Relaxing the actin cytoskeleton for adhesion and movement with Ena/VASP

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At cell–cell contacts, as well as at the leading edge of motile cells, the plasticity of actin structures is maintained, in part, through labile connections to the plasma membrane. Here we explain how and why *Drosophila* enabled/vasodilator stimulated phosphoprotein (Ena/VASP) proteins are candidates for driving this cytoskeleton modulation under the membrane.

The actin cytoskeleton and cell–cell adhesion

Ena/VASP proteins, a family of multi-functional actin-modulating proteins, are implicated in cell–cell contact maturation (Scott et al., 2006), as are the actin polymerization nucleating proteins, formins and the Arp2/3 complex (Verma et al., 2004; Zigmond, 2004). Although it is intuitively clear why an assembly process like actin polymerization can drive protrusion, it is less obvious why actin dynamics are also integral to more static processes like cell–cell adhesion. However, the emerging picture is that actin polymerization is important for pushing adjacent membranes together, with the actin network being subsequently remodeled to maintain the contact and allow it to evolve. One interesting question is how the branched structures formed by the Arp2/3 complex, which nucleates daughter filaments from the sides of existing filaments, are refashioned into the belts of aligned actin filaments that lie parallel to the plasma membrane and reinforce cell–cell contacts.

Such a reinforcement is essential, as indicated by the fact that cell–cell adhesion vanishes in the presence of drugs affecting the dynamics of the actin cytoskeleton. In fact, physical measurements of the adhesion forces between individual cadherins indicate that this association is extremely weak, and does not add up to the value for cell–cell adhesion. The missing force could be supplied by the actin cytoskeleton (Mège et al., 2006). Given that the actin cytoskeleton is far more rigid than the cell membrane, what would a cell gain in the composite structure, with adhesion molecules anchoring membranes together, and actin belts underneath the membrane? An analogy can be drawn to polymer adhesion, where peeling (for example, pulling up on a piece of tape) is easier than lifting off (for example, separating two rigid pieces of plexiglass). In the cell, reinforcement by the actin cytoskeleton would rigidify the membrane, placing cellular systems in a lifting-off regimen instead of a peeling regimen, and thus rendering cell adhesions more robust.

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Abbreviations used in this paper: Ena/VASP, *Drosophila* enabled/vasodilator stimulated phosphoprotein; NPF, nucleation promoting factor.

What is Ena/VASP doing anyway?

Ena/VASP proteins are localized at actin cytoskeleton hot spots in the cell (the leading edge, filopodia tips, cell-substrate contacts, cell–cell contacts), and are additionally associated with the movement of several viral and bacterial pathogens, as well as objects mimicking these pathogens (for review see Krause et al., 2003; Plastino and Sykes, 2005). Nevertheless, there is considerable controversy concerning the mode of action of Ena/VASP proteins. The main points of disagreement are summarized in Table I, and there is a partial convergence concerning only two points: (1) Ena/VASP proteins increase the protrusion of the leading edge, and the speed of propulsion of the bacteria *Listeria monocytogenes* and of objects like beads and droplets; and (2) Ena/VASP decreases the frequency of actin filament branches formed by the Arp2/3 complex in actin networks in cells and on moving surfaces. Concerning the first point, the only note of caution appears to be associated with the behavior of whole cells: Moeller et al. (2004) see decreased overall cell motility when VASP is depleted from the leading edge of cells, whereas Bear et al. (2002) see the opposite. This is not particularly surprising given the complexity of processes at work for whole cell movement, and the fact that VASP is involved not only in leading edge dynamics, but in the formation of stress fibers and cell-substrate adhesions. What is more important is that, in both cases, kymographs of protruding edge movement show more jagged profiles when VASP is present, supporting the general thesis that VASP promotes protrusion and propulsion. A similar result is observed in very different experimental conditions during repulsion from ephrin ligands (Evans et al., 2007).

Concerning the observation that VASP decreases the frequency of actin filament branches formed by the Arp2/3 complex, the only real contradictory results come from fluorescence microscopy observations of phalloidin-stabilized filaments. Using almost identical assays involving ActA-activated Arp2/3 complex in the presence or absence of VASP, Boujemaa-Paterski et al. (2001) conclude that VASP does not affect branching and

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Table I: **What is Ena/VASP doing anyway**?

