishment of ATP through the adenylate kinase reaction results in increasing levels of AMP. Binding of AMP to AMPK results in a conformational shift that makes AMPK a better substrate for its upstream kinase, LKB1 [27,28], and a poorer substrate for protein phosphatases [29]. The resultant increase in phosphorylation at Thr172 on AMPK's T-loop potently activates AMPK which then mediates a wide variety of cell responses aimed at restoring the energy charge of the cell and/or allowing the cell to cope with the heightened energy stress under which it has been subjected [30]. In epithelial cells, LKB1 acts through AMPK to control epithelial polarity and cell–cell adhesion [31], possibly by driving changes in cellular actin organization [32]. How AMPK alters actin organization and dynamics in cells remains poorly understood, though VASP has been demonstrated to be a target of AMPK, specifically at one of the well-characterized PKA target sites [26]. Here we examine whether AMPK might phosphorylate VASP at additional locations and find that VASP

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phosphorylation by AMPK occurs primarily at a residue outside of the three well-characterized AMPK sites. We also show phosphorylation reduces VASP-actin binding and that inhibition of AMPK activity alters VASP localization at cell–cell adhesions.

2. Materials and methods

2.1. Protein biochemistry

Murine His-VASP fusion proteins were produced in plasmid-transformed bacteria. After growing and inducing, with IPTG, under conditions optimized for each protein, bacteria were harvested by centrifugation at 4 °C. Pellet was resuspended in ice cold Buffer A (100 mM NaCl, 20 mM Tris, 10 μg/ml antipain, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM PMSF, 1 mM DTT, pH7.4) and homogenized by sonification on ice. The resulting extract was clarified by centrifugation at 50,000*g* for 15 min at 4 °C. Proteins were recovered from extracts with iminodiacetic acid-agarose beads that had been charged with Ni^{2+} ions and equilibrated in Buffer A. Bound proteins were eluted with Buffer A containing imidazole, then dialyzed into Buffer A. Protein concentrations were determined by Bradford method [19]. Peptides containing the control or mutated putative AMPK phosphorylation site (RRRTTLPRMKSSSSVTT and RRRTTLPRMKASSSVTT, respectively) were commercially synthesized to 85% purity. In vitro phosphorylation was performed by mixing target protein/peptide with LKB1/STRAD/MO25 complex and with or without AMPK in phosphorylation buffer (40 mM Hepes, 0.2 mM SAMS, 80 mM NaCl, 8% glycerol, 0.8 mM EDTA, 0.8 mM DTT, 5 mM $MgCl_2$, 0.2 mM ATP (with or without ATP containing $\gamma^{32}P$), 0.2 mM AMP, pH 7.0). To analyze phosphorylation of VASP proteins, samples were separated by electrophoresis in SDS–polyacrylamide gels, which were stained with GelCode Blue and analyzed by autoradiography. To analyze peptide phosphorylation, recombinant $\alpha2\beta2\gamma2$-AMPK was activated by incubation with recombinant LKB1-STRAD-MO25 (Upstate-Millipore) at 30 °C for 60 min in reaction buffer (40 mM HEPES, 0.2 mM AMP, 80 mM NaCl, 8% glycerol, 0.8 mM EDTA, 0.8 mM DTT, 5 mM $MgCl_2$, 0.2 mM ATP, pH 7.0). Control or mutated VASP peptides were then incubated in reaction buffer with activated AMPK + LKB1 or LKB1 alone plus labeled [γ-^{32}P]ATP in a total volume of 25 μl for 30 min at 30 °C. Fifteen microliter of the reaction was then transferred to P81 filter paper. The filter paper was then washed five times with 1% phosphoric acid to wash out the labeled ATP. The papers were washed in distilled water, then acetone, and then air-dried. Labeled phosphate incorporated into the peptides was then measured by scintillation counting. For actin binding studies, proteins were added to samples containing G-actin in Buffer A, which was then supplemented with $MgCl_2$ and ATP to drive actin filament formation. After 1 h, samples were subjected to differential centrifugation at 100,000*g* for 7 min. Supernatant and pellet fraction were separated in SDS–polyacrylamide gels by electrophoresis. Gels were transferred to PVDF and VASP protein detected by Western blot analysis with VASP antibodies.

