Junctional adhesion molecules (JAMs): more molecules with dual functions?

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Summary

Junctional adhesion molecules (JAMs) are members of an immunoglobulin subfamily expressed by leukocytes and platelets as well as by epithelial and endothelial cells, in which they localize to cell-cell contacts and are specifically enriched at tight junctions. The recent identification of extracellular ligands and intracellular binding proteins for JAMs suggests two functions for JAMs. JAMs associate through their extracellular domains with the leukocyte β2 integrins LFA-1 and Mac-1 as well as with the β1 integrin α4β1. All three integrins are involved in the regulation of leukocyte-endothelial cell interactions. Through their cytoplasmic domains, JAMs directly associate with various tight junction-associated proteins including ZO-1, AF-6, MUPP1 and the cell polarity protein PAR-3. PAR-3 is part of a ternary protein complex that contains PAR-3, atypical protein kinase C and PAR-6. This complex is highly conserved through evolution and is involved in the regulation of cell polarity in organisms from *Caenorhabditis elegans* and *Drosophila* to vertebrates. These findings point to dual functions for JAMs: they appear to regulate both leukocyte/platelet/endothelial cell interactions in the immune system and tight junction formation in epithelial and endothelial cells during the acquisition of cell polarity.

Key words: JAMs, Leukocyte-endothelial cell interaction, Tight junctions, Cell polarity, PDZ domain

Introduction

Cell-cell interactions are regulated by adhesion receptors on opposing cells. These receptors are frequently associated at the cytoplasmic surface with signalling molecules that respond to binding of extracellular ligands. One of the largest families of such adhesion receptors is the immunoglobulin (Ig) superfamily. Recent estimations based on the human genome sequence predicted that 765 human genes encode Ig domains (Lander et al., 2001). Unsurprisingly, Ig-superfamily molecules are conserved through evolution and can be found in flies and worms, which are predicted to have 153 and 70 different Ig-like-domain-containing proteins, respectively (Rubin et al., 2000).

Members of the CD2 subgroup of the Ig superfamily are characterized by two Ig-like domains: a membrane-distal V-type Ig-domain and a membrane-proximal C_2-type Ig-domain (Williams and Barclay, 1988; Barclay et al., 1997). Some of the more recently identified members of this family – the junctional adhesion molecules (JAMs) – are expressed by leukocytes and localize to tight junctions (TJs) of epithelial and endothelial cells. Ig-superfamily molecules are widely used in the regulation of immune responses, and the involvement of JAMs in leukocyte-endothelial cell interactions is conceptually not new. The localization of JAMs to TJs and the identification of intracellular scaffolding proteins associated with JAMs, however, suggest that Ig-superfamily members are involved in TJ formation in endothelial cells and epithelial cells. Here, we elaborate the concept of JAMs as molecules with dual functions: mediators of leukocyte-endothelial cell interactions and regulators of cell polarity.

Immunoglobulin-like molecules at tight junctions

The first Ig-like molecule identified at TJs of both endothelial and epithelial cells was junctional adhesion molecule (JAM) (Martin-Padura et al., 1998). It is now called JAM-A* but is also referred to as JAM-1 or the 106 antigen (Malergue et al., 1998) and was originally characterized as the F11 platelet receptor for a stimulatory anti-platelet antibody (Kornecki et al., 1990; Naik et al., 1995). The human orthologue of JAM-A is also expressed on a variety of circulating leukocytes, including monocytes, neutrophils, and B- and T-lymphocytes (Williams et al., 1999; Liu et al., 2000). The two closely related molecules JAM-B (also known as VE-JAM/mJAM-3/hJAM2) and JAM-C (also known as mJAM-2/hJAM3) (Palmeri et al., 2000; Cunningham et al., 2000; Aurrand-Lions et al., 2000; Aurrand-Lions et al., 2001b; Arrate et al., 2001; Liang et al., 2002) are expressed by endothelial cells in different organs and are prominently expressed by high endothelial venule (HEV) endothelial cells. Like JAM-A, JAM-C is also expressed by various human leukocyte subsets and platelets (Arrate et al., 2001; Liang et al., 2002; Johnson-Leger et al., 2002; Santoso et al., 2002). Although the subcellular localization of JAM-B and JAM-C has not been addressed by ultrastructural analysis,

