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Intracellular mediators of JAM-A–dependent epithelial barrier function

Ana C. Monteiro and Charles A. Parkos

Epithelial Pathobiology Research Unit, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia

Abstract

JAM-A is a critical signaling component of the apical junctional complex, a structure composed of several transmembrane and scaffold molecules that controls the passage of nutrients and solutes across epithelial surfaces. Observations from JAM-A–deficient epithelial cells and JAM-A knockout animals indicate that JAM-A is an important regulator of epithelial paracellular permeability, however the mechanism(s) linking JAM-A to barrier function are not understood. This review highlights recent findings relevant to JAM-A–mediated regulation of epithelial permeability, focusing on the role of upstream and downstream signaling candidates. We draw on what is known about proteins reported to associate with JAM-A in other pathways and on known modulators of barrier function to propose candidate effectors that may mediate JAM-A regulation of epithelial paracellular permeability. Further investigation of pathways highlighted in this review may provide ideas for novel therapeutics that target debilitating conditions associated with barrier dysfunction, such as inflammatory bowel disease.

Keywords

JAM-A; barrier function; scaffold proteins; apical junctional complex (AJC); permeability

Introduction

The epithelial barrier is a critical component of tissue homeostasis as it selectively controls the passage of nutrients and solutes across mucosal surfaces while deterring the passage of pathogens and toxins. Functional regulation of the epithelial barrier is determined by a collection of tight junction (TJ) and adherens junction (AJ)-associated transmembrane and scaffold proteins termed the apical junctional complex (AJC). One transmembrane component of the TJ is Junctional Adhesion Molecule-A (JAM-A). Evidence suggests that JAM-A does not directly regulate barrier by forming a “seal” between cells but rather functions as a signaling molecule with divergent downstream target proteins.¹² Indeed, recent reports have implicated JAM-A–mediated signaling events in regulating a diverse array of epithelial functions including epithelial proliferation, migration and barrier function.³⁴² While the evidence linking expression of JAM-A to TJ regulation and barrier maintenance is accepted, insights into downstream mechanisms linking JAM-A to regulation of barrier are limited. In this review, we summarize current findings that are relevant to how JAM-A might control barrier function, focusing on the role of upstream and downstream signaling components linking JAM-A to paracellular permeability. We draw on what is

Correspondence: Charles A. Parkos Emory University Pathology and Laboratory Medicine 615 Michael St. Atlanta, Georgia 30322.

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known about JAM-A effectors from other pathways to speculate on attractive candidate molecules that regulate the epithelial barrier.

JAM-A expression affects epithelial permeability

The importance of JAM-A in regulating barrier function is best illustrated by reported observations in JAM-A deficient intestinal cell lines and knockout (KO) animals.²⁵ In cell lines, it has been clearly shown that siRNA mediated loss of JAM-A expression results in enhanced permeability as determined by transepithelial resistance (TER) and paracellular flux of labeled dextran. Such observations have been confirmed in multiple epithelial and endothelial cell types,^{1, 5-8} including primary rat alveolar epithelial cells, which exhibit decreased TER after treatment with JAM-A shRNA (Mitchell and Koval, personal communication). *In vivo*, JAM-A deficient mice have a leaky colonic epithelium.² Additionally, JAM-A-deficient mice are more susceptible to dextran sulfate sodium (DSS)-induced colitis compared to control mice, presenting with a higher disease activity index and more severe weight loss. Intriguingly, JAM-A KO mice also present with increased mucosal infiltration of leukocytes in the colonic mucosa and altered levels of pro-inflammatory cytokines.² This finding is consistent with current views that link impaired barrier function with increased susceptibility to mucosal inflammation. Future studies should determine whether enhanced permeability and leukocyte infiltration are also observed in other epithelial compartments of JAM-A deficient mice.

Epithelial barrier function is established by a complex series of poorly understood signaling events that culminate in the formation of mature TJs. Evidence suggests that JAM-A is important in early events required for TJ assembly. Such early events begin with emergence of nascent puncta containing the AJ proteins E-cadherin and nectin, providing initial points of cell-cell contact that recruit other junctional proteins necessary to establish a mature apical junctional complex. JAM-A appears to be an early mediator of this process since it is recruited along with occludin immediately after puncta are established.⁹ These observations are consistent with our findings demonstrating that antibody blockade of JAM-A in cultures of subconfluent epithelial cells delays the development of a tight barrier, as determined by TER measurements (unpublished observations).