2.2. Cell culture and cell-based assays

MDCK cells were maintained in DMEM with 10% fetal bovine serum. Compound C treatment was maintained for 24 h at 40 μM. For fluorescence experiment, cells were seeded at the appropriate density on collagen-coated coverslips. Cells were fixed with paraformaldehyde on ice for 15 min and then stained with VASP primary antibodies (Calbiochem) and Alexa dye-conjugated secondary antibodies. Cell–cell junction formation was measured using hanging drop assays. Trypsinized MDCK cells were resuspended in medium at 250,000 cells/ml. Drops of cell suspension were placed on the inner surface of a cell culture dish and cultured for 2 h. Drops were then spread onto a glass coverslips, either directly or following trituration, and photographed. The number of cells in small (<50), medium (50–100), and large (>100) cell aggregates were counted from three independent experiments.

3. Results and discussion

A previous report indicates that AMPK phosphorylates VASP at T278 [26], one of the well characterized PKA target phosphorylation sites [23]. However, the site of AMPK phosphorylation reported in that study does not match the consensus target sequence of AMPK (Fig. 1A), which contains hydrophobic residues at the −5 and +4 positions and a basic residue at the −4 or −3 position [33]. We first sought to confirm that VASP is a target of AMPK-dependent phosphorylation by performing an in vitro kinase assay on purified VASP using either AMPK with the AMPK kinase LKB1 or, as a control, just LKB1. After completion of the assay, proteins were separated in SDS–polyacrylamide gels, which were then stained and subjected to autoradiography. VASP displayed radioactivity that shows it was indeed phosphorylated in these assays (Fig. 1B). Importantly, VASP phosphorylation only occurred when AMPK was present in the in vitro kinase reaction, but not when only LKB1 was present, demonstrating that AMPK, and not LKB1, phosphorylates VASP. This verifies that VASP is indeed an AMPK substrate.

The previous report used monoclonal antibodies against phosphorylated PKA sites in VASP to detect VASP phosphorylation [26]. However, this approach does not address whether additional sites might also be targeted by AMPK. To address this, AMPK phosphorylation of PKA phosphorylation-resistant mutants of VASP was assessed. Purified VASP bearing point mutations at each of the well-characterized PKA target sites (VASP S153A, VASP S235A, and VASP T274A), as well as purified VASP containing all three substitution mutations (VASP 3 × A), were subjected to AMPK phosphorylation in vitro with radioactive ATP. After incubation, proteins in each reaction were separated in SDS–polyacrylamide gels, which were then stained and subjected to autoradiography. All VASP proteins tested, comprising all those with mutations at any or all PKA phosphorylation target sites, bear radioactivity that indicates phosphorylation (Fig. 1C). Since the mutants bear one or more PKA sites that cannot receive phosphorylation and since none of these mutations, alone or together, can prevent VASP phosphorylation by AMPK, these results clearly demonstrate that AMPK phosphorylates VASP outside of the three well-characterized PKA target sites.

Though AMPK can phosphorylate VASP at a site that is outside of the well-characterized PKA target sites, it remains unclear whether this phosphorylation occurs only in addition to phosphorylation at the well-characterized PKA sites. It is possible that the novel site of AMPK-dependent phosphorylation is of minor importance compared to the reported AMPK phosphorylation at VASP T278 [26]. Conversely, the sensitivity of the monoclonal antibody detection strategy employed in demonstrating AMPK phosphorylation at VASP T278 [26] allows for the possibility that VASP phosphorylation is rare at this site, but that AMPK phosphorylation of VASP outside of the well-characterized PKA sites is more common. This would suggest that phosphorylation at the novel position is more critical to any AMPK-dependent regulation of VASP. We sought to determine the extent of AMPK phosphorylation at different sites within the VASP protein by performing tryptic digests on VASP proteins that had been subjected to in vitro phosphorylation with AMPK. A control reaction containing VASP of the wildtype sequence was used for comparison. Following in vitro phosphorylation and subsequent tryptic digestion, the resulting protein fragments were separated in SDS–polyacrylamide gels. Autoradiography of the gels revealed that two peptides from wildtype VASP