*In this review we use the new nomenclature for JAMs, which applies identical names for the respective mouse and human orthologues (Muller, 2003). JAM-A corresponds to JAM, JAM-1, F11-receptor or the 106 antigen (Martin-Padura et al., 1998; Malergue et al., 1998; Ozaki et al., 1999; Liu et al., 2000; Sobocka et al., 2000). JAM-B corresponds to mouse JAM-3 (Aurrand-Lions et al., 2000) and human JAM2/VE-JAM (Cunningham et al., 2000; Palmeri et al., 2000; Liang et al., 2002). JAM-C corresponds to mouse JAM-2 (Aurrand-Lions et al., 2000; Aurrand-Lions et al., 2001b) and to human JAM3 (Arrate et al., 2001). The two recently identified JAM-like molecules JAM4 (Hirabayashi et al., 2003) and JAM-Like (JAML) (Moog-Lutz et al., 2003) are more closely related to ESAM and CAR.

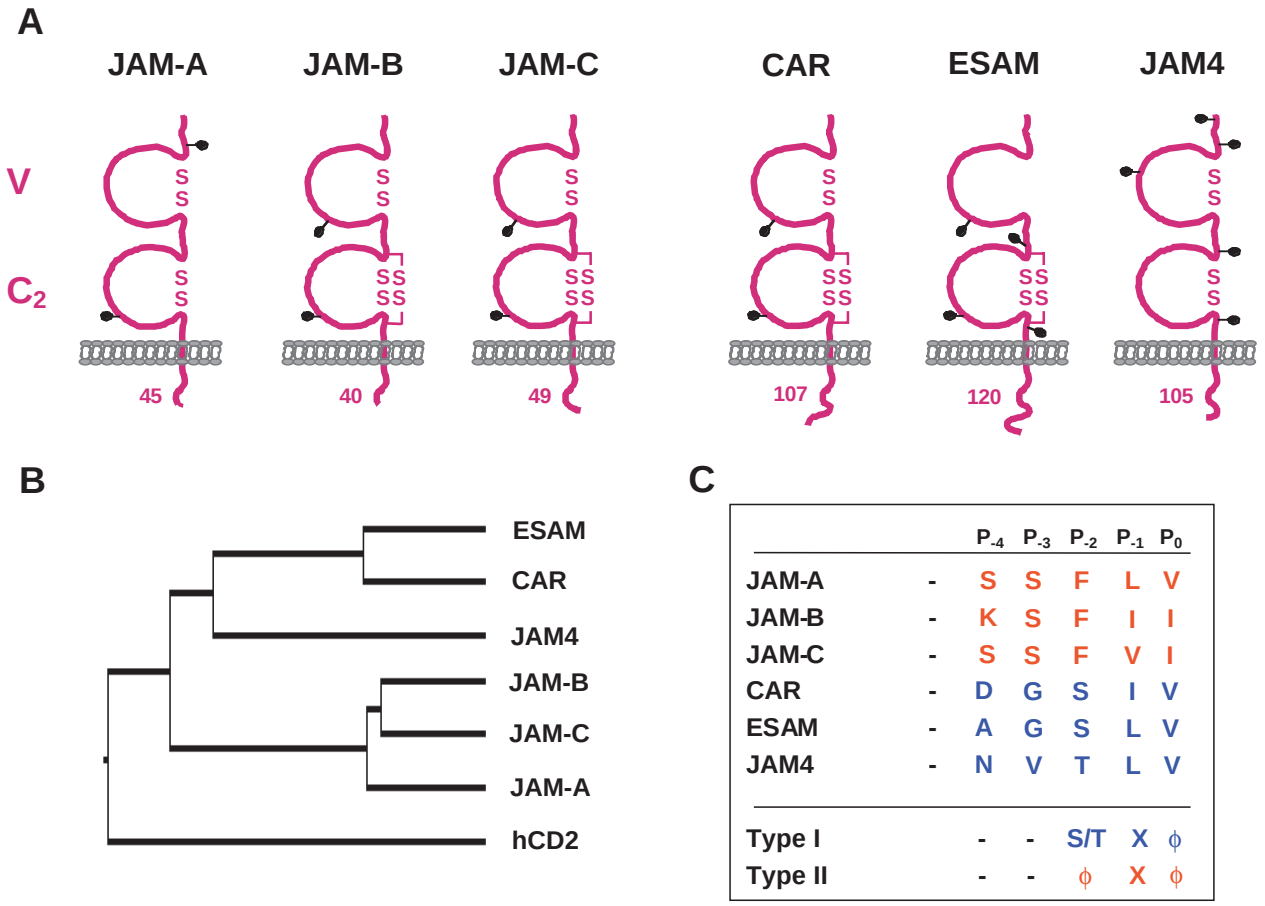

Fig. 1. Ig-SF proteins at TJs. (A) All Ig-SF proteins present at TJs belong to the CD2 subfamily with a membrane-distal V-type Ig-domain and a membrane-proximal C_2-type Ig-domain. Putative *N*-linked glycosylation sites are illustrated by dots. Disulfide bridges and putative additional intramolecular disulfide bridges formed by conserved cysteine residues in the C_2-type Ig-domain are indicated. The sizes of the cytoplasmic domains (mouse molecules) are indicated at the bottom of each molecule. TJ localization has so far been shown for JAM-A, JAM-C, CAR, ESAM and JAM4 (see text for details). (B) A phylogenetic tree (Clustal W program, residue weight table PAM250; human CD2 is used as an outgroup) indicates that JAM-A, JAM-B and JAM-C, as well as CAR, ESAM and JAM4, form individual subfamilies. (C) All six Ig-SF molecules end in canonical PDZ domain binding motifs, which are of type II for JAM-A, JAM-B and JAM-C, and type I for CAR, ESAM and JAM4. The subcellular localization of two proteins with a similar structural organization, BT-IgSF (Suzu et al., 2002) and JAML (Moog-Lutz et al., 2003), has not yet been addressed; these molecules are not included in this figure. P, position of the amino acids with respect to the C-terminal aa, which is designated P_0; ϕ, hydrophobic residue; X, any residue.

the finding that ectopic expression in polarized epithelial cells results in the co-distribution of JAM-C with the TJ marker ZO-1 suggests that it also associates with TJs (Aurrand-Lions et al., 2001b). The subcellular localization of JAM-B in endothelial cells is less clear, because it is not specifically enriched at TJs on ectopic expression in MDCK epithelial cells (Aurrand-Lions et al., 2001a).