Dimerization of JAM-A is necessary for regulation of barrier

JAM-A is composed of a short cytoplasmic tail, a single-pass transmembrane region and two extracellular Ig-like loops. A number of reports, including crystallography data,¹⁰ indicate that JAM-A forms functionally significant homodimers through ionic interactions within a conserved motif in its distal most Ig loop (D1).⁵¹¹ These reports suggest that such ionic interactions within the D1 loop of JAM-A mediates homodimerization in a *cis* configuration (on the surface of the same cell); however, structural data from murine JAM-A¹² suggests that JAM-A also homodimerizes in *trans*, or across cells, at distinct but yet undefined site(s) in the same D1 loop. We observed that mutagenesis of residues promoting *cis*-dimerization of JAM-A or treatment with a monoclonal antibody that binds to the dimerization domain results in attenuated JAM-A-dependent regulation of epithelial cell migration.¹¹ Interestingly, the same dimerization disrupting antibodies delay barrier development in monolayers of epithelial cells with disassembled AJC after transient calcium depletion (calcium switch). These observations suggest that JAM-A homodimerization is necessary for assembly of functional TJs.⁵¹ Studies on JAM-A-mediated effects on cell migration eventuated in a model of JAM-A function involving dimerization-mediated activation of a signaling module that leads to cell migration. While mechanistic insights detailing how JAM-A dimerization leads to regulation of paracellular permeability are not understood, some clues are provided by the above model and from reports implicating

known JAM-A–interacting molecules with the regulation of barrier function. From these observations, we can begin to assemble a potential model by which JAM-A dimerization controls epithelial barrier function.

Several stimuli that alter epithelial barrier also affect JAM-A expression

Although there are limited studies on the signaling pathways that link JAM-A to regulation of barrier, there are several recent reports describing paracrine and autocrine cues that affect junctional integrity and alter JAM-A expression and localization (Fig. 1). Such studies underscore the fluidity of the AJC, which is constantly restructured to accommodate physiological events, such as leukocyte transmigration across epithelia and varying demands of fluid and nutrient absorption in the gastrointestinal tract.

Cytokine-mediated internalization of epithelial TJ proteins exacerbates inflammatory conditions by maintaining an open entryway for leukocytes to the inflamed region and enhancing leukocyte exposure to luminal antigens. Inflammatory cytokines such as INF- γ and TNF- α enhance permeability of endothelial and epithelial barriers by inducing internalization of JAM-A and other AJC proteins,^{13,14} while local administration of TGF- β and TNF- α to the blood testis barrier also induces clathrin-dependent internalization of AJC proteins that include JAM-A, occluding, and N-cadherin.¹⁵ Conversely, cues that enhance barriers may be protective against chronic inflammation, again by altering the AJC. For example, estrogen, thought to have anti-inflammatory effects in the gut,^{16,17} was reported to reduce the permeability of the intestinal epithelium *in vivo* and *in vitro* by upregulating TJ protein levels of JAM-A and occludin.¹⁸ CD-24, a ligand for p-selectin implicated in epithelial restitution in mouse models of inflammatory bowel disease (IBD), was also reported to enhance barrier function of the oral epithelium by upregulating JAM-A and claudins 4 and 15 in a src-kinase dependent manner.¹⁹ These examples indicate a potential reciprocal influence of inflammatory signals on mucosal permeability, which may act to perpetuate a pathological inflammatory response.