	NO	YES
1. Ena/VASP enhances protrusion and propulsion speed		VASP targeted to leading edge = lamellipodia protrusion speed increases (Bear et al., 2000, 2002). Results confirmed by CALI[a] against EGFP (Vitriol et al., 2007). FAT1 knockdown to reduce VASP at leading edge = kymograph leading edge smoother (Moeller et al., 2004). *Listeria* (Loisel et al., 1999). Beads (Samarin et al., 2003; Plastino et al., 2004b). Soft beads (Trichet et al., 2007).
2. Ena/VASP inhibits formation of actin branches by the Arp2/3 complex		In solution: fluorescence microscopy, phalloidin-stabilized (Skoble et al., 2001) (conflicting study: [Boujemaa-Paterski et al., 2001], see text). In cells, electron microscopy of the leading edge (Bear et al., 2002). Comets on beads, Arp2/3 to actin ratio (Samarin et al., 2003). Comets on beads, electron microscopy (Plastino et al., 2004b).
	NO	**YES**
3. Ena/VASP nucleates actin polymerization	By pyrene assay[b], high (physiological) salt concentration (Barzik et al., 2005). *Listeria* mutants that do not recruit the Arp2/3 complex do not accumulate actin (Skoble et al., 2000). On mitochondria that target Ena/VASP proteins via the poly-proline repeats of ActA (Bear et al., 2000).	By pyrene assay[b], low salt concentration (Hüttelmaier et al., 1999; Schirenbeck et al., 2006). On beads coated with the ActA domain that binds VASP (Fradelizi et al., 2001; Plastino et al., 2004a). In conjunction with zyxin, observed by targeting zyxin to mitochondria in cells (Fradelizi et al., 2001).
4. Ena/VASP enhances barbed end elongation	By pyrene assay[b] using F-actin seeds (Bear et al., 2002). By pyrene assay[b] with actin NPFs free in solution (Samarin et al., 2003).	By pyrene assay[b] using monomeric actin (Skoble et al., 2001). By pyrene assay[b] using F-actin seeds (Barzik et al., 2005). By pyrene assay[b] with actin NPFs immobilized on beads (Samarin et al., 2003). By measuring actin incorporation into comet tails on moving beads (Plastino et al., 2004b).
5. Ena/VASP protects filament barbed ends from capping		
–Anti-capping	By pyrene assay[b] (Samarin et al., 2003).	By pyrene assay[b] (Bear et al., 2002; Barzik et al., 2005).
–Uncapping	By pyrene assay[b] (Schirenbeck et al., 2006). Lack of capture of capped barbed ends by beads coated with VASP (Bear et al., 2002)[c].	

[a]Chromophore-assisted laser inactivation.
[b]The pyrene assay is a method of monitoring the change in the amount of F-actin in a solution by following the fluorescence of pyrenyl-actin, which increases when pyrenyl-actin molecules are incorporated into the filament.
[c]In a similar study, Samarin et al. (2003) observed capture of capped barbed ends by beads coated with VASP. However, these authors concluded that this activity was due to VASP's F-actin binding activity and not to uncapping activity.

Skoble et al. (2001) arrive at the opposite conclusion. It is of note that some of the same authors of Boujemaa-Paterski et al. (2001) later published that VASP clearly decreased branch frequency in comet tails growing on bead surfaces (Samarin et al., 2003). The one difference in the two experimental set-ups of Skoble et al. (2001) and Boujemaa-Paterski et al. (2001) is the inclusion of phalloidin throughout the procedure in the case of Skoble et al. (2001), which may favor the formation of shorter filaments. Why this might change the end conclusion remains an open question.