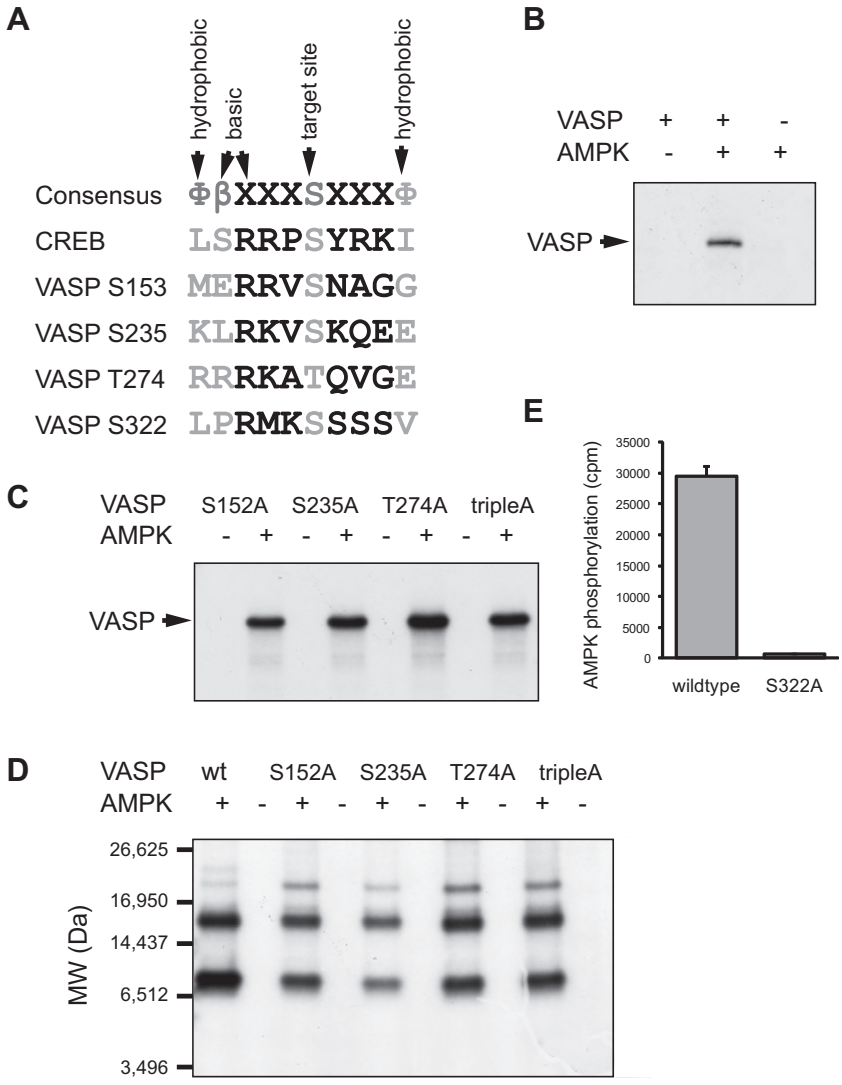

Fig. 1. VASP phosphorylation by AMPK. (A) Alignment of the consensus phosphorylation target recognition sequence for AMPK with a known target site from CREB and with the murine VASP PKA/PKG phosphorylation sites at S153, S235, and T274, as well as with the putative novel AMPK site at serine 322. (B and C) An autoradiograph of purified VASP proteins subjected to in vitro AMPK kinase assays and then separated in SDS–polyacrylamide gels. (D) An autoradiograph of an SDS–polyacrylamide gel containing separated protein fragments from tryptically digested VASP proteins that were subjected to in vitro phosphorylation by AMPK. (E) Radioactivity of peptides phosphorylated by AMPK in vitro. Target and substituted labels refer to the wildtype target peptide sequence and the control peptide containing the S322 alanine substitution, respectively.

bear significant phosphorylation. Peptides of the identical molecular weight are also phosphorylated in all of the VASP mutants, including that bearing mutations at all three well-characterized PKA sites. Since the same trypsin cleavage products are phosphorylated in wildtype VASP and in all PKA phosphorylation-resistant mutants, AMPK phosphorylation of VASP must occur predominantly outside of the three well-defined PKA target sites.