Other studies implicating a role of paracrine signaling in JAM-A expression provide mechanistic insights into JAM-A recruitment to TJs, which may be important for JAM-A stability and function. Studies using immortalized primary pancreatic duct cells²⁰ revealed that inclusion of fetal bovine serum (FBS) after serum starvation enhanced the expression and TJ localization of several TJ proteins including JAM-A, occludin, ZO-1 and several claudins in a PKC-dependent manner. Cells formed no functional barrier during serum starvation but develop a functionally tight barrier after the addition of serum. Interestingly, inhibition of PKC reduced JAM-A expression and TER to that of serum-free levels. In serum-free media, addition of TPA, a DAG pharmacomimetic that activates typical PKCs, enhanced levels and TJ localization of ZO-1, ZO-2, and occludin, however JAM-A expression and TER remained unchanged. The study does not further explore the pathway regulating JAM-A expression, but it is tempting to speculate that JAM-A recruitment to TJs may be dependent on an atypical PKC, one not activated by TPA/DAG. This is consistent with observations of JAM-A association with aPKC²¹ in the context of cell polarity. An understudied aspect of JAM-A is related to the multiple potential phosphorylation sites on the relatively short cytoplasmic tail that may be important for JAM-A recruitment and function, five of which are likely targets for PKC, as determined by ntePhosK analysis. Notably, JAM-A has been shown to be phosphorylated by PKC in platelets.²² Furthermore, during the final editorial review of this manuscript, Ebnet and colleagues published a study demonstrating that the cytoplasmic tail of JAM-A is indeed phosphorylated by aPKC ζ at serine 285 to affect tight junction assembly and epithelial barrier function.²⁴ Further investigation of JAM-A phosphorylation by aPKC may provide additional insights on mechanisms controlling the stability and localization of JAM-A to the TJ, which may be an

important event in the transition from nascent to mature TJ formation leading to a stable epithelial barrier.

Studies of other barrier forming pathways have clearly demonstrated that cytoskeletal dynamics play an important role in barrier function. One study has provided a potential link between JAM-A, cytoskeletal dynamics and barrier function. Mice lacking guanylyl cyclase C (GCC), a transmembrane receptor to endogenous ligands that modulates epithelial chloride conductance, demonstrate an intriguingly similar phenotype to JAM-A KO mice and have altered phosphorylation of actin-associated proteins. Compared to wild-type mice, GCC-null animals have a more permeable gut mucosa, increased levels of pro-inflammatory cytokines and large amounts of lymphocytes in the intestinal epithelial compartment, suggesting a concomitant inflammatory phenotype.²³ Importantly, GCC-null mice and GCC-deficient colonic epithelial cells have decreased levels of JAM-A and claudin-2 with increased phosphorylation of myosin light chain (pMLC), suggesting that the barrier deficiency observed in the GCC-null mice may be related to the loss of JAM-A and claudin-2 as well as the phosphorylation of MLC. Notably, MLC phosphorylation has been implicated in increased epithelial permeability by inducing contraction of the epithelial acto-myosin ring and the enlargement of the intercellular space, thereby enhancing epithelial leak.²⁵ However, Han *et al.* propose instead that pMLC is important for TJ assembly by recruiting JAM-A and other proteins to the AJC. Future studies are required to clarify the relationship between JAM-A signaling and acto-myosin contraction to better understand the role of cytoskeletal dynamics in JAM-A-dependent regulation of epithelial permeability.

Putative signaling effectors downstream of JAM-A that regulate barrier

While the importance of JAM-A in endothelial and epithelial barrier function is appreciated,⁸⁷⁵¹ downstream pathways linking JAM-A to paracellular permeability are unknown. As mentioned above, signaling pathways regulating JAM-A dependent cell migration have been described.⁴ From these studies, it is reasonable to postulate that similar pathway(s) may regulate barrier function. In migration studies, it was found that JAM-A associates with the scaffold protein afadin and the guanine nucleotide exchange factor PDZ-GEF2 resulting in the activation of the small GTPase Rap1a, stabilization of $\beta 1$ integrins and enhanced cell migration. While these effector molecules have not been reported to directly affect barrier in epithelia, afadin, PDZ-GEFs, and Rap1 have been widely implicated in regulation of endothelial barrier, as will be discussed below.

Afadin, a large PDZ-containing scaffold protein shown to associate with JAM-A,⁴ has been strongly implicated in the regulation of barrier function. Mice with intestinal epithelial-targeted loss of afadin have increased intestinal permeability²⁶ and a phenotype similar to that observed with JAM-A KO mice. JAM-A KO mice have normal intestinal mucosal architecture but a leaky colonic epithelium, increased mucosal lymphoid follicles and enhanced susceptibility to acute injury-induced colitis.² While complete genomic deletion of afadin is lethal, mice with intestinal epithelial targeted loss of afadin (cKO) are viable, have a similar increase in gut permeability, and exhibit seemingly normal intestinal morphology. Similarly, afadin cKO mice show enhanced susceptibility to acute injury-induced colitis. Although the above parallels between JA KO and afadin cKO animals are consistent with afadin regulation of barrier function downstream of JAM-A, afadin has also been regarded as a cadherin-associated scaffold that mediates outside-in signaling after nectin-driven nascent junctions are initiated.²⁷ While the latter observation might implicate afadin in controlling barrier downstream of nectin, mice lacking nectins-2 and -3 in the intestinal epithelium have no increase in intestinal permeability compared to wild type counterparts.²⁶ Furthermore, the intestinal epithelium of such nectin-deficient mice has normal localization

of afadin. These findings suggest that intestinal barrier function and junctional localization of afadin can occur by nectin-independent mechanism(s).