Moving on in Table I (entries 3 and 4), we come to the controversy concerning Ena/VASP nucleation and barbed end elongation activity. The pyrene assay figures in both the "yes" and "no" columns concerning these two points, highlighting the inherent problem with this bulk polymerization assay, which measures the change in total F-actin content of the sample, but gives no information as to the number of filaments, their length, or morphology (branched vs. unbranched). As such, enhanced nucleation, enhanced filament elongation, and increased filament branching can all give the same signal by pyrene assay. At low salt concentrations in the pyrene assay, Ena/VASP shows nucleation activity due to its electrostatic interactions with G-actin (Hüttelmaier et al., 1999; Schirenbeck et al., 2006). However, this effect is not observed at high (physiological) salt by the same assay, shedding doubt on the biological relevance of this nucleation activity (Barzik et al., 2005). Nevertheless, VASP-recruiting beads in cell extract or mitochondria recruiting Ena/VASP via the poly-proline domain of zyxin in HeLa and Vero cells are seen to accumulate a cloud of actin (Fradelizi et al., 2001; Plastino et al., 2004a). Inversely, *Listeria* impaired for Arp2/3 complex recruitment, but still capable of binding Ena/VASP, do not amass actin around them when introduced into host cells, although this depends on the cell type (Skoble et al., 2000). Likewise, mitochondria recruiting Ena/VASP via the poly-proline repeats of the ActA protein in mouse fibroblasts do not accumulate actin (Bear et al., 2000). One can perhaps reconcile these conflicting data in cells and cell extracts by concluding that, although not a bona fide nucleator, Ena/VASP proteins can recruit actin seeds from cytosol and exercise barbed end polymerization enhancement, as discussed later, and that the efficiency of this process appears to be dependent on cell type.

However, the story is not completely clear concerning Ena/VASP's role in barbed end polymerization enhancement (Table I, entry 4). To evaluate this by pyrene assay, it is essential to either work at high salt concentrations and/or use F-actin seeds to avoid confusing elongation and nucleation effects. Using F-actin seeds

and high salt conditions, Barzik et al. (2005) report that VASP gives a slight increase in barbed end elongation, whereas Bear et al. (2002) report no effect. The Skoble et al. (2001) enhancement of barbed end elongation by VASP may be partly due to nucleation effects, as these studies were done in low salt conditions, using monomeric actin. When F-actin seeds are formed in situ via the ActA protein and the Arp2/3 complex, Samarin et al. (2003), like Bear et al. (2002), find that addition of VASP gives no difference in polymerization kinetics by pyrene assay. Importantly, when the exact same assay is done with ActA immobilized onto the surface of polystyrene beads, addition of VASP gives a drastic increase in the formation of F-actin by pyrene assay. Also using the bead system, but by measuring actin incorporation per unit time, Plastino et al. (2004b) likewise conclude that VASP enhances barbed end elongation at bead surfaces (In this study, beads supporting Arp2/3 complex-dependent actin polymerization at their surface and additionally recruiting VASP display comets that are four times less dense than beads that do not recruit VASP. However, because these beads move seven times more rapidly, that means that per unit time, approximately twice as much actin is incorporated at the surface of the bead recruiting VASP.). Overall, these examples illustrate the care that must be taken in relating the bulk pyrene assay, where the biochemistry of individual filaments is under study, and macroscopic assays (beads, cells, *Listeria*). These latter systems probe the collective behavior of filaments and integrate both biochemical and physical effects into the overall picture of actin network dynamics on objects in motion.

From the preceding discussion, we can conclude that, when evaluated in biologically relevant conditions, Ena/VASP proteins on their own have little or no genuine nucleation or barbed end elongation activity. However, in the presence of profilin or capping proteins, barbed end polymerization enhancement can be observed (for example in cell extracts, see Plastino et al. [2004b]). This enhancement could be due to Ena/VASP recruitment of profilin-actin, as suggested by recent structural studies (Ferron et al., 2007; Kursula et al., 2008) or due to Ena/VASP's barbed end protection activity (Bear et al., 2002; Barzik et al., 2005). Although often cited as the mode of action of Ena/VASP proteins, the data in support of barbed end protection are far from unanimous (Table I, entry 5), in part due to some confusion as to how protection could take place: prevention of capping (anti-capping activity) and removal of capping protein from barbed ends (uncapping activity). In the pyrene assays, anti-capping experiments are those where F-actin seeds are either mixed simultaneously with capping protein and VASP (Bear et al., 2002; Barzik et al., 2005) or first with VASP then capping protein (Samarin et al., 2003). Both Barzik et al. (2005) and Bear et al. (2002) see an anti-capping activity for VASP as indicated by an increase in F-actin by pyrene assay for increasing quantities of VASP in the presence of capping protein. On the other hand, Samarin et al. (2003) using a single concentration of VASP (in the range of that used in the other studies) do not see an effect of VASP competition with capping protein, neither by following pyrene increase kinetics nor by looking at final F-pyrenyl-actin concentration. This discrepancy remains unexplained, the only real difference in the Samarin et al. (2003) experimental set-up being the use of human instead of mouse VASP.