Recently, three additional Ig-superfamily members have been identified at TJs: the coxsackie and adenovirus receptor (CAR) (Cohen et al., 2001), endothelial cell-selective adhesion molecule (ESAM) (Nasdala et al., 2002) and JAM4 (Hirabayashi et al., 2003). All three molecules are structurally related and share with the JAMs a similar overall organization: two Ig-like domains, a single transmembrane domain and a cytoplasmic tail that ends in a canonical PDZ domain-binding sequence (Fig. 1). On the basis of amino acid sequence similarity the JAM-A, JAM-B and JAM-C molecules are more related to each other than to ESAM, CAR and JAM4, and vice versa (Fig. 1B). This closer relationship among members of the two subgroups is illustrated by two structural differences: first, the predicted cytoplasmic tails are 40-50 residues for JAM-A, JAM-B and JAM-C but 105-120 residues for ESAM, CAR and JAM4; and second, the five C-terminal aa residues of JAM-A, JAM-B and JAM-C represent class II PDZ domain binding motifs, whereas for ESAM, CAR and JAM4 they represent class I PDZ domain binding motifs (Fig. 1C). The two subgroups therefore probably associate with different cytoplasmic proteins and have distinct functions.

What role do the Ig-superfamily members play at TJs? On ultrathin section electron micrographs, TJs appear as discrete sites of apparent fusions between the outer leaflets of the plasma membranes of adjacent cells, which, on freeze-fracture replica electron micrographs appear as intramembranous particle strands, the TJ strands (Tsukita et al., 2001). Two types of integral membrane proteins, occludin and claudins, seem to be the molecular basis for TJ strands (Tsukita et al., 2001).

Both occludin and claudins have four membrane-spanning domains and two extracellular loops (Tsukita et al., 2001). Claudins, when ectopically expressed in fibroblasts, induce the formation of TJ strands (Furuse et al., 1998), and occludin is incorporated into claudin-based tight junction strands (Furuse et al., 1998). In the same system, JAM-A does not induce the formation of TJ strands (Itoh et al., 2001). This strongly suggests that the function of JAM-A, and probably the other Ig-SF members at TJs, is different from that of occludin/claudins.