In addition to afadin, the guanine nucleotide exchange factor PDZ-GEF2 has been reported to associate with JAM-A and mediate β 1 integrin dependent epithelial cell migration,⁴ presumably through activation of the small GTPase Rap1a. Despite this observation, the role for PDZ-GEF2 or the closely related PDZ-GEF1 in regulating epithelial barrier is not understood. Loss of PDZ-GEF1/2 in epithelial and endothelial cells has been shown to affect the composition and architecture of the AJ so that its morphology resembles nascent puncta,^{28,29} suggesting that PDZ-GEF1/2 may be important in the maturation of initial puncta into functionally developed AJCs. On the other hand, the differences in AJ architecture described in these reports were only detectable when cells were recultured at low confluence, suggesting that the role of PDZ-GEFs in barrier function may be limited to early events in junction formation. Additionally, loss of PDZ-GEF2 in epithelial cells did not affect the localization of the TJ proteins occludin or ZO-1,²⁸ supporting the idea that altered AJ morphology does not necessarily translate to defects in TJ composition. In endothelial cells, Pannekoek *et al.* reported that PDZ-GEF1/2 depletion resulted in decreased transendothelial impedance, a proxy measure of barrier function,²⁹ but no analogous functional observations were reported for epithelial cells. Such observations support the concept of distinct regulatory mechanisms governing endothelial and epithelial barrier, which may be explained in part by the fact that the AJC in endothelial cells differs from that of epithelial cells by having a less defined separation between AJs and TJs.³⁰ It is therefore important to confirm the functional importance of PDZ-GEFs in epithelial barrier function so as to determine whether they may act downstream of JAM-A to affect epithelial permeability.

As is the case for PDZ-GEFs, the small GTPase Rap1 has been implicated in downstream signaling events from JAM-A mediated regulation of cell migration. Interestingly, Rap1a/b have been widely implicated in regulation of endothelial barrier function^{31,29,32} but much less is known regarding the role of Rap1 as an effector of barrier in epithelial cells. In epithelial cells, Rap1a has been implicated in mediating trans-dimerization of E-cadherin³² and in organization of E-cadherin along cell-cell contacts.^{33,28} However, inactivation of Rap1 by the guanine nucleotide activating protein RapGAP does not affect the localization of ZO-1 to cell-cell contacts,³³ suggesting that TJ formation does not require Rap1 in epithelial cells. Furthermore an *in vivo* study on the role of the oxidized phospholipid OXPAPC in lung epithelial permeability reported no changes in transepithelial resistance after downregulation of Rap1.³⁴ Additionally, Rap1 null *C. elegans* display normal epithelial architecture of the epidermis and gut.³⁵ Notably, most studies claiming a role for Rap1 in regulating endothelial and epithelial barrier are in fact describing functions of proteins known to alter the activation of Rap1, such as EPAC, RapGAP, and PDZ-GEF1/2.^{29,33} Since these mediators lack specificity for Rap1,^{36–39} the possibility of involvement of other small GTPases has not been excluded. The paucity of data directly relating Rap1 to functional measures of epithelial permeability leaves more questions than answers regarding a link between Rap1 and JAM-A dependent regulation of barrier function.