Uncapping experiments refer to those where F-actin is in contact with capping protein before VASP is added. In the pyrene assay, where F-actin seeds are preincubated with capping protein, Schirenbeck et al. (2006) see no effect of VASP on either polymerization or depolymerization of pyrenyl-F-actin. Another very visual way of evaluating uncapping activity is to coat beads with Ena/VASP proteins, mix them with capped filaments, and then evaluate filament capture by the beads. However, using this exact same assay, two different studies give opposite results (Bear et al., 2002; Samarin et al., 2003). Samarin et al. (2003) report that VASP coated beads capture uncapped and capped filaments to an equal extent. Using a gentler method that involves flowing in filaments and washes over paramagnetic beads, stuck to a coverslip by a magnetic field, Bear et al. (2002) see a clear inhibition of filament capture by VASP coated beads when filaments are capped. The drawback of these assays, in both cases, is that they are treated in a descriptive manner, without quantification, and are thus difficult to compare. We also have reservations about using centrifugation steps for this kind of analysis (as in Samarin et al., 2003), as this may compact filaments around beads irrespective of barbed end decoration. However, overall there is no evidence for a real uncapping activity of Ena/VASP proteins, as the results of Samarin et al. (2003) could equally be explained by Ena/VASP's F-actin side-binding activity (Bachmann et al., 1999; Hüttelmaier et al., 1999).

Whether Ena/VASP proteins act via anti-capping or barbed end polymerization enhancement with profilin-actin, the end result is the same: enhanced G-actin incorporation at growing filament ends and enhanced actin-based motility. We also must bear in mind that Ena/VASP proteins most certainly have additional roles, beyond polymerization enhancement. This is illustrated, for example, by the change in architecture of actin filaments in the presence of Ena/VASP proteins (Bear et al., 2002; Plastino et al., 2004b), and by the requirement for Ena/VASP bundling and G-actin binding activities for filopodia formation (Mejillano et al., 2004; Schirenbeck et al., 2006; Applewhite et al., 2007).

The interplay of Ena/VASP proteins and the Arp2/3 complex

Ena/VASP proteins and the Arp2/3 complex partially colocalize in the cell and are both associated with enhanced actin dynamics, yet as previously mentioned, Ena/VASP appears to antagonize the formation of Arp2/3 complex-based branches. It is interesting, therefore, to look at the interplay of these two molecules in a controlled manner. This can be accomplished using in vitro bead systems, mentioned in the previous discussion, that reproduce Arp2/3 complex-based actin polymerization on surfaces, in the presence of increasing surface recruitment of Ena/VASP. The actin comet tails that form on the surface provide then a read-out of Ena/VASP modulating activity.

Increasing the amount of VASP recruited to hard bead surfaces produces an abrupt transition between two regimes of movement: a low-speed phase (1.5 μm/min) with full comets composed of branched filaments and a high-speed phase (10.0 μm/min) with partially hollow comets composed of unbranched filaments, aligned in the direction of movement (Plastino et al., 2004b). Although speed increases by a factor of 7 between the two phases, comet density measurements indicate that actin

incorporation per unit time only increases by a factor of 2 (Plastino et al., 2004b; see also previous section). The abrupt speed shift, the change in filament organization, and the discrepancy between actin incorporation and speed suggests a global and drastic change in how actin filaments act on the surface to produce propulsion in the presence of VASP.

The somewhat surprising conclusion from this is that more actin is not necessarily better for movement, i.e., a hollow comet propels an object more effectively than a full comet. But why do hollow comets form in the first place? On a convex surface like a bead or bacteria, pulling forces exist at the comet center that could rip the actin filaments from the surface to produce hollowed-out comets (Plastino et al., 2004b). These comets would support more rapid movement because retarding forces at the comet center that oppose forward movement would be diminished. Hollow comets and enhanced speeds are observed only when Ena/VASP is present at the surface of beads, leading to the idea that these proteins weaken the attachment between the actin network and the actin-polymerizing proteins absorbed on the surface, thus promoting rip-off of the actin network.

