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Spatial segregation between cell–cell and cell–matrix adhesions

Mithila Burute and Manuel Thery

Cell–cell adhesion (CCA) and cell–matrix adhesion (CMA) play determinant roles in the architecture and function of epithelial cells. CCA and CMA are supported by transmembrane molecular complexes that dynamically interact with the extracellular environment and the cell cytoskeleton. Although those complexes have distinct functions, they are involved in a continuous crosstalk. In epithelia, CCA and CMA segregate in distinct regions of the cell surface and thereby take part in cell polarity. Recent results have shown that the two adhesion systems exert negative feedback on each other and appear to regulate actin network dynamics and mechanical force production in different ways. In light of this, we argue that the interplay between these regulatory mechanisms plays an important role in the spatial separation of cell–cell and cell–matrix adhesions components in distinct regions of the cell surface.

Address
Laboratoire de Physiologie Cellulaire et Végétale, Institut de Recherche en Technologies et Sciences pour le Vivant, CNRS/UJF/INRA/CEA, 17 rue des martyrs, 38054, Grenoble, France

Corresponding author: Thery, Manuel (manuel.thery@cea.fr)

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Introduction

The microenvironment of a cell is made of extra-cellular matrix (ECM) and neighboring cells. Cells adhere to the ECM and their neighbors through spatially distinct regions of their surface, which contain molecular complexes interacting with extracellular ligands on one side and regulating and interacting with the cytoskeleton on the other side. The best characterized CMA complexes comprise transmembrane proteins integrins, directly binding to ECM proteins such as fibronectin, laminin and collagen and recruiting actin binding and regulatory proteins (such as talin, paxilin and focal adhesion kinase (FAK)) [1]. The most studied CCA complex comprise the transmembrane cadherins, which form homophilic bonds between neighbor cells and recruit actin binding and regulatory proteins of the catenin family [2]. This review focuses on integrin-based and cadherin-based cell adhesion, though other types of adhesion complexes also exist. These two types of adhesion complexes are remarkably similar. They have in common several structural components, they can bind actin filaments, they can utilize some of the same signaling pathways and act as mechanical sensors [3]. Despite this, they contribute differently to cell and tissue architecture. In addition to its well-known role of structural support, ECM regulates the intra-cellular level of contraction [4,5], transmits mechanical forces over long distances [6•], and acts as a basement and signaling platform for epithelia [7]. For example, CMA signaling regulates lamellipodial activity at the front of migrating cells [1] and the 3D organization of CMA regulates the confined migration processes of individual cells [8,9] and cell groups [10]. CMA signaling also regulates the orientation of epithelial cell polarity [11,12] as well as branching morphogenesis of several organs [13]. CCA regulates epithelia shape and remodeling [2] and propagate polarity signals [14]. CMA and CCA both act as cues for cell apico-basal polarity orientation [15] and the expression level of their components regulates the degree of polarization during epithelial morphogenesis [16].

These two adhesion systems appear not to act independently. Rather, their functions are connected by a permanent crosstalk [17]. CCA and CMA can upregulate and downregulate one another depending on the context [18,19•]. Spatial segregation of CMA and CCA seems to act as and/or result from a major morphogenetic force shaping cells and tissues. Although this segregation has been observed in many conditions, very few studies have been directly dedicated to find the underlying mechanism. Here we review recent examples in which CMA and CCA segregation has been observed *in vivo* and then describe the negative local feedbacks they exert on each other and finally propose a mechanism for their spatial segregation based on their mechanical interaction.

Spatial segregation in tissues

It has been appreciated for a long time that the expression of CCA and CMA components is increased during the epithelial morphogenesis and that they segregate in opposed locations [15]. Recently the list of organs displaying such a spatial segregation has been extended, which further confirmed the universal nature of this feature in multicellular organisms.

In mice, liver bile duct formation proceeds with the formation of new tubes along the portal vein. During lumen formation, cadherin localization on the portal side precedes the localization of laminin on the opposite basal pole [20,21•]. During pancreatic tubulogenesis, CMA and

CCA also appeared separated in the cells forming the early luminal structures [22] (Figure 1).