ZO-1 is an important TJ-associated scaffold protein and one of three zonula-occludens proteins. ZO-1 has three PDZ domains (PDZ1–3), an Src homology 3 domain (SH3), and a guanylate kinase homology domain (GUK) and has been reported to associate with JAM-A.⁴⁰ A recent crystallography study identified PDZ3 as the putative binding pocket in ZO-1 responsible for JAM-A association, and that this association required the presence of the SH3 domain.⁴¹ Interestingly, mice lacking ZO-1 or ZO-2 do not survive, suggesting that both proteins are required for embryonic development.^{42,43} ZO-3 null mice, however, have little or very subtle phenotypic differences compared to their wild-type counterparts,

suggesting a redundant role of ZO-3 in epithelial function.⁴¹ Cell culture studies have further defined barrier-inducing roles of ZO proteins through simultaneous silencing of ZO-1, 2 and 3 followed by their replacement one at a time.^{44,45} Epithelial cells lacking all three ZO proteins have no TJs, highlighting the importance of these scaffold proteins to barrier formation. Interestingly, addition of either ZO-1 or ZO-2 to ZO-null cells is sufficient for establishing TJs. Additionally, it was found that protein levels of JAM-A, claudins, and occludin are unchanged in ZO-depleted cells, suggesting that ZO-1 or 2 are likely necessary and sufficient for recruitment of TJ components to the AJC during barrier formation, but that synthesis of TJ proteins occurs independently of ZO-1/2 expression.⁴⁶ ZO-1 has also been shown to associate with claudins via PDZ1, occludin through its GUK domain, afadin via its SH3 domain, and with other ZO proteins via PDZ2, allowing it to cluster several scaffold proteins, potentially leading to TJ maturation. It is possible that ZO-1, afadin, PDZ-GEFs, and other PDZ-containing scaffold proteins associate with transmembrane PDZ motif-containing proteins, such as JAM-A, to direct maturation and maintenance of barrier function. Ebnet and others have reported co-association between JAM-A and ZO-1 from cell lysates,^{47,40} and Nomme *et al.* have shown *in vitro* direct association of these proteins via crystallography studies.⁴⁰ However, there are no reports showing a direct association of JAM-A and ZO-1 in cells. Additionally, there is limited data on whether ZO-2 can associate with JAM-A or participate in the same signaling module that ZO-1 and JAM-A are reported to share. While the role of ZO-1 and -2 proteins in barrier formation are well appreciated, the mechanisms linking ZO-1 and -2 to JAM-A mediated regulation of barrier function require further elucidation.

The GEFS and scaffold proteins discussed above are attractive candidate mediators regulating JAM-A dependent barrier function. However, potential distal signaling elements downstream of JAM-A, which are intimately associated with regulation of epithelial permeability, merit careful consideration. In particular, cytoskeletal dynamics and claudin composition of TJs directly affect paracellular permeability (Fig. 2).^{48–49} The tetraspan TJ forming claudins are classified as either leaky or tight and dimerize across the apical intercellular space to form channels that control the permeability of monolayers.^{47,51,49} It was previously reported that JAM-A null mice and JAM-A-depleted intestinal epithelial cells demonstrate enhanced levels of the leaky claudins 10 and 15,² but not of claudin 2 or occludin, suggesting that JAM-A affects the claudin composition of TJs. It is not known how JAM-A does this, however previous studies indicate that JAM-A affects levels of β 1 integrin by maintaining its stability at the cell surface.⁵² Given the likely overlap in function of some of the signaling elements discussed above, it is reasonable to hypothesize that JAM-A may regulate barrier function through effects on the stability of certain claudin family members at the TJ. Clearly, further studies should help to answer this important question.

JAM-A associated AJC scaffold proteins, such as afadin and ZO molecules, have actin binding domains that allow for communication between the AJC and the apically positioned actin-myosin ring, a critical component of barrier integrity.²⁵ The association of the AJC with cytoskeletal components is necessary for maintaining AJC structure.^{53,54} An attractive potential mechanism for JAM-A regulation of barrier would involve signaling through effectors such as afadin to induce cytoskeletal changes that control paracellular flux and epithelial permeability. JAM-A modulates epithelial cell migration, a process dependent on dynamic restructuring of actin, and induces activation of Rap GTPases, which have important cytoskeletal regulatory properties.^{11,55} Analogous JAM-A-dependent pathways that regulate barriers are therefore easily envisioned and require further exploration.

