



Published in final edited form as:

*Curr Opin Cell Biol.* 2009 October ; 21(5): 684–693. doi:10.1016/j.ceb.2009.06.004.

## Control of cell adhesion dynamics by Rap1 signaling

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### Summary

Individual cells in their particular environments adhere to the extracellular matrix (ECM) and their neighbors via integrin- and cadherin-containing complexes, respectively. The dynamics of these interactions regulate the formation and maintenance of complex tissues. An expanding body of evidence accentuates the role of the small Rap1 GTPase and its associated signaling network in many of these processes. In this review we will discuss more recently revealed roles of Rap1 signaling by primarily focusing on functions of the Rap1 effectors RIAM, KRIT-1/CCM1 and AF-6/Afadin in junctional regulation of the vascular system and in epithelial cells. Furthermore, we will describe novel findings on the Rap activator PDZ-GEF in the regulation of cell-cell adhesion between epithelial cells and within a stem cell niche.

### Introduction

Integrin-mediated adhesion to the ECM and cadherin-mediated adhesion between cells within developmental and physiological compartments are dynamically regulated. Consequently, failures in efficient adhesion control can result in pathogenic conditions. Interestingly, signaling through the small Rap1 GTPase has been implicated in both integrin- and cadherin-mediated adhesion events. As a member of the Ras superfamily of small GTPases, Rap1 is subject to a GTP/GDP exchange cycle in which dedicated Rap1 specific guanine nucleotide exchange factors (GEFs) promote the exchange of GDP to GTP, thereby converting Rap1 into an active signaling competent form. The latter in turn triggers spacio-temporally defined effector signaling that can stimulate a variety of cellular events. Rap1 GTPase activating proteins (GAPs), on the other hand, terminate the signal output through Rap1 by enhancing its intrinsic rate of GTP hydrolysis. Several Rap1 GEFs, such as C3G, and members of the Epac, CalDAG-GEF and PDZ-GEF subfamilies have been identified, which have been implicated in the regulation of adhesion complexes [1,2]. Also, several Rap1 effectors have been ascribed to adhesion complexes [1,2]. In particular, recent light has been shed on the RIAM, KRIT-1/CCM1 and AF-6 proteins and the molecular machineries they impinge on. In this review we will exemplify these by illustrating them in the experimental model systems that enabled their discovery. These include genetically tractable organisms as well as specifically tailored cell culture systems.

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## Emerging mechanisms for Rap1 signaling in integrin activation

Rap1 has been implicated in a variety of integrin-mediated 'inside-out' signaling events. In particular, Rap1 regulates integrin  $\beta 1$ ,  $\beta 2$  and  $\beta 3$  subunits to affect both integrin affinity and avidity (clustering) [1]. A recent series of observations has unraveled a Rap1-dependent mechanism that could explain how agonist-induced stimulation of platelets modulates their interaction with extracellular ligands. The details of this mechanism likely will be applicable to a number of cell types. Due to their property to spread and aggregate, platelets protect the vascular system against damage. Agonists, like thrombin, are released or activated at sites of vascular injury and activate G-protein coupled receptors and protein kinase C $\alpha$  (PKC $\alpha$ ) to trigger the release of intracellular Ca<sup>2+</sup> [3]. As a result  $\alpha$ IIb $\beta$ 3 integrins increase their affinity for extracellular ligands, such as Willebrand factor, fibrinogen or fibronectin to enable a clotting reaction and the formation of a thrombus. Han et al. were able to reconstitute the integrin activation process by engineering Chinese Hamster Ovary (CHO) cells that stably express  $\alpha$ IIb $\beta$ 3 integrin dimers, ectopic talin and PKC $\alpha$  and found them to respond to the PKC agonist Phorbol Myristate Acetate (PMA) [4••]. Talin gets recruited to integrin cytoplasmic tails in the activation process and establishes one of numerous integrin-cytoskeletal linkages [5]. The relevance of talin for cell-to-matrix adhesion is reflected by the inability of talin-1 deficient embryonic stem cells and fibroblasts to properly link integrins to the actin cytoskeleton, assemble focal adhesions, and spread on a permissive ECM substrate [6,7]. Moreover, conditional disruption of talin in platelets or megakaryocytes blocks  $\alpha$ IIb $\beta$ 3 activation and results in a bleeding diathesis in mice [8]. Talin is built of an N-terminal globular head and a C-terminal rod-like tail domain [5]. Within its head portion, talin harbors a FERM-domain that can associate with integrin  $\beta$  cytoplasmic tails. An additional integrin binding motif, as well as vinculin and actin binding sites, reside in the rod part of the protein. According to current models, the integrin interacting motifs in talin are functionally masked in cells with disengaged integrins, probably due to an intra- or inter-molecular interaction [9]. In its autoinhibited conformation, talin localizes to the cytoplasm and only after agonist stimulation translocates to integrin complexes [10]. Hence, the question how talin becomes 'unlocked' and stabilized in its open conformation, and thus rendered competent for productive integrin interaction is of central importance. Rap1 had been shown to be activated in platelets in response to thrombin stimulation [11], and, indeed, expression of Rap1GAP in reconstituted CHO cells inhibits  $\alpha$ IIb $\beta$ 3 integrin activation in response to PMA. Conversely, constitutively active Rap1 can bring about integrin activation independently of PKC $\alpha$  and in the absence of PMA. In this process, Rap1 strictly depends on talin, since Rap1-induced integrin activation is blocked by an integrin binding-deficient talin mutant and can be bypassed by an 'activated' talin fragment derived from its head domain. Rap1 causes talin to translocate from the cytoplasm into integrin complexes. Surprisingly, however, Rap1 does not stimulate talin phosphorylation or proteolytic cleavage between talin's head and rod domains, which are two vividly discussed modes of talin activation [4••,9]. Thus, one explanation for Rap1's effect on talin could be the induction of a conformational change that allows for translocation and integrin activation.