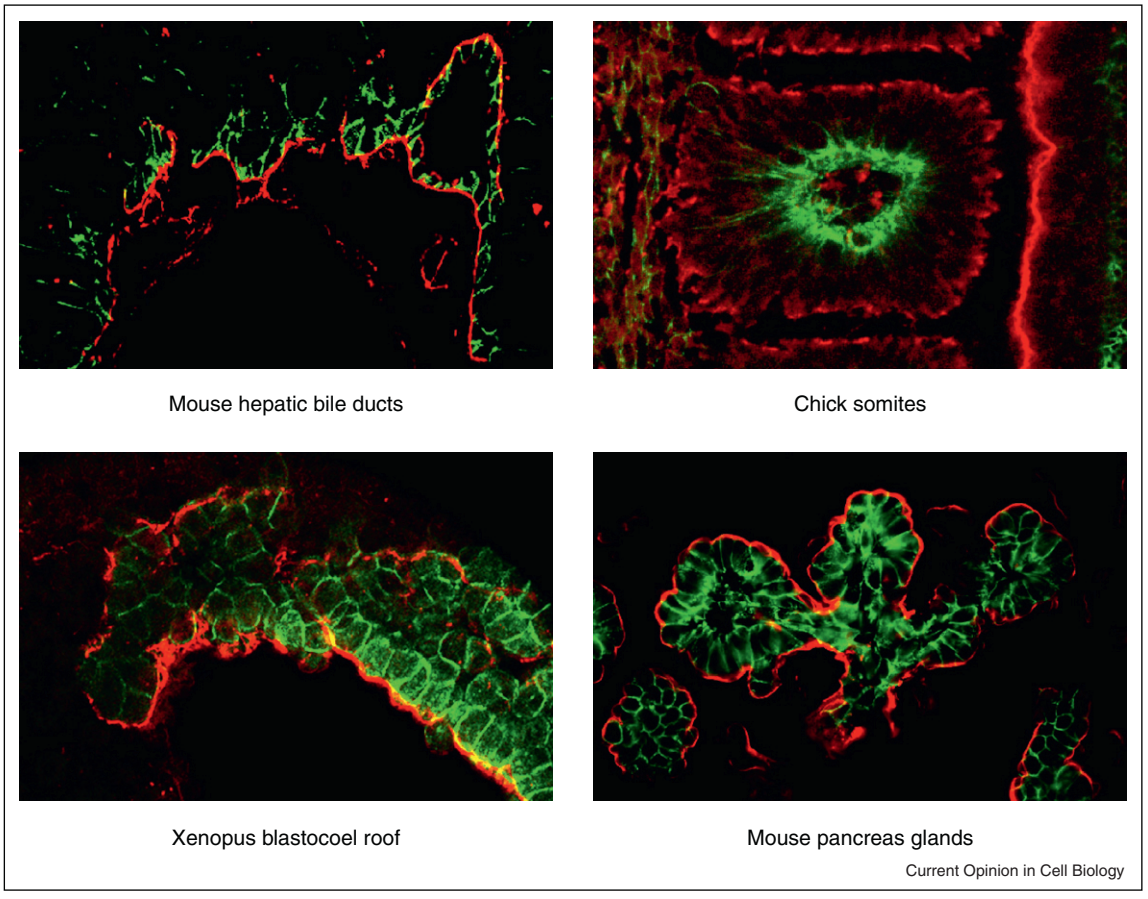
The direct effect of one adhesion system on the expression and location of the other has been reported during various morphogenetic events. During mouse lung and salivary gland morphogenesis, local engagement of cell–ECM adhesions reduce the expression of E-cadherin, which contributes to CCA disassembly and induces cleft formation [23,13]. During arteriolar morphogenesis in mice, the beta1 integrin deletion mutants exhibit upregulation of cadherins, extended cell–cell contacts and a lack of lumen [24], which suggests that assembly of CCA along short lateral contacts depends on the engagement of CMA along endothelial cell basal surface. Similarly, during bone formation, cell adhesion to collagen on basal surface seems to contribute to proper CCA formation on the cell's lateral surfaces [25]. During chick embryo somitogenesis, basal fibronectin assembly induces the restricted localization of cadherins at the apical surface [26•,27]. Conversely, tissue tension that requires cadherin adhesion on lateral surfaces of blastocoel cell roof cells mediates fibronectin assembly on upper surface during xenopus gastrulation [28,29] (Figure 1).