Interestingly, a consensusn AMPK target site is found nearer the –COOH terminus of the amino acid sequence of human VASP (Fig. 1A). That this site is fully conserved among all mammalian VASP homologues supports that this site might have biological significance. We first sought to confirm that the AMPK target sequence from mammalian VASP homologues can indeed be phosphorylated by AMPK. A synthetic amino acid sequence of the putative AMPK target site in VASP was subjected to in vitro phosphorylation with AMPK. As a control, a second synthetic amino acid sequence that bears a single serine-alanine substitution at the predicted target serine was also subjected to the same reaction. Incubation of the target peptide with AMPK resulted in high levels of target peptide phosphorylation (Fig. 1E). However, the control peptide bearing the alanine substitution at the serine 322 position demonstrated much lower levels of phosphorylation. Since peptides were synthesized to only 85% purity, the low levels of phosphorylation of the control peptide may be accounted for by peptide impurities generated during synthesis. Importantly, phosphorylation of both peptides requires AMPK; the presence of only LKB1 (AMPK kinase) in the kinase assays was not sufficient to generate a phosphorylated peptide product. We conclude that AMPK can phosphorylate VASP at serine 322.

How does VASP phosphorylation at serine 322 alter VASP's biochemical activities? We answered this question by analyzing actin filament binding of phosphorylated and unphosphorylated VASP. VASP was subjected to in vitro phosphorylation in reactions that did or did not contain AMPK, then subjected to actin sedimentation assays that separate actin filament binding proteins from those that do not bind F-actin (Fig. 2A). Small amounts of VASP were used in these experiments to ensure as much VASP protein as possible would be phosphorylated; Western blotting was then used to determine VASP distribution in the pellet (F-actin-binding) and supernatant (soluble protein) fractions. Unphosphorylated VASP