Ena/VASP may exercise this effect by reducing filament branching, which in turn reduces the number of growing barbed ends abutting the surface and transiently interacting with actin nucleation promoting factors (NPFs) there. However, the exact nature of anti-branching activity remains unclear. Perhaps VASP protects the sides of the growing ends of filaments from interaction with the Arp2/3 complex or actively interferes with Arp2/3 complex branching ability (although VASP does not interact physically with the Arp2/3 complex; Boujemaa-Paterski et al., 2001). By whatever mechanism, the fact that VASP reduces filament branching by the Arp2/3 complex is one of the few points on which there is general agreement (Table I, entry 2), although definitive experiments involving direct measurements on single filaments in real time are still lacking, and should be the object of future studies.

Actin cytoskeleton moves proteins in membranes

Proteins associated with the plasma membrane can be pushed and pulled around by the actin filaments to which they are transiently attached, creating microdomains of proteins, as observed in the maturation of cadherin adhesions (Mège et al., 2006), the formation of podosomes, and in in vitro liposome systems (Co et al., 2007). We saw in the previous section that a massive reorganization of the actin network on beads is observed in the presence of VASP, begging the question as to how Ena/VASP affects protein microdomains. This is especially important given the context that Ena/VASP proteins are necessary for the formation of cadherin macroclusters in maturing cell–cell contacts (Scott et al., 2006).

On fluid surfaces (liposomes or oil droplets) undergoing actin comet-based motion, NPFs that activate the Arp2/3 complex are dragged (convected) rearward as the object advances (Giardini et al., 2003; Upadhyaya et al., 2003; Boukellal et al., 2004). However, the attachment is transient and can release, whereupon the NPFs are free to diffuse and repopulate the surface, until they associate with another filament. In very general terms, the degree of NPF polarization on a fluid surface is therefore a measure of how the actin network associates with the surface. If the attachment is very firm, or if the reattachment of diffusing NPFs is very favorable, NPFs will be highly polarized under the comet on the fluid surface. This is what is experimentally observed in the absence of VASP recruitment, while the recruitment of VASP to fluid surfaces decreases the partitioning effect (Trichet et al., 2007). VASP therefore appears to weaken the convective effect and/or strengthen the two-dimensional diffusive effect, in keeping with an overall decrease in attachment between the actin network and the surface.

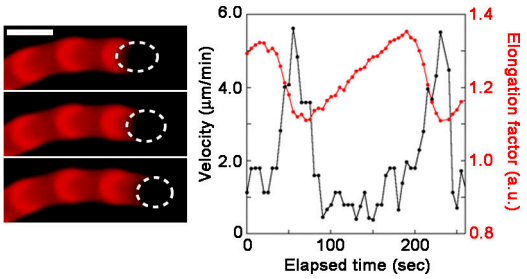

Figure 1. **Slow-and-go movement in the presence of VASP reflects cycles of near-complete separation of the actin comet from the surface.** On the left, an oil droplet just before a jump (top), during a jump (middle), and after a jump (bottom). Note the relaxation of the elongated shape upon release of contacts with the actin comet, which then appears as a rind of bright actin left behind. Actin is fluorescently labeled. Bar, 5 μm. On the right, in graphic form, the variation of the elongation of the droplet in relation to the velocity curve. Note that just before the velocity jump the elongation factor plummets, indicating that the breakage of links occurs first and then the drop jumps forward as retarding connections are released.

The importance of reduced NPF clustering and weakening of network–surface attachment is reflected in the appearance of events of near complete separation of the comet from the surface engendering slow-and-go motion, which only happens upon VASP surface recruitment (Trichet et al., 2007; and Fig. 1). In this regimen, just before the "go" phase, we can see that the rind of actin detaches from the droplet surface, and the drop begins to relax to a round shape as contact with the comet dissolves. The round shape (as quantified by a reduced elongation factor in Fig. 1) corresponds to the peak in the velocity cycle. The elongation/speed cycle can repeat with regular periodic behavior, giving droplets that bounce back and forth between round and elongated shapes, as the actin network forms and gives way concomitant with the reorganization of NPFs on the fluid droplet surface. The take-home message from jumping droplets is therefore that the physical parameters of convection and diffusion can be determining factors in actin dynamics and organization on fluid surfaces like a cell membrane, and that VASP magnifies these physical effects by playing on the attachment of filaments to the surface.