Further illuminating the mechanism is the finding that Rap1's ability to activate integrins in CHO cells hinges on the function of RIAM as an effector molecule [4••]. RIAM (for Rap1-GTP-interacting adaptor molecule), originally isolated by yeast-two-hybrid screening, is characterized by an RA-domain, which mediates its interaction with Rap1 [12•]. The protein sequence located N-terminally of RIAM's RA domain harbors a proline-rich patch bracketed by two potential coiled-coil regions. Further C-terminal of the RA-domain reside a PH-domain and a second proline-rich region (Figure 1A). RIAM belongs to the MRL (Mig-10/RIAM/Lamellipodin) family of adaptor proteins, with members being present in organisms ranging from *C. elegans* and *Drosophila* to humans. When overexpressed, RIAM can induce cells to spread and form lamellipodia as well as activate  $\beta 1$  and  $\beta 2$ -containing integrin complexes

[12•]. Interestingly, RIAM co-immunoprecipitates with talin and both proteins co-localize to focal adhesions (FA) early in their formation. Importantly, ectopic expression of RIAM actively triggers talin-dependent integrin activation [4••]. Conversely, RIAM depletion in reconstituted CHO cells effectively decreases both Rap1<sup>V12</sup> and thrombin receptor/Protease Activated Receptor (PAR)-induced integrin activation. Also RNAi against RIAM in Jurkat T-cells inhibits integrin adhesion to extracellular fibronectin [4••,12•,13•]. The nature of the Rap1/RIAM interaction, however, suggests some level of complexity, since RIAM - in at least one setting - was able to modulate the distribution of Rap1 itself. RIAM's down-regulation by RNAi coincided with a displacement of endogenous Rap1 from the plasma membrane [12•]. In a different set of experiments, expression of a fusion between a small motif in RIAM's N-terminus predicted to form an amphipathic helix and the C-terminal membrane-targeting CAAX box of Rap1, is sufficient to translocate talin to integrin complexes [14]. While the intermolecular dependencies are not completely understood, it is likely that RIAM provides a scaffolding function by binding to Rap1, which enables subsequent talin tethering and integrin activation (Figure 1B). Watanabe and colleagues corroborated this model by live cell experiments, in which they used elegant bimolecular fluorescence complementation (BiFC) microscopy with split moieties of the Venus protein. Expression of the N-terminal half of Venus fused to the N-terminus of talin and the C-terminal half of Venus fused to the  $\alpha$ IIB integrin subunit reconstitutes Venus-specific fluorescence when an integrin-activating signal is relayed via talin. This bimolecular complementation signal is enhanced by the presence of constitutively active Rap1<sup>V12</sup> or over-expressed RIAM. Conversely, the signal can be dampened by RNAi-mediated down-regulation of RIAM in cells whose PAR receptors are activated by the addition of thrombin [13•]. The above assay represents a sophisticated experimental tool that could be employed to examine the contribution of individual RapGEF proteins, and varied to further decipher the role of Rap1 signaling networks in various integrin activation events. Although not investigated yet in Rap1/RIAM/talin-dependent  $\alpha$ IIB $\beta$ 3 integrin activation in thrombin-responsive CHO cells or platelets, a relevant RapGEF required for Rap1 activation could be CalDAG-GEF1. CalDAG-GEF1 is responsive to Ca<sup>2+</sup> and diacylglycerol (DAG), and knock-down or knock-out of CalDAG-GEF1 interferes with  $\alpha$ IIB $\beta$ 3 activation [15,16].