Cell–matrix adhesions locally weaken cell–cell adhesions

In the next two paragraphs we review recent works in which some results suggest that the two adhesion systems can negatively affect each other by various means and in many different and unrelated conditions. According to this view, the spatial segregation of the two adhesion systems may rely on their mutual exclusion by a process of local negative feedback (Figure 2A).

The local negative regulation of CCA by CMA has been directly shown in various contexts by several distinct approaches. Covering the apical poles of a monolayer of epithelial cells with ECM induced the formation of apical membrane protrusions leading to the disruption of CCA localized close to these apical poles and to the reassembly of CCA in ECM free regions at the opposite cell side [30]. Similarly, the formation of CMA in cancer cells prevents the proximal formation of E-cadherin

Figure 1



Several examples of the spatial segregation of CCJ (green) and CMA (red) in mouse hepatic bile duct (E-cadherin in green and laminin in red) [21•], in chick somites (N-cadherin in green and laminin in red) [26•], in *Xenopus* blastocoel roof (N-cadherin in green and fibronectin in red) [29] and in mouse pancreas (E-cadherin in green and laminin in red) [22].

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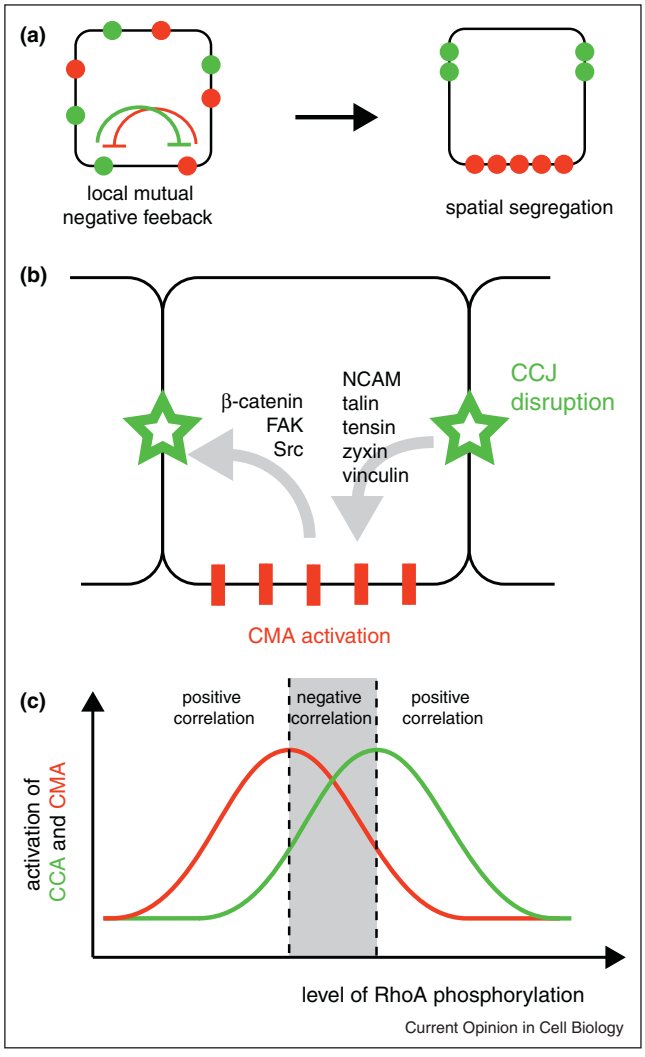
Figure 2



(A) The negative feedback CCA (green) and CMA (red) exert locally on each other can account for their spatial segregation. **(B)** Description of the players involved in CCA disruption upon CMA activation and vice versa. **(C)** Schematic illustration of the possibility for RhoA, or other RhoGTPases, to exert opposite effects on CCA and CMA despite similar activation curves. Positive correlation means that both CCA and CMA are activated, or inactivated, by an increase of RhoA. Negative correlation means that one gets activated while the other is inactivated.

complexes when cells are cultured on micropatterned substrate coated with ligands for both types of adhesions [31]. With an increase of cell spreading area on ECM, the rigidity modulus of a cadherin-mediated contact is reduced [19•].

CMA can activate Src, which in turn phosphorylates FAK. FAK relocalization to CCA results in the phosphorylation of β-catenin and the disruption of β-catenin association with the cadherin complex [32,33] (Figure 2B). The same Src pathway is involved in VEGF-induced vascular permeability [34]. In colon cancer cells, integrin associated Src activity is enhanced and perturbs E-cadherin localization [35]. *In vivo*, in squamous cell carcinoma, CMA activate FAK, which in turn activates E-cadherin internalization, CCA weakening and tumor cell dispersal [36].