Detaching/reorganizing activity of Ena/VASP proteins in dynamic actin structures and in cell–cell adhesions

Perhaps as a mechanism of direction seeking, cell edges undergo cycles of protrusion and retraction. In a recent study (Giannone et al., 2007), the switch between a protrusive phase and a retraction phase is observed to be accompanied by a wave

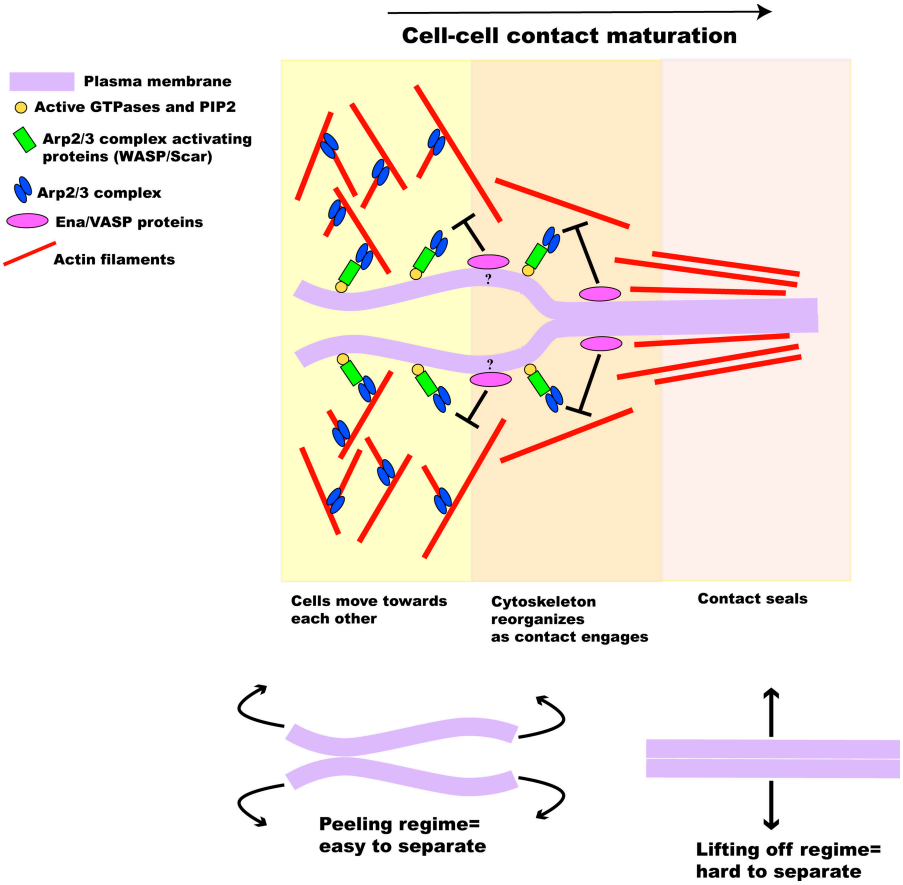

Figure 2. **Schematic representation of the possible interplay of the Arp2/3 complex and VASP for the remodeling of the branched networks that drive cells together (left) into the belts of parallel filaments that reinforce cell–cell contacts (right).** The cartoons at the bottom represent in graphic form the "peeling regimen" that is easy to undo, and the "lifting off regimen" that is hard to separate, representing, respectively, the forming contact and the mature contact reinforced by belts of actin parallel to the membrane. The question mark in the scheme indicates that it is not clear how Ena/VASP proteins are recruited to the membrane: some possibilities include via lamellipodin (Krause et al., 2004), via WASP (Castellano et al., 2001), or via αII-spectrin (Benz et al., 2008).

of detached actin network that travels back from the leading edge. In this scenario, the leading edge actin network is decomposed into two layers of actin: the lamellipodium (LP) that detaches from the membrane in periodic waves and the lamellum (LM) that underlies the LP and remains attached. Partial detachment of the actin network from the leading edge cell membrane, such as observed with jumping droplets, therefore appears to be a very real component of cell behavior, and this effect may be due to Ena/VASP proteins. Indeed, as with droplets, Ena/VASP remains associated with the plasma membrane during the rupture event (Giannone et al., 2007). It is of note that in an earlier study of cell protrusion and retraction, VASP was clearly lost from the leading edge during retraction phases (Rottner et al., 1999). However, the retraction events in the latter case were on a much larger scale, involving a collapse of several microns. In this case, there may not be enough remaining actin cytoskeleton to correctly target VASP to the membrane. In the droplet system, comet detachment is never complete (the droplet speed remains greater than zero), so VASP localization in total absence of barbed ends cannot be evaluated.