Comparative experiments showed that the closely related RIAM homologue Lamellipodin (Lpd) has no effect on Rap1's potential to activate integrins, and an interaction between Rap1 and Lpd *in vivo* so far has not been demonstrated [13•,17]. Since also Lamellipodin contains a well-conserved RA-domain it is possible that it enters into a complex with another Ras family GTPase. Such an interaction might either regulate adhesion events or be otherwise required. It is interesting to note that many integrin-mediated events that induce gross morphological changes in cells, like e.g. motility in migration processes, are associated with an expansion and/or treadmilling of the integrin linked actin cytoskeleton. Both, RIAM and Lpd stimulate actin polymerization through their affinity to members of the Ena/VASP family, which catalyze actin assembly and branching [12•,17]. Also, the *C. elegans* MRL protein Mig-10 uses its affinity to Ena/VASP to promote axon guidance and outgrowth [18]. Depletion of RIAM can markedly increase the cellular ratio between monomeric G-actin and filamentous F-actin [12•,17]. It is thus tempting to speculate that the talin-activating role of RIAM is coupled to local changes in actin assembly. Many of the constituents in integrin complexes actively associate with actin to stabilize the interaction with ECM ligands. Noteworthy, the only MRL representative in *Drosophila*, Pico, also has the ability to shift G:F-actin ratios toward the polymerized state due to a direct effect on Ena [19•]. Importantly, this in turn stimulates the serum response factor (SRF) to provoke tissue growth. In agreement with this, loss of *Pico* inhibits growth of the entire organism, while ectopic activity of *Pico* can produce over-growth phenotypes [19•]. These findings taken together demonstrate an unanticipated versatility within the MRL family and imply that actin-related activities proximal to adhesion complexes could be coupled to growth regulation of the whole organism.

Rap1 signaling can engage different mechanisms of integrin activation depending on the specific complex and cell type under consideration. Besides RIAM, also the Rap1 effector RapL has been implicated in integrin activation. In lymphoid cells, Rap1 promotes the translocation of RapL to  $\alpha$ L $\beta$ 2-type LFA-1 integrin receptors, which consequently become activated [20,21]. In *RapL* deficient mice, lymphocyte adhesion and migration as well as lymphocyte homing are drastically impaired [22]. Another level of complexity is given by the fact that different RapGEFs can activate Rap1 to promote integrin-mediated adhesion. As indicated above, CalDAG-GEF1 affects integrin adhesion in platelets and megakaryocytes [15,16]. However, in different biological contexts other GEFs mediate Rap1 activation or even work in concert with each other. For example, in fibroblasts that lack C3G, integrin-mediated adhesion is ineffective and cells show an ectopic migration behavior. CalDAG-GEF1 and Epac can both compensate for C3G in these circumstances [23]. Expression of a dominant-negative C3G protein also interferes with cell scattering and adhesion induced by hepatocyte growth factor (HGF) [24]. Interestingly, also the *Drosophila* paralogue of the PDZ-GEF proteins mediates integrin-dependent cell shape changes of hemocytes in the fly embryo [25]. Thus, different GEFs may control Rap1 in its capacity to activate the integrin-ECM adhesion switch and the precise requirements and contributions will have to be further dissected.