RhoGTPase, RhoA and Rac1 have similar contributions CMA and CCA formation [3]. Rac1 is involved in initial formation and RhoA contributes to maturation, lengthening and strengthening of the adhesions [37,38•,39]. Excessive activation of RhoA or Rac1 induces junction disruption [3]. But how Rho GTPases are involved in the crosstalk between CCA and CMA is not clearly established. At first glance, they seem to have the same effect on both adhesions. For example, increase in the level of RhoA phosphorylation first activate and then disrupt the two types of cell adhesions, giving a 'bell shape' to CCA and CMA activation curves (Figure 2c). But if these similar curves are slightly shifted, a given variation of Rho concentration in the intermediate regime, between the two activation maxima, would have opposite effect on CCA and CMA and thereby mediate a negative correlation between the two types of adhesion (Figure 2C).

Abl kinases are also involved in both CCA and CMA formation and maintenance. Abl kinases support stabilization of CCA [40] and inhibition of β1-integrin mediated laminin assembly at the same time [11] and thus could also be key regulators of their crosstalk.

Noteworthy, the CMA-CCA crosstalk can be either dominated or dampened by CMA maturation in response to ECM rigidity [41,42,31,43•].

Cell–cell adhesions locally impair cell–ECM adhesions

Several examples directly showed that CCA locally impairs CMA formation and downstream signaling. In epithelial cells plated on micropatterned surfaces of cadherins and ECM, cadherin engagement prevents the formation of CMA at the same location, and reduces downstream signaling responsible for membrane protrusion formation in close-by CMA [44•]. The formation of CCA between two individual myocytes leads to the disassembly of the CMA that were present close to the contact region [43•]. When vascular smooth muscle cell density is increased, the formation of CCA is increased while the expressions of talin and vinculin required for CMA maturation and production of traction forces are reduced [41].

Downregulation of CMA by CCA is also indirectly revealed by the CMA formation in response to CCA disruption. Downregulation of CCA components, such as E-cadherin or a-catenin, correlates with increased cell migration on ECM [45,46]. The role of CCA weakening is particularly critical to epithelium to mesenchyme transition (EMT) during which CMA is activated. E-cadherin downregulation is required to potentiate the effect of TGF-β and promote metastatic growth [42]. Upon

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E-cadherin loss of function, NCAM is overexpressed and translocated into lipid rafts where it activates FAK resulting into CMA assembly [47]. *NCAM-dependent activation of CMA formation is modulated by polysialic acid* [48]. Noteworthy, during EMT, E-cadherins are replaced by N-cadherins. During Xenopus gastrulation, tension on N-cadherins stimulate CMA displacement away from CCA [28,29]. In mouse astrocytes, N-cadherins maintain cell polarity by preventing the formation of CMA adjacent to cell–cell contact [49]. In some neuronal tumors, N-cadherin level is reduced resulting into enhanced CMA activity and increased cell migration [49].

In various physiological contexts, CCA disruption and CMA formation might be coupled through the regulated distribution of common structural components. Tensin relocalization from CCA to CMA in response cell attachment with fibronectin reduces the strength of CCA [50]. Zyxin, vinculin and talin are well characterized CMA components. However they are also localized to CCA where they regulate the strength of the CCA [51,52•,53]. This suggests that in the case of CCA disruption zyxin, vinculin and talin may be released from CCA and relocalize to CMA that would be subsequently reinforced (Figure 2B).