Another Ena/VASP-rich structure in the cell, the filopodium, is also a site of actin cytoskeleton rearrangements. Recent observations using cryo-electron tomography show that filaments at the tip of growing filopodia are arranged in a cone structure (Medalia et al., 2007). This radial structure must collapse, similar to the closing of an umbrella, to form the parallel filaments that make up the body of the mature filopodium. What, if any, role Ena/VASP might play in this rearrangement is unknown, but Ena/VASP family proteins are essential for correct filopodia formation (Krause et al., 2003).

In addition to its role in actin remodeling for cell motility, Ena/VASP is being increasingly implicated in cell–cell adhesion processes. Ena/VASP proteins localize with E-cadherin/catenin-enriched puncta between primary keratinocytes (Vasioukhin et al., 2000), and disruption of Ena/VASP function perturbs cadherin-associated actin bundles in CHO cells (Scott et al., 2006). In the developing *Drosophila* embryo, Ena is implicated in actin filament formation at apical adherens junctions between cells of the egg chamber (Baum and Perrimon, 2001). A recent study in *Drosophila* shows that Ena/VASP proteins are essential for epithelial zippering during dorsal closure (Gates et al., 2007), and the Ena/VASP-like protein in *Xenopus* seems to promote cell–cell adhesion during morphogenesis of the otic vesicle epithelium (Wanner and Miller, 2007). Along the same lines, Ena/VASP has been shown to be required for endothelial barrier function and correct embryogenesis in mice (Furman et al., 2007), and has been shown to stabilize cell–cell adhesion for a decrease in endothelial permeability in endothelial cells in culture (Benz et al., 2008). Overall, it appears that many cell–cell adhesion processes depend on Ena/VASP proteins for correct execution.

At first glance, it seems contradictory that a protein implicated in de-adhesion of the actin cytoskeleton from the membrane could play an important role in cell–cell adhesion.

However, it is now known that the link between the actin cytoskeleton and cell–cell contacts is not static, but very transient (Yamada et al., 2005). In fact, the ternary complex of β-catenin/α-catenin/actin does not exist in a stable fashion: dimeric α-catenin binds to actin but not β-catenin, whereas monomeric α-catenin binds to β-catenin but not actin (Drees et al., 2005). In this context, a detaching activity for Ena/VASP in cell–cell adhesion processes could help maintain a slack connection between the actin cytoskeleton and membrane, a feature that may be essential for morphogenetic changes during cell and tissue development.

Along the same lines, Ena/VASP may be important for the formation of mature contacts in the first place, as the Arp2/3-branched actin arrays that drive cells together must collapse into the actin belts that characterize mature cell–cell junctions (Fig. 2). Dimeric α-catenin has been hypothesized to play a role in this process, due to its inhibition of Arp2/3-complex dependent branching probably via competition for sites on F-actin (Drees et al., 2005). Due to their anti-branching activity, Ena/VASP proteins may play an overlapping role with dimeric α-catenin in this regard. A similar conclusion was arrived at in the study of epidermal morphogenesis of mutant *Caenorhabditis elegans*, where the presence of UNC-34 (the *C. elegans* Ena/VASP homologue) partially compensated for impaired α-catenin function (Sheffield et al., 2007). It is of note that collapse of unbranched filaments parallel to the plasma membrane has been observed upon over-recruitment of Ena/VASP at the leading edge of moving fibroblasts (Bear et al., 2002). Fig. 2 illustrates how this collapse, tuned by Ena/VASP, could be responsible for the "peeling off" to "lifting off" transition, defined in the first section, which fortifies cell–cell adhesions.

Conclusion

Overall, it is apparent that detachment and remodeling of actin filaments under the plasma membrane and concomitant reorganization of plasma membrane proteins is important for key cellular processes, including the cell shape changes associated with the developing embryo. Ena/VASP proteins are prime candidates for powering some of these changes in the actin network, although simple barbed end elongation enhancement, whether by anti-capping activity or by profilin-actin recruitment, does not seem to explain all the available data. We propose that an additional, global effect of Ena/VASP proteins in the cell is the detachment of the actin cytoskeleton from the surface where polymerization is occurring. We hypothesize that the detaching activity of Ena/VASP may originate in its anti-branching activity, which reduces the number of growing barbed ends abutting the surface. Anti-branching is additionally implicated in the observed remodeling of Ena/VASP-associated networks into parallel arrays.

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