### Diverse roles for Rap1 in the endothelium

Research in different arenas has revealed functions for Rap1 in the endothelium and in cells that interact with it. For example, Rap1 regulates adhesion among endothelial cells and also has functions in lymphoid cells that are involved in transendothelial migration [1,26]. Tight regulation of angiogenesis and permeability of differentiated blood vessels is essential to the homeostasis of any higher organism. Consequently, failures in establishing a normal vasculature, in adjusting its architecture to varying oxygen requirements and in maintaining its integrity can have detrimental consequences [27]. Also, the microvasculature in developing tumors appears to be especially refractile to mechanical stress with a compounding effect on overall tumor etiology [28]. Central for endothelial function are VE-cadherin-based adherens junctions (AJ), and Rap1 signaling has been implicated in their differentiation and maintenance [2]. Among the Rap1GEFs, Epac promotes endothelial barrier formation in response to cAMP. The Epac-specific cAMP analog 8CPT-2'-O-Me-cAMP (007) is able to activate Rap1 to increase permeability of cultured endothelial monolayers in a VE-cadherin-dependent manner [29–32]. The structural basis for Epac activation, its translocation to the membrane and activation of Rap1 has recently been revealed by crystallographic and detailed microcopic studies [33,34]. In human umbilical vein endothelial cells (HUVECs) and human arterial endothelial cells (HAECs), VE-cadherin also recruits the scaffolding protein MAGI-1 to AJs. The MAGI-1 protein is linked to  $\beta$ -catenin and PDZ-GEF1, another RapGEF [35]. These interactions are relevant for VE-cadherin mediated adhesion since depletion of MAGI-1 in HUVECs reduces their adhesive potential [35]. Moreover, vascular development within the yolk sac and embryo is disrupted in PDZ-GEF1 mutant mice [36]. Thus, different aspects of AJ maturation and stability in the endothelium may be regulated by different Rap1 activating mechanisms.

So far, the selectivity of RapGEFs towards specific Rap1 effectors has been insufficiently resolved. However, two effectors that reside in AJs are the KRIT-1/CCM1 and AF-6/Afadin proteins. Recent evidence has clearly implicated KRIT-1/CCM1 in the morphogenesis and homeostasis of the vascular system. Mutations in the human *KRIT-1* gene (*KRIT-1* stands for Rap1/Krev1 interaction trapped 1; [37]) are a cause of cerebral cavernous malformations (CCMs) [38]. CCMs are vascular dysplasias arising spontaneously or due to familial, autosomal, dominant mutations in 3 genes that so far have been found causative for the disease, *KRIT-1/CCM1*, *MGC4607/CCM2* and *PDCD10/CCM3* [38–41]. Hallmarks of CCMs are densely packed conglomerates of thin-walled dilated vessels that are enveloped by a collagen

layer and often leak blood. CCMs develop mainly in the central nervous system [42,43], however, they also occur systemically in other parts of the body [44]. Animal studies in mice and zebrafish demonstrated that exclusive loss of CCM function in the endothelial compartment of mice and zebrafish results in heart, arterial and venous malformations [27]. However, in light of the low frequency with which hemorrhagic lesions occur in mice heterozygous for *KRIT-1*, it has been suggested that additional mutations are necessary for a phenotypic onset. Indeed, in a recently developed mouse model the incidence rate of vascular lesions evoked by *KRIT-1* heterozygosity is significantly elevated in a *p53*<sup>-/-</sup> background. Because the remaining wild type allele of *KRIT-1* remained unaffected in the destabilized genome, either a *p53*-dependent mechanism or yet unidentified mutations must interact with mutant *KRIT-1* in mice [45]. This model may empower the search for cooperating players by genetic means.

The KRIT-1 protein is present at low amounts in AJs within the human endothelium [46] and in cultured arterial (BAECs) and venous (UVECs) endothelial cells [47••]. Importantly, the level of KRIT-1 in endothelial AJs correlates with the permeability of endothelial cell monolayers. KRIT-1 immunoprecipitates in a complex with VE-cadherin,  $\beta$ -catenin, p120catenin and AF-6, and its concentration in AJs is augmented in the presence of constitutively active Rap1<sup>V12</sup> [47••]. BAECs treated with thrombin loose KRIT-1 from their AJs, which is reversed by addition of the cAMP analog 8CPT-2'O-Me-cAMP, supporting the notion that Epac and Rap1 stimulate KRIT-1's translocation to cell-cell contacts. Structurally, KRIT-1 is lacking a consensus RA-domain that characterizes most Ras/Rap effectors. Instead, it features 4 centrally located ankyrin repeats and a C-terminal FERM-domain (Figure 1A), the latter of which has been demonstrated to mediate KRIT-1's localization to AJs as well as its interaction with Rap1. These binding characteristics evoke a possible mechanism in which Rap1 binding increases the accessibility of KRIT-1's FERM domain to adherens junctional partners which in turn may promote junctional maturation and/or integrity [47••].