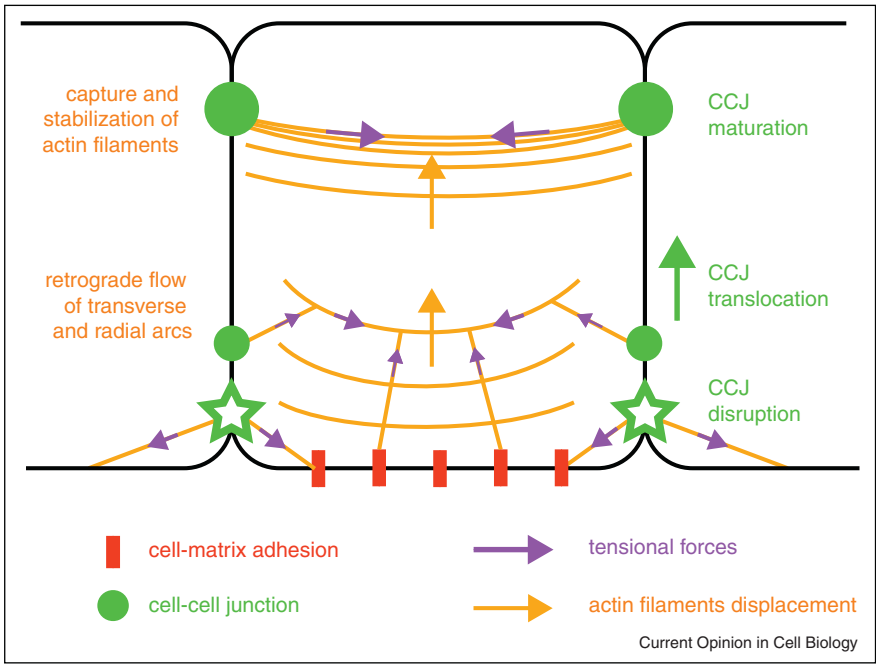
Interestingly, Plakoglobin, a CCA component, has been shown to stimulate ECM expression and therefore CMA formation [54]. When Plakoglobin is locally recruited on CCA subjected to external tension, it reorients the intermediate filament network and promotes the formation of membrane protrusions at the opposite cell pole [55•]. Although in this case, local CMA disruption is not involved, the possibility for cells to secrete and adhere to ECM seems to be limited to the diametrically opposed cell side.

The above examples show that in many conditions associated to epithelium remodeling (tubulogenesis, EMT, cancer, ...) one adhesion system can dismantle or repulse the other. The signaling pathways involved in these regulations could, at lower activation levels, contribute to a local negative regulation and result into spatial segregation between CMA and CCA (Figure 2). Yet the mechanism supporting this segregation still has to be elucidated. In parallel to the cross signaling, several examples suggest that structural mechanisms participate in the spatial organization of cell adhesions. Notably, the two types of adhesions differently regulate the actin network. Hence, we argue that the coupling of these different actin-regulating processes could participate in CMA and CCA spatial segregation.

Actin network dynamics and force transmission to cell–matrix adhesion sites

CMA assembly, growth and maturation processes are associated with distinct mechanisms controlling actin dynamics [1]. Recent studies have shown that upon

Figure 3



Speculative description of a mechanism supporting CCA displacement away from CMA. Force production on trans-cellular stress fibers lead to CCA disruption close to the basal surface. Retrograde flow of actin transverse arcs formed on CMA is coupled to CCA through radial fibers. Transmission of acto-myosin contractile forces through these fibers pulls CCA away from CMA. The accumulation of actin filaments at the apical pole and the production of acto-myosin forces along these cortical bundles induce CCA strengthening and maturation respectively.

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engagement with the extra-cellular matrix, integrins induce actin filament growth. Lateral interactions and translocation of integrins promote their clustering and early adhesion formation [56]. Nascent CMA are then associated with Rac activation and the formation of membrane protrusions (lamellipodia, filopodia) based on actin polymerization and formation of a dendritic network [57,58]. At a later stage, CMA maturation and the increase of acto-myosin contraction are inter-dependent [56,57,59–61].

In migrating cultured cells, the subcellular localization of mature CMA determines the spatial transition between the dendritic network of actin filaments next to plasma membrane and the network of actin bundles in the cell interior [62]. The compression of this dendritic network nucleated at the plasma membrane leads to filament alignment and formation of transverse arcs [63•,64]. Acto-myosin contraction drives the retrograde movement of these arcs toward cell interior. As these arcs move inward, they bundle with CMA-associated actin filaments and induce the formation of radial fibers through which they transmit contractile forces to the extra-cellular matrix [65,66,63•] (Figure 3).