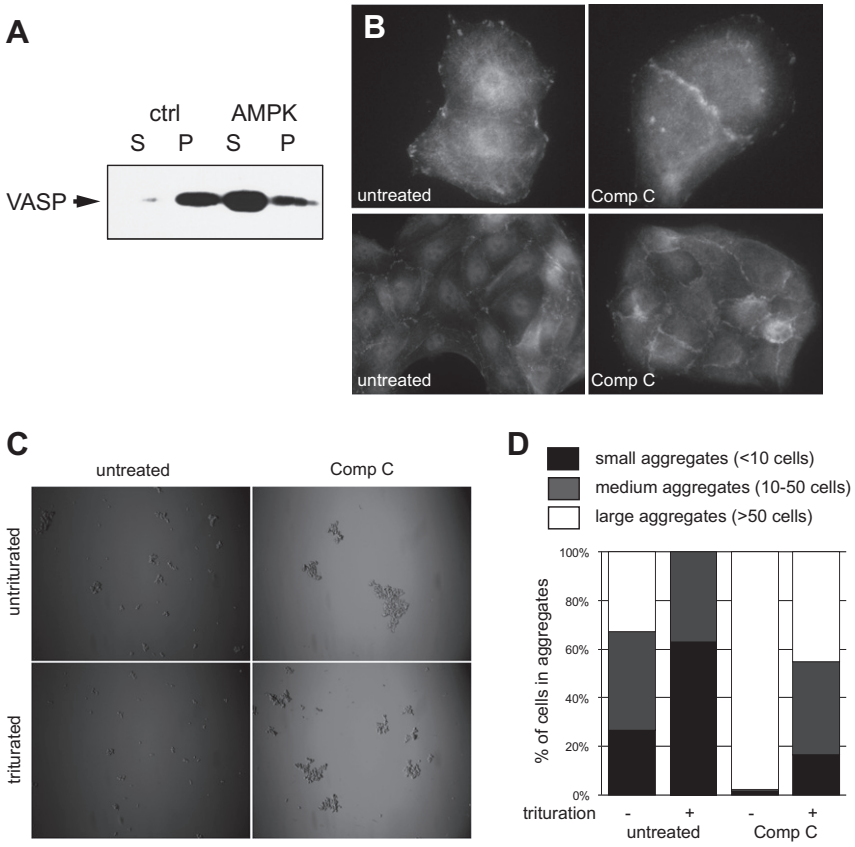

Fig. 2. Biochemical and cellular effects of VASP phosphorylation by AMPK. (A) Western blot analysis of the supernatant and pellet fractions from actin sedimentation assays containing unphosphorylated or AMPK-phosphorylated VASP. (B) Cellular distribution of VASP in control and Compound C-treated MDCK cells. Upper panels show a two cell colony, lower panels show larger colonies. (C) Aggregates of MDCK cells formed after two hours in suspension, before and after subjection to trituration forces. Cells were culture with or without Compound C. (D) Distribution of control and Compound C-treated MDCK cells in small, medium, and large aggregates in hanging drop assays, with and without trituration.

appears almost exclusively in the pellet fraction, as expected for an actin filament binding protein. In contrast, VASP that had been phosphorylated in vitro with AMPK is observed predominantly in the supernatant fraction, indicating that F-actin associations are disrupted. Clearly AMPK phosphorylation reduces the ability of VASP to bind F-actin filaments, an effect that is similar to phosphorylation at the well-defined PKA target sites [34].

We finally sought to demonstrate cellular effects resulting from changes in VASP phosphorylation by AMPK. MDCK cells were treated with the AMPK inhibitor Compound C and VASP localization determined by immunofluorescence. Compared to untreated control cells, Compound C treated cells showed a striking accumulation of VASP at cell–cell contacts (Fig. 2B). In contact between two cells, VASP levels at cell–cell junctions are much higher than in untreated control cells. In larger colonies of cells, VASP appears at discrete points along cell–cell contacts of untreated control cells, while Compound C-treated cells accumulate VASP at higher levels and with a more dispersed distribution along the junction. These data suggest that AMPK phosphorylation might participate in VASP turnover at cell–cell contacts, perhaps by allowing release of VASP from actin structures at cell–cell contacts. Several reports indicate that VASP functions in cell–cell adhesion. We therefore examined if Compound C treatment alters the properties of cell–cell adhesion by measuring cell aggregate formation and the resistance of aggregates to trituration forces. (Fig. 2C–D) After 2 h in suspension, MDCK cells treated with Compound C aggregate into larger groups than those formed in suspension culture of control cells. Further, groups of Compound C-treated cells are much less easily disrupted into smaller groups or single cells than groups of untreated control cells. These data suggest that AMPK negatively regulates VASP function during cell–cell adhesion, a result that is consistent with increased VASP levels at cell–cell contacts in Compound C treated cells.

The interface of cellular signaling networks with actin regulatory machinery is fundamental to understanding cellular processes that rely on tight control of actin organization. Results presented here outlining a novel site for VASP phosphorylation by AMPK, as well as biochemical and cellular effects of phosphorylation at this site, provide new insight into how cells use the LKB1-AMPK signaling pathway to control actin dynamics that underlie cell–cell adhesion.

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References

[1] J.T. Parsons, A.R. Horwitz, M.A. Schwartz, Cell adhesion: integrating cytoskeletal dynamics and cellular tension, Nat. Rev. Mol. Cell Biol. 11 (2010) 633–643.

[2] J.E. Bear, J.J. Loureiro, I. Libova, R. Fassler, J. Wehland, F.B. Gertler, Negative regulation of fibroblast motility by Ena/VASP proteins, Cell 101 (2000) 717–728.