Endothelial junctions, like epithelial junctions, are strengthened by a prominent actin cytoskeleton and the extent of actin polymerization in the proximity of junctions governs the permeability of the endothelium [32,48,49]. In fact, over-expression of KRIT-1 elevates the levels of junction-associated F-actin whereas depletion of KRIT-1 provokes a loss of F-actin at AJs [47••]. How KRIT-1 interacts with junctional partners and triggers actin polymerization are pertinent questions. First clues are provided by observations that KRIT-1 is found in a complex with other CCM proteins [47••,50–52]. In particular, a phospho-tyrosine binding (PTB) domain in CCM2 couples to a consensus NPxY motif in KRIT-1, the relevance of which is testified by a familial missense mutation in CCM2 that specifically disrupts this interaction [51,53]. It should be noted that CCM2 binds in an analogous way to ICAP1, a  $\beta$ 1 integrin interacting protein [54], suggesting that CCM proteins might have distinct functions in VE-cadherin and integrin-dependent adhesion complexes [53,55]. Like in KRIT-1 depleted cells, the AJ-associated cortical actin cytoskeleton cannot be maintained in CCM2-depleted cells, and instead stress fibers are formed, with the result of an impaired barrier function [56]•. The CCM2 mutant cytoskeletal phenotype correlates with an increase in RhoA activity and elevated stress signaling via JNK. RhoA and Rac1 both coimmunoprecipitate with CCM2 from endothelial cell extracts, and, importantly, inhibition of RhoA activity with simvastatin, a compound which blocks RhoA isoprenylation, partially eliminates the amount of stress fibers and suppresses permeability defects in cells with depleted CCM2. Interestingly, in CCM2<sup>-/-</sup> mice treated with VEGF, simvastatin normalizes the vascular permeability response [56]. Thus, it is tempting to speculate that the CCM protein complex is activated by Rap1 to locally dampen RhoA signaling during vascular modeling.

An interesting functional link has recently been revealed by the analysis of the *heart of glass* (*Heg1*) gene in mice and zebrafish. Similar to CCM mutants, *Heg1*<sup>-/-</sup> animals experience

serious defects in heart and blood vessel development and interestingly *Heg1* knockdown in the zebrafish synergizes with that of *KRIT-1* and *CCM2* [57]. Although no vascular defects in *Heg1*<sup>-/-</sup> mice were observed before midgestation, *Heg1*<sup>-/-</sup>; *CCM2*<sup>+/-</sup> compound mutant zebrafish die earlier with an abnormal heart and vasculature [58••]. Transmission electron microscopy revealed that *Heg1*<sup>-/-</sup>; *CCM2*<sup>+/-</sup> mutant endothelia display significantly shortened AJs, suggesting a direct role for *Heg1*/*CCM* signaling in junctional formation and maintenance. In addition, depletion of *CCM2* in cultured HUVECs interferes with spontaneous lumen formation of single cells [56,58••]. In zebrafish embryos, *Heg1* loss of function, in combination with a reduced dosage at the *CCM2* locus, inhibits endothelial cells to form lumenized tubes [58••].

Interestingly, biochemical experiments showed that *CCM2* is recruited into a complex with *Heg1* in a *KRIT-1*-dependent manner. Owing to its ability to form a complex with AJ proteins like  $\beta$ -catenin and AF-6, *KRIT-1* may function to bridge the intracellular complexes associated with the AJ and the *Heg1* membrane protein. (Figure 1C). Based on the above phenotypical and biochemical findings, it is tempting to speculate that a Rap1 regulated *CCM*/*Heg1* complex affects AJ maturation and lumen formation in the differentiating vasculature. Rap1 in endothelial junctions might even undergo consecutive interactions with AF-6 and *KRIT-1* to regulate VE-cadherin function and mediate crosstalk between *Heg1* and VE-cadherin. It also will be interesting to see whether a human *Heg1* orthologue will play a role in the etiology of *CCMs*.

## Lessons from invertebrates

### Rap1 functions in cell shape determination

Many developmental episodes in embryogenesis and organogenesis rely on the precise orchestration of cell shape changes, among which gastrulation offers often striking examples. To accomplish gastrulation processes, cells need to invaginate, intercalate, elongate or migrate, all of which likely require AJs to be modeled through changes in their associated proteins, including cytoskeletal components [59,60]. Specifically, work in *Drosophila* embryogenesis indicated that Rap1 participates in developmental processes that are distinguished by diverse shape alterations. These include the ventral invagination of the presumptive mesoderm, dorsal closure (DC) of the bilateral ectoderm, and the migration of primordial germ cells [61]. Although direct mechanistic proof is still lacking, it seems likely that signal transduction through Rap1 at least in part impinges on E-cadherin function to modulate these processes. Significantly, observations in proliferating epithelia indicate that Rap1 activity promotes AJ continuity. Rap1 accumulates in the cytokinetic furrow to specifically remodel the invaginating AJs on the mother cell's membrane into separated junctional rings surrounding both sister cell's apical membrane domains, thereby guarding AJ continuity over the entire tissue [62••].