JAMs and the regulation of inflammatory responses

Extracellular ligands: *cis*- and *trans* interactions

JAM-A, JAM-B and JAM-C undergo homophilic binding, molecules on adjacent cells interacting in *trans*. This is indicated by their subcellular localization on ectopic expression in CHO or MDCK cells: they localize exclusively to cell contacts between transfected cells but not to sites where transfected cells face nontransfected cells (Martin-Padura et al., 1998; Cunningham et al., 2000; Aurrand-Lions et al., 2001a; Aurrand-Lions et al., 2001b; Ebnet et al., 2001; Ebnet et al., 2003). In addition, homophilic interaction between recombinant soluble JAM-A and native transmembrane JAM-A has been shown biochemically (Bazzoni et al., 2000a). The homophilic interaction can be inhibited by an anti-JAM-A antibody that binds to an epitope close to the N-terminus of the mature protein (Bazzoni et al., 2000a) as well as by a peptide that corresponds to the N-terminal 23 residues of the mature protein (Babinska et al., 2002a). This suggests that the homophilic *trans* interaction is mediated through the membrane-distal V-type Ig-like domain of JAM-A and that a region at the N-terminus of the molecule is necessary.

The crystal structure of recombinant soluble JAM-A indicates that it forms U-shaped dimers in *cis* mediated through a dimerization motif that is present in the V-type Ig-domain (Kostrewa et al., 2001; Prota et al., 2003). Pairs of *cis* dimers from adjacent cells contact each other via their V-type Ig-domains. *Cis*-dimer formation probably precedes the *trans* interaction and thus would be required for homophilic adhesion (Kostrewa et al., 2001). The *cis*-dimerization motif is conserved between all three JAMs, which suggests that JAM-B and JAM-C might dimerize in a similar way. Interestingly, *cis*-dimerization as a prerequisite for homophilic *trans*-interactions has also been shown for nectins – Ig-superfamily adhesion molecules with three Ig-domains located at adherens junctions (Takai and Nakanishi, 2003).

Whether the homophilic interactions between JAMs contribute to cell adhesion is not clear: this has yet to be described in cell aggregation assays. However, some indirect evidence suggests that such interactions might contribute to cell adhesion. JAM-A-positive platelets bind to immobilized JAM-A, and this binding can be blocked by soluble recombinant JAM-A or a JAM-A peptide (Babinska et al., 2002b); the effect, however, could also be due to an interaction of JAM-A with an as-yet-undefined ligand on platelets. Further evidence for a role of JAMs in supporting cell adhesion is the observation that JAM-B-transfected CHO cells adhere to immobilized recombinant JAM-B (Cunningham et al., 2000).

The homophilic interactions between JAMs have functional consequences for cell-cell contact integrity. JAM-A or JAM-C expression in heterologous CHO cells reduces the paracellular permeability to macromolecules such as FITC dextran (Martin-Padura et al., 1998; Aurrand-Lions et al., 2001b). More importantly, however, antibodies raised against JAM-A or soluble recombinant JAM-A significantly delay the recovery of transepithelial electrical resistance (TER) during TJ formation in epithelial cell monolayers (Liu et al., 2000; Liang et al., 2000). Homophilic interactions between JAM molecules thus appear to contribute to the formation of cell-cell contacts and possibly to the formation of functional TJs.

CAR, ESAM and JAM4 also undergo homophilic binding because they localize to cell-cell contacts on ectopic expression in cell types that do not express the proteins endogenously (i.e. CHO cells or MDCK cells) (Cohen et al., 2001; Hirata et al., 2001; Nasdala et al., 2002; Hirabayashi et al., 2003). In addition, CAR or JAM4 expression in heterologous CHO cells reduces the paracellular permeability (Cohen et al., 2001; Hirabayashi et al., 2003). Interestingly, CAR, ESAM and JAM4 support cell aggregation, which indicates that they mediate robust homotypic cell adhesion (Cohen et al., 2001; Honda et al., 2000; Hirata et al., 2001; Hirabayashi et al., 2003). As pointed out previously, cell aggregation mediated through JAMs has not been reported yet, which indirectly suggests that CAR, ESAM and JAM4, besides being structurally different, are also functionally distinct from JAM-A, JAM-B and JAM-C.