[3] M. Krause, E.W. Dent, J.E. Bear, J.J. Loureiro, F.B. Gertler, Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration, Annu. Rev. Cell Dev. Biol. 19 (2003) 541–564.
[4] L. Trichet, C. Sykes, J. Plastino, Relaxing the actin cytoskeleton for adhesion and movement with Ena/VASP, J. Cell Biol. 181 (2008) 19–25.
[5] A.S. Kris, R.D. Kamm, A.L. Sieminski, VASP involvement in force-mediated adherens junction strengthening, Biochem. Biophys. Res. Commun. 375 (2008) 134–138.
[6] J.A. Scott, A.M. Shewan, N.R. den Elzen, J.J. Loureiro, F.B. Gertler, A.S. Yap, Ena/VASP proteins can regulate distinct modes of actin organization at cadherin-adhesive contacts, Mol. Biol. Cell 17 (2006) 1085–1095.
[7] V. Vasioukhin, C. Bauer, M. Yin, E. Fuchs, Directed actin polymerization is the driving force for epithelial cell–cell adhesion, Cell 100 (2000) 209–219.
[8] C. Furman, A.L. Sieminski, A.V. Kwiatkowski, D.A. Rubinson, E. Vasile, R.T. Bronson, R. Fassler, F.B. Gertler, Ena/VASP is required for endothelial barrier function in vivo, J. Cell Biol. 179 (2007) 761–775.
[9] K. Rottner, B. Behrendt, J.V. Small, J. Wehland, VASP dynamics during lamellipodia protrusion, Nat. Cell Biol. 1 (1999) 321–322.
[10] C. Haffner, T. Jarchau, M. Reinhard, J. Hoppe, S.M. Lohmann, U. Walter, Molecular cloning structural analysis and functional expression of the proline-rich focal adhesion and microfilament-associated protein VASP, EMBO J. 14 (1995) 19–27.
[11] M.D. Hansen, M.C. Beckerle, Opposing roles of zyxin/LPP ACTA repeats and the LIM domain region in cell–cell adhesion, J. Biol. Chem. 281 (2006) 16178–16188.
[12] P.M. Benz, C. Blume, J. Moebius, C. Oschatz, K. Schuh, A. Sickmann, U. Walter, S.M. Feller, T. Renne, Cytoskeleton assembly at endothelial cell–cell contacts is regulated by alphaII-spectrin-VASP complexes, J. Cell Biol. 180 (2008) 205–219.
[13] L. Pasic, T. Kotova, D.A. Schafer, Ena/VASP proteins capture actin filament barbed ends, J. Biol. Chem. 283 (2008) 9814–9819.
[14] D. Breitsprecher, A.K. Kiesewetter, J. Linkner, C. Urbanke, G.P. Resch, J.V. Small, J. Faix, Clustering of VASP actively drives processive, WH2 domain-mediated actin filament elongation, EMBO J. 27 (2008) 2943–2954.
[15] D. Breitsprecher, A.K. Kiesewetter, J. Linkner, M. Vinzenz, T.E. Stradal, J.V. Small, U. Curth, R.B. Dickinson, J. Faix, Molecular mechanism of Ena/VASP-mediated actin-filament elongation, EMBO J. 30 (2011) 456–467.
[16] S.D. Hansen, R.D. Mullins, VASP is a processive actin polymerase that requires monomeric actin for barbed end association, J. Cell Biol. 191 (2010) 571–584.
[17] M. Barzik, T.I. Kotova, H.N. Higgs, L. Hazelwood, D. Hanein, F.B. Gertler, D.A. Schafer, Ena/VASP proteins enhance actin polymerization in the presence of barbed end capping proteins, J. Biol. Chem. 280 (2005) 28653–28662.
[18] J.E. Bear, T.M. Svitkina, M. Krause, D.A. Schafer, J.J. Loureiro, G.A. Strasser, I.V. Maly, O.Y. Chaga, J.A. Cooper, G.G. Borisy, F.B. Gertler, Antagonism between Ena/VASP proteins and actin filament capping regulates fibroblast motility, Cell 109 (2002) 509–521.
[19] C. Bachmann, L. Fischer, U. Walter, M. Reinhard, The EVH2 domain of the vasodilator-stimulated phosphoprotein mediates tetramerization F-actin binding, and actin bundle formation, J. Biol. Chem. 274 (1999) 23549–23557.