Actin network dynamics and force transmission at cell–cell contacts

The formation of a cell–cell contact triggers actin cytoskeleton assembly [67]. Extension and retraction of lamellipodia over adjacent cells leads to the formation of interconnecting actin filaments whose remodeling by fascin and myosin eventually lead to the assembly of CCA [68]. Arp2/3 [69], N-WASP [70] and α-actinin [71] nucleate, recruit and stabilize actin filaments along CCA. Rac-induced actin-network polymerization promotes cell–cell contact area growth and Rho activation promotes further CCA maturation [37,38•]. Furthermore, CCA are reinforced upon application of external or internal stress [72,73•,38•]. The application of tension can lead to the recruitment of vinculin [52•] and additional actin filaments through VASP and EPLIN [74•,75,76], which strengthen cell–cell adhesion [77]. However, excess forces can result in junction disassembly [78,79]. Abl kinase [40] and Cdc42 are involved in the fine regulation of that threshold [80].

Thus, mature CCA anchor acto-myosin bundles [81] (Figure 3). Myosin IIb recruits actin filament along the junctions [82] and Myosin IXA supports the formation of actin bundles orthogonal to the junction [79]. Both myosin types ensure cell–cell contact integrity by resisting destructive orthogonal forces on the CCA.

Coupling of actin dynamics associated with CMA and CCA

The nucleation, stabilization, capture and disassembly of actin filaments have to be integrated at the cell level to ensure the stationary state of the entire network. The cytoskeletal forces applied on CCA and CMA also have to be balanced to ensure cell mechanical stability. These forces may be responsible for adhesion maturation as well as for their rupture or displacement in the membrane. The spatial distribution of forces in the actin network and the spatial arrangement of filament nucleation, bundle assembly and bundle stabilization processes may be responsible for CMA and CCA displacement away from each other. As cells come into contact and assemble CCA, traction force on CMA close to the contact region get turned into tugging force at cell–cell contacts that result into local CMA disassembly [43•]. The magnitude of the tugging force at cell–cell contacts is proportional to that of cell traction forces exerted through CMA [83•]. How the magnitude of these forces relate to CCA positioning with respect to CMA has been studied in a minimal system of two cells in which CMA is confined on ECM micropatterns of controlled geometry [84•]. In this system, CCA are subjected to high tugging forces when they are close to CMA sites and lower forces when positioned away from them [84•]. As a consequence, the contact plane is moved away from CMA sites and cells adopt a stationary position in which the cell–cell contact is as far as possible from CMA. Thereby the steady state of multicellular organizations corresponds to the minimization of the overall magnitude of tensional forces [84•].

How force production on CCA lead to such a controlled junction displacement and cell positioning remain to be elucidated. There are at least two ways to apply forces on CCA [85,86]. Contractile acto-myosin bundles can mediate forces orthogonal [71,87–90] or parallel to the junction [91]. Mechanical forces applied orthogonally to the CCA can be transmitted to the CMA sites through radial actin bundles (Figure 3). Such a configuration may occur in a flat epithelium such as the vascular endothelium [87,89]. This configuration could also occur at CCA close to basal surfaces of simple epithelia [71,88,51]. Since integrins may support higher forces than cadherin on comparable substrate stiffness [73•], mechanical force could lead to CCA disruption near CMA sites [92,51] (Figure 3). In addition, at the apical pole of epithelial cells, the retrograde movement of transverse arcs linked to radial bundles orthogonal to the CCA produces tensional forces on CCA [90,93•]. We speculate that the retrograde movement of transverse arcs and radial bundles from CMA (described above) exert forces on CCA responsible for their rupture and displacement away from CMA (Figure 3). Indeed, actin network dynamics has been shown to be responsible for a basal-to-apical flow of CCA in moving epidermal cells [94]; and apical enrichment of actin filaments is necessary for the maintenance of the apical localization of CCA in intestinal cells [70]. We suggest that the actin flow initiated by bundle formation at CMA sites and their retrograde movement

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could pull CCA away from CMA. The accumulation of these contractile actin bundles at CCA distant from CMA could contribute to the strengthening and stabilization of CCA (Figure 3).

Conclusion

The complete mechanism supporting the spatial segregation of CCA and CMA remains elusive. Future insights should be expected from the analysis of actin network dynamics and its relationship with mechanical force production. In addition, the coupling between CCA components renewal at the membrane and force production [70,95] could play a key role in epithelial morphogenesis [96–99]. Unravelling the mechanisms supporting spatial segregation of cell adhesions during epithelial morphogenesis, which is tightly coupled to apico-basal polarity, could greatly improve our understanding of organogenesis and oncogenesis.

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