Evidence from *Drosophila* DC has connected Rap1 to the Canoe (Cno) protein, the fly orthologue of AF-6 [63•]. Both genes genetically cooperate in embryos proceeding through DC. *cno* loss of function and expression of dominant negative Rap1<sup>N17</sup> both provoke penetrant 'dorsal open' phenotypes in which the dorsalward migration of the bilateral ectoderm is blocked. Importantly, over-expression of a wild-type *cno* transgene can rescue all ectodermal defects produced by Rap1<sup>N17</sup>, while an N-terminal deletion mutant of Cno, which lacks the two RA-domains (Figure 1A) required for Rap1 interaction, can not. These data indicate that Cno operates downstream of Rap1 in DC by serving a bona fide effector function [63•]. More recently, in genetic interaction and epistasis experiments, *DPDZ-GEF*, the fly PDZ-GEF counterpart, has been placed upstream of Rap1 in DC. In agreement with this, *DPDZ-GEF* loss-of-function is associated with abnormalities in DC, which can be overcome by ectopic expression of both Rap1 and Cno. Interestingly, this newly defined *DPDZ-GEF*/*Rap1*/*Cno* module regulates MyosinII function(s) in DC. MyosinII-dependent cell stretching and the

assembly of a force-generating MyosinII purse-string cable in the dorsal most rows of cells within the bilateral ectoderm are disrupted by a dysfunction of the module. This relationship is corroborated by strong genetic interactions between *DPDZ-GEF* and *cno* with the MyosinII encoding *zipper* gene [64•]. MyosinII in other morphogenetic processes, such as extension of the germband in *Drosophila* embryogenesis, modulates E-cadherin plasticity directly [65]. Therefore, while Rap1 signaling during DC, and maybe also at earlier stages does not significantly affect the levels of AJ proteins, it may modulate AJ plasticity via MyosinII. How Rap1 signaling affects MyosinII function in DC and whether MyosinII is indeed involved in AJ remodeling during embryonic closure processes are important issues, especially in light of the remarkable resemblances between DC and certain wound healing processes in mammals. Intriguingly, the mammalian PDZ-GEF2 protein recently has been implicated in the maturation of AJs of A549 lung carcinoma and primary endothelial cells [66•]. Depletion of PDZ-GEF2 results in a zipper-like morphology of AJs with frequent gaps between the AJ strands on juxtaposed cells. Compared to wild type cells, PDZ-GEF2 deficient cells have lowered levels of surface E-cadherin. This phenotype is reminiscent of immature AJs that form after initial cell-cell contact. In PDZ-GEF2-depleted cells, however, the AJs are not transitioning to a mature, wild type morphology with opposing strands running in parallel and forming a tightly sealed apical belt [66•]. The differences in effects on E-cadherin levels between the PDZ-GEF2 knock-down study in human lung carcinoma cells and those observed in *DPDZ-GEF* mutant *Drosophila* may be related to the fundamentally different mechanisms that regulate AJ organization in mammalian cells grown in culture and *Drosophila* embryogenesis.

Another paradigm that allowed recent insight into Rap1's functions in epithelial morphogenesis is the ventral closure (VC) process in the *C. elegans* embryo. Surprisingly, in the nematode, zygotic elimination of *Rap1* produces a significant fraction of viable and fertile animals that proceed through all developmental stages whereas the remaining lethal fraction of embryos displays a defective hypodermis [67••,68]. Frische and co-workers performed an RNAi screen to identify factors that cooperate with *Rap1* and that when markedly reduced give rise to synthetic lethality. This approach identified several components of the Ral-1/exocyst pathway, including *RalGDS* (encoding a RalGEF), *RalA*, *Exo84* and *sec5*. RNAi against these exocytosis-relevant molecules in *Rap1* null embryos provokes a synthetic block in hypodermal migration. Interestingly, in *Rap1* mutant embryos with reduced *RalA*, *Sec5* or *Exo84* expression,  $\alpha$ -catenin was not detectible in defined adherens junctional structures. Thus, in *C.elegans* the exocyst in conjunction with Rap1 is required for delivery of AJ components to the basolateral membrane [67••]. The question of whether and how Rap1 could be biochemically linked to RalA/exocyst function still needs to be answered. The exocyst certainly has additional functions separate from Rap1, raising the possibility that Rap1 signaling could either activate a specific subcomplex of the exocyst or converge with it further downstream to control AJ levels and hypodermal migration. A candidate for a relevant Rap1 activator in *C. elegans* VC is Pxf-1, the worm PDZ-GEF orthologue. Pxf-1 is strongly expressed in the hypodermis and *pxf-1* mutants display a highly abnormal hypodermis that resembles that of defective *Rap1*; *Rap2* double mutant embryos. Importantly, this phenotype can be rescued with a constitutively active *Rap1* transgene [68]. Until now, however, interactions between *pxf-1* and the RalA/exocyst pathway have not been reported. Polarized secretion is an absolute requirement for the generation of the embryonic epithelium in *Drosophila*, and vice versa, VC of the *C. elegans* embryo also relies on a purse-string-like structure in a row of 6 cells that follows the two pairs of leading cells [60]. Future work should reveal whether DC in *Drosophila* and VC in *C. elegans* have more in common than is currently known or whether both processes evolved on the basis of separate strategies.