[20] A. Schirenbeck, R. Arasada, T. Bretschneider, T.E. Stradal, M. Schleicher, J. Faix, The bundling activity of vasodilator-stimulated phosphoprotein is required for filopodium formation, Proc. Natl. Acad. Sci. USA 103 (2006) 7694–7769.
[21] J. Skoble, V. Auerbuch, E.D. Goley, M.D. Welch, D.A. Portnoy, Pivotal role of VASP in Arp2/3 complex-mediated actin nucleation actin branch-formation, and listeria monocytogenes motility, J. Cell Biol. 155 (2001) 89–100.
[22] P.M. Benz, C. Blume, S. Seifert, S. Wilhelm, J. Waschke, K. Schuh, F. Gertler, T. Munzel, T. Renne, Differential VASP phosphorylation controls remodeling of the actin cytoskeleton, J. Cell Sci. 122 (2009) 3954–3965.
[23] E. Butt, K. Abel, M. Krieger, D. Palm, V. Hoppe, J. Hoppe, U. Walter, CAMP- and cGMP-dependent protein kinase phosphorylation sites of the focal adhesion vasodilator-stimulated phosphoprotein (VASP) in vitro and in intact human platelets, J. Biol. Chem. 269 (1994) 14509–14517.
[24] A.K. Howe, B.P. Hogan, R.L. Juliano, Regulation of vasodilator-stimulated phosphoprotein phosphorylation and interaction with Abl by protein kinase A and cell adhesion, J. Biol. Chem. 277 (2002) 38121–38126.
[25] J.J. Loureiro, D.A. Rubinson, J.E. Bear, G.A. Baltus, A.V. Kwiatkowski, F.B. Gertler, Critical roles of phosphorylation and actin binding motifs, but not the central proline-rich region, for Ena/vasodilator-stimulated phosphoprotein (VASP) function during cell migration, Mol. Biol. Cell 13 (2002) 2533–2546.
[26] C. Blume, P.M. Benz, U. Walter, J. Ha, B.E. Kemp, T. Renne, AMP-activated protein kinase impairs endothelial actin cytoskeleton assembly by phosphorylating vasodilator-stimulated phosphoprotein, J. Biol. Chem. 282 (2007) 4601–4612.
[27] J.S. Oakhill, Z.P. Chen, J.W. Scott, R. Steel, L.A. Castelli, N. Ling, S.L. Macaulay, B.E. Kemp, beta-Subunit myristoylation is the gatekeeper for initiating metabolic stress sensing by AMP-activated protein kinase (AMPK). Proc. Natl. Acad. Sci. USA 107 (2010) 19237–19241.
[28] S.A. Hawley, J. Boudeau, J.L. Reid, K.J. Mustard, L. Udd, T.P. Makela, D.R. Alessi, D.G. Hardie, Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade, J. Biol. 2 (2003) 28.
[29] S.P. Davies, N.R. Helps, P.T. Cohen, D.G. Hardie, 5'-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC, FEBS Lett. 377 (1995) 421–425.
[30] D.G. Hardie, AMP-activated protein kinase: a cellular energy sensor with a key role in metabolic disorders and in cancer, Biochem. Soc. Trans. 39 (2011) 1–13.
[31] N. Amin, A. Khan, D. St Johnston, I. Tomlinson, S. Martin, J. Brenman, H. McNeill, LKB1 regulates polarity remodeling and adherens junction formation in the Drosophila eye, Proc. Natl. Acad. Sci. USA 106 (2009) 8941–8946.
[32] L. Miranda, S. Carpentier, A. Platek, N. Hussain, M.A. Gueuning, D. Vertommen, Y. Ozkan, B. Sid, L. Hue, P.J. Courtoy, M.H. Rider, S. Horman, AMP-activated protein kinase induces actin cytoskeleton reorganization in epithelial cells, Biochem. Biophys. Res. Commun. 396 (2010) 656–661.
[33] M.C. Towler, D.G. Hardie, AMP-activated protein kinase in metabolic control and insulin signaling, Circ. Res. 100 (2007) 328–341.
[34] B. Harbeck, S. Huttelmaier, K. Schluter, B.M. Jockusch, S. Illenberger, Phosphorylation of the vasodilator-stimulated phosphoprotein regulates its interaction with actin, J. Biol. Chem. 275 (2000) 30817–30825.