Heterophilic interactions in *trans* among JAM family members have been shown between JAM-B and JAM-C (Arrate et al., 2001; Liang et al., 2002) (Fig. 2). In addition, JAM-A, JAM-B and JAM-C interact with integrins on adjacent cells: JAM-A binds to the β2 integrin leukocyte function-associated antigen-1 (LFA-1, integrin αLβ2) (Ostermann et al., 2002), JAM-B interacts with T-cell-associated integrin very late antigen-4 (VLA-4, integrin α4β1) (Cunningham et al., 2002), and JAM-C associates with the β2 integrin Mac-1 (integrin αMβ2) (Santoso et al., 2002). As we discuss below, these heterophilic interactions probably regulate leukocyte-endothelial cell and leukocyte-platelet interactions.

JAMs and leukocyte-endothelium interactions

The first evidence for a putative role for JAMs in the regulation of leukocyte-endothelial cell interactions came from the observation that an antibody directed against JAM-A inhibits monocyte transendothelial migration in vitro and reduces monocyte and neutrophil recruitment in two models of inflammation in vivo (Martin-Padura et al., 1998; Del Maschio et al., 1999). This antibody binds to the N-terminal region of the JAM-A dimer and exclusively recognizes the dimeric form (Bazzoni et al., 2000a). This indicates that leukocytes bind to JAM-A dimers expressed by endothelial cells.

Recently, Ostermann et al. reported that LFA-1 on leukocytes binds JAM-A on endothelial cells (Ostermann et al., 2002) (Fig. 2). LFA-1 is used by various leukocyte subsets to adhere firmly to endothelial cells by binding to intercellular adhesion molecule (ICAM)-1 and ICAM-2 (Hogg et al., 2003). The JAM-A–LFA-1 interaction supports the adhesion of T cells to endothelial cells as well as T-cell and neutrophil transendothelial migration (Ostermann et al., 2002). The predominant localization of JAM-A at cell-cell contact sites on endothelial cells does not necessarily mean that JAM-A is not