## Adherens junction formation in a stem cell niche

Adherens junctional control by Rap1 not only establishes cohesion between identical cell types in a single tissue, but also provides for adhesion between cells of different developmental lineages. An example is that of stem cell adhesion in their somatic niche. In both *Drosophila* males and females, homozygous loss of the Rap1 activator *DPDZ-GEF* causes sterility in rarely surviving adults [69,70••]. While *DPDZ-GEF* needs to be better analyzed in mutant ovaries, the investigation of *DPDZ-GEF* in the male germ line has allowed for a more comprehensive view. Here, both germ line stem cells (GSCs), and their encysting somatic stem cells (SSCs) adhere to the hub, a cluster of postmitotic somatic cells [71]. *DPDZ-GEF* deficiency results in a stem cell loss phenotype in which selectively GSCs and SSCs are lost whereas the hub remains relatively intact. In agreement with this, *DPDZ-GEF* mutant germ cells can be seen to drift away from the hub and lose their identity, probably owing to a lack of stem cell regulating factors, such as ligands of the Jak/Stat and TGF $\beta$  family that are produced by the hub. A mild drifting effect produced by partial loss of *DPDZ-GEF* expression is markedly amplified by either *Rap1* or *Rap21* (encoding the fly Rap2 orthologue) heterozygosity [70••]. Importantly, the hub/stem cell interface in wild type testis displays high levels of AJ components [72]. These are markedly reduced in *DPDZ-GEF* mutant testis, whereas AJs between hub cells appear unaffected. Expression of constitutively active Rap1<sup>V12</sup> is able to significantly rescue both AJ protein levels at the interface and stem cell loss. The defects are also counteracted by ectopic expression of E-cadherin, which strongly suggests a scenario in which *DPDZ-GEF*/Rap signaling recruits AJ components to the hub/stem cell interface [70••]. *DPDZ-GEF* mutant females carry supernumerary spermathecae, the receptacles that store the male sperm. Although not investigated with cellular resolution yet, genetic evidence also here suggests a signal transducing mechanism that operates via *DPDZ-GEF* and Rap GTPases to control AJs [73]. Interestingly, AJs at the hub/stem cell interface are sensitive to *DPDZ-GEF* loss, suggesting an as yet not understood asymmetry in junction formation. Knox and Brown have demonstrated an indispensable role for *Rap1* in AJ remodeling between sister cells late in cytokinesis. *Rap1* mutant cells fail to locally reconstitute AJs and start to abnormally disperse within the epithelium [62••]. Certainly, adhesion of stem cells to their niche and AJ remodeling in the final stages of cytokinesis are vastly different from each other, one depending on adhesion of one cell type to another, and the other representing a fundamental element of epithelial cell proliferation. It will, however, be interesting to see if future research will identify common factors that regulate AJs in both developmental processes. One obvious candidate for a Rap1 effector in both processes is Cno/AF-6, which localizes to epithelial AJs [63•,74]. Underscoring the relevance of AF-6 for AJ biology are observations that AF-6 gene disruptions in mice cause severe failures in AJ formation and/or maintenance [75,76]. AF-6 with its PDZ-domain binds to the cytoplasmic tail of Nectins, and Cno at least *in vitro* can associate with Echinoid, a Nectin-like protein in *Drosophila* AJs [77,78]. In addition, AF-6 interacts with the actin regulator profilin and associates with F-actin filaments [79,80]. In light of these interactions, the role of AF-6 and Cno in AJs will have to be further defined.