Possible mechanisms that may differentiate divergent JAM-A signaling modules

A major challenge has been to understand how JAM-A mediates diverse cellular functions, such as altered permeability,⁵⁴ cell migration, and enhanced proliferation.³ It is assumed that functional specificity of JAM-A signaling is determined by the interaction of JAM-A with specific scaffold proteins, which may in turn be differentially distributed to subcompartments of epithelial cells. Since previous observations have demonstrated that JAM-A concentrates at the AJC but also along the basolateral membrane,⁵⁶ it is possible that JAM-A dimerization or phosphorylation may determine the localization of JAM-A in either compartment, allowing for specific activation of a particular JAM-A–dependent signaling cascade. For example, dimerization of JAM-A in a *cis* but not *trans* configuration, as would be expected in spreading or subconfluent cells, might favor proliferation and/or migration. However, *trans* interactions between cells, as would be expected in confluent epithelia, would favor close apposition of PDZ-bound molecules that promote barrier forming and perhaps even senescence-inducing cues (Fig. 3).

Likewise, JAM-A phosphorylation events may be important for determining subcellular localization. JAM-A localizes to nascent puncta with E-cadherin, nectin, and ZO-1 to initiate AJC formation, however it also co-localizes with ZO-1 in mature TJs away from the AJ proteins E-cadherin and nectin. It is possible that JAM-A phosphorylation event(s) could facilitate movement of JAM-A towards TJ maturation after puncta establishment (Fig. 4). Strong support for this latter proposed mechanism was very recently shown in a report published while this manuscript was in editorial review. In particular, aPKC ζ -mediated phosphorylation of ser285 in the JAM-A cytoplasmic tail was shown to regulate JAM-A localization to mature TJs.²⁴ It will be interesting to investigate whether such phosphorylation events alter binding affinities between JAM-A and different scaffold proteins, thereby determining activation of a specific JAM-A–dependent cascade in epithelial cells. These two proposed mechanisms may be key elements determining specificity of JAM-A dependent cues, and are attractive areas to be investigated as novel therapeutic targets may emerge that can target JAM-A–dependent function.

Conclusions

JAM-A is a transmembrane, TJ-associated Ig superfamily member that regulates epithelial barrier function, cell migration, and proliferation. Current evidence indicates that JAM-A dimerization is necessary for functional regulation of barrier, however the mechanisms linking JAM-A to barrier function have not been elucidated. While it has been shown that JAM-A regulates cell migration through dimerization-dependent clustering of the cytoplasmic scaffold molecules afadin and PDZ-GEF2, resulting in activation of Rap1a to stabilize cell surface β 1 integrin, the involvement of these pathways in regulation of barrier is unclear. Moreover, association of JAM-A with cellular components known to affect epithelial permeability, such as claudins, ZO-1/2, and the epithelial cytoskeleton remains to be established. Understanding mechanisms that regulate epithelial permeability downstream of JAM-A may be useful in identifying therapeutic targets for diseases associated with barrier dysfunction and may lead to novel approaches towards trans-epithelial drug delivery. Finally, future studies should aim to elucidate mechanisms that determine JAM-A expression and its subcellular localization, since such cues may be key to deciphering how divergent JAM-A–modulated signaling cascades are differentially induced. Identification of effectors that specifically modulate epithelial permeability downstream of JAM-A without affecting proliferation, migration, or other pathways could lead to the development of specific pharmaceutical interventions with fewer off-target effects.

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Figure 1.
Examples of cues that affect epithelial barrier function and alter JAM-A expression.

Figure 2.

Possible downstream mechanisms linking JAM-A dimerization to barrier function. While PDZ-GEF2, Afadin, and Rap1 have been reported to associate with JAM-A, their potential roles in regulation of epithelial barrier require further elucidation. Other key components reported to affect epithelial barrier, including ZO-1, claudins, and the epithelial cytoskeleton, may also act downstream of JAM-A; however, association(s) with JAM-A have not yet been established.

Figure 3.

Model of how JAM-A may differentially induce divergent signaling modules based on JAM-A dimerization. When JAM-A homodimerizes exclusively in *cis*, as may be the case in subconfluent epithelial sheets, a proliferative or migratory pathway may be initiated. When JAM-A homodimerizes in *cis* and *trans*, as may be the case in confluent, fully polarized epithelial monolayers, JAM-A may associate with other scaffold proteins that promote barrier function and possibly senescence.

Figure 4.

Model proposing how JAM-A may be recruited to differential subcellular compartments based on JAM-A phosphorylation. In the upper part of the figure, unphosphorylated JAM-A preferentially localizes to the basolateral membrane, where it can participate in events such as initiation of nascent puncta. JAM-A phosphorylation by an atypical PKC may then lead to its co-localization with TJ associated proteins and maturation of the AJC (lower panels).