## Conclusions

Rap1 function is integral to signaling pathways that regulate various aspects of different adhesion systems. Different Rap1 GEFs often operate in both integrin and cadherin-based junctional complexes suggesting still unappreciated cross-talking mechanisms. Through its ability to activate several key effector proteins such as RIAM, KRIT-1/CCM and AF-6/Cno, Rap1 can help assemble adhesion-relevant protein complexes. The functions of individual GEFs and effectors are beginning to be resolved and open up experimental opportunities for further expansion into the Rap1 signaling network. Genetic modeling of diseases, like CCM in mice and zebrafish and mutational analysis in invertebrates start to yield *in vivo* evidence for particular Rap1 responsive machineries. Integrated with advanced cell biological



techniques and biochemical assays, they will help to further unravel the Rap1 signaling network in biologically and clinically important processes.

## Acknowledgments

We are grateful for Jim Duffy for help with preparation of the figure. Our work described in this review was supported by NIH grant R01-CA096882 to L.V.A., and B.B. is a recipient of an NIH postdoctoral training grant (S T32-CA009176). We apologize to those colleagues whose work we did not discuss here.

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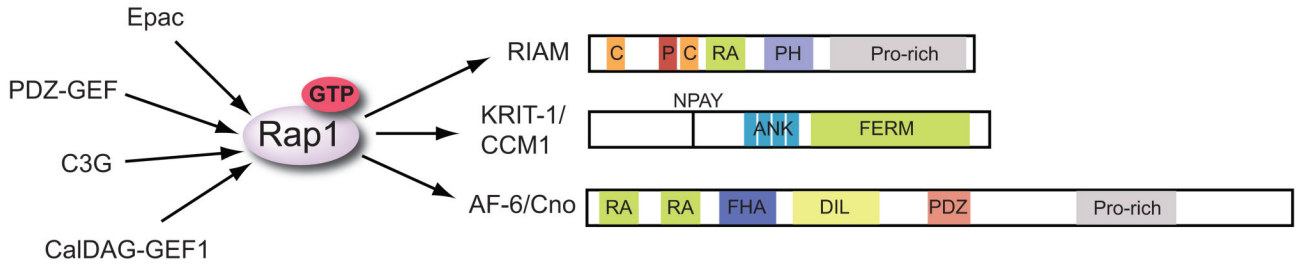
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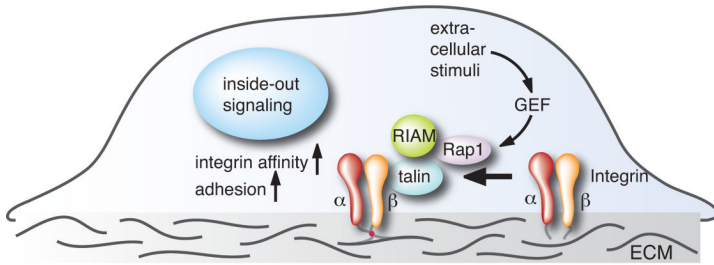
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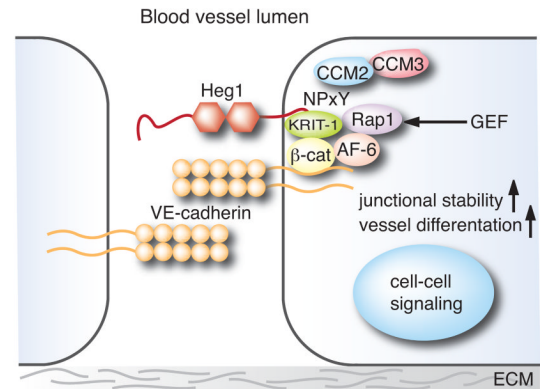
(a)



(b)



(c)



**Figure 1.**

(A) Rap1 is activated by a group of highly specific GEFs that perform a GDP/GTP nucleotide exchange reaction. Loaded with GTP, Rap1 is competent to associate with effector proteins to initiate downstream signaling. The RIAM, KRIT/CCM1 and AF-6 protein are distinguished by various scaffolding domains: C, coiled-coil; P, proline-rich; RA, Ras associating; PH, Pleckstrin homology; ANK, Ankyrin repeat; FERM, band 4.1/Ezrin/Radixin/Moesin homology; FHA, fork head associated; DIL, dilute/Myo5 homology and PDZ, PSD-95/Discs large/ZO-1 domains. (B) Schematic diagram of the RIAM and talin-dependent effect of Rap1 on integrin complexes and their increased affinity to ECM molecules. (C) Schematic view of the proteins that act downstream of Rap1 in endothelial junctions. All diagrams are incomplete depictions of Rap1-interacting proteins and complexes and restricted to the molecules referred to in the text.