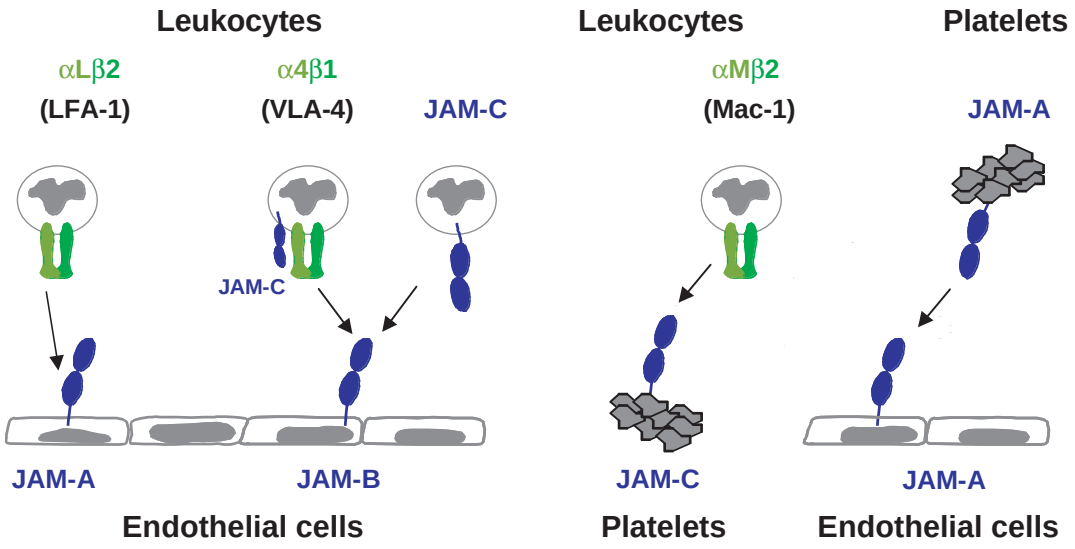

Fig. 2. The role of JAM-A, JAM-B and JAM-C in leukocyte-platelet-endothelial cell interactions. Homophilic and heterophilic interactions of JAM-A, JAM-B and JAM-C suggest that various heterotypic cell-cell interactions might be supported by JAMs. Binding of leukocytes to endothelial cells might be supported by integrins LFA-1 and α4β1 binding to JAM-A and JAM-B, respectively, on endothelial cells. Note that the α4β1-mediated association with JAM-B seems to be enhanced by JAM-C co-expression with α4β1 on the same leukocyte. Leukocyte binding to endothelial cells might also be mediated by leukocyte JAM-C binding to endothelial JAM-B. Leukocyte adherence to platelet deposits is mediated by the leukocyte integrin Mac-1 binding to JAM-C on platelets. Platelet binding to endothelial cells might be supported by JAM-A homophilic interactions. The associations illustrated in this figure have been shown in different experimental systems. Static cell-substrate adhesion assays with recombinant proteins immobilized on plastic were used to show the interaction between JAM-C and JAM-B (Arrate et al., 2001; Liang et al., 2002), α4β1 and JAM-B (Cunningham et al., 2002), Mac-1 and JAM-C (Santoso et al., 2002), and JAM-A and JAM-A (Babinska et al., 2002a). Static cell-cell adhesion assays showed the association between LFA-1 and JAM-A (Ostermann et al., 2002), Mac-1 and JAM-C (Santoso et al., 2002) and between JAM-A and JAM-A (Babinska et al., 2002a). An interaction in cell-cell adhesion assays under flow conditions has so far been shown for LFA-1 and JAM-A (Ostermann et al., 2002), as well as for Mac-1 and JAM-C (Santoso et al., 2002).

accessible to leukocytes. Treatment of endothelial cells with a combination of tumour necrosis factor (TNF)-α and interferon (IFN)-γ induces the redistribution of JAM-A away from cell-cell contacts (Ozaki et al., 1999), which indicates that, under inflammatory conditions, JAM-A can be made available at the apical surface for LFA-1-mediated leukocyte binding.

Several anti-JAM-A antibodies have no effect on leukocyte transendothelial or transepithelial migration (Lechner et al., 2000; Liu et al., 2000; Shaw et al., 2001), and the rate of transmigration does not seem to be affected by TNF-α/IFN-γ-induced JAM-A redistribution (Shaw et al., 2001). Therefore, the mechanism by which JAM-A regulates leukocyte transendothelial migration is still unclear and needs further investigation

The concept of a role for endothelial cell-expressed JAMs in regulating leukocyte-endothelial cell interactions is further supported by other reports. JAM-C, when ectopically expressed in endothelioma cell lines, increases the rate of lymphocyte transmigration and this increase can be blocked by pre-incubating the endothelial cells with anti-JAM-C antibodies (Johnson-Leger et al., 2002). JAM-C might promote lymphocyte migration through homophilic JAM-C–JAM-C interactions. JAM-B interacts with JAM-C (Arrate et al., 2001; Liang et al., 2002) (Fig. 2). Although the relevance of this interaction for transendothelial migration has not been addressed experimentally, it opens up the possibility that endothelial cell-specific JAM-B serves as a ligand for JAM-C on leukocytes. JAM-B also interacts with the integrin α4β1 expressed by T lymphocytes (Cunningham et al., 2002). The α4β1 integrin mediates the adhesion of memory T cells to inflamed endothelium through its interaction with vascular cell adhesion molecule (VCAM)-1 (Butcher and Picker, 1996), and the α4β1–JAM-B interaction could strengthen this interaction.

JAMs and leukocyte-platelet-endothelium interactions

JAM-A and JAM-C are expressed by platelets. As mentioned above, JAM-A was originally defined as the target of an antibody (F11) that stimulates platelet aggregation (Kornecki et al., 1990; Naik et al., 1995; Wang et al., 1995). Nonstimulated platelets show increased binding to JAM-A-transfected CHO cells compared with mock-transfected CHO cells (Naik et al., 2001); in addition, nonstimulated platelets bind to cytokine (TNF-α or IFN-γ)-stimulated human umbilical vein endothelial cells (HUVEC), and this binding is partially blocked by recombinant soluble JAM-A or JAM-A peptides (Babinska et al., 2002a). These findings suggest that JAM-A could contribute to binding of platelets to activated endothelium.

Platelets also express JAM-C, which binds to the leukocyte integrin Mac-1 (Santoso et al., 2002). Mac-1 is strongly expressed on neutrophils and monocytes and mediates their adhesion to activated endothelium at sites of inflammation (Springer, 1995). On platelets, JAM-C cooperates with a second ligand for Mac-1, glycoprotein Ibα (GPIbα), in Mac-1 binding (Santoso et al., 2002) to facilitate platelet-leukocyte interactions.

The findings that JAM-A and JAM-C might be involved in platelet aggregation, and in the binding of platelets to the endothelium and leukocytes, have important physiological implications. JAM-A and JAM-C on platelets may significantly contribute to the formation of platelet plaques at the surface of nondenuded blood vessels in inflamed tissues but also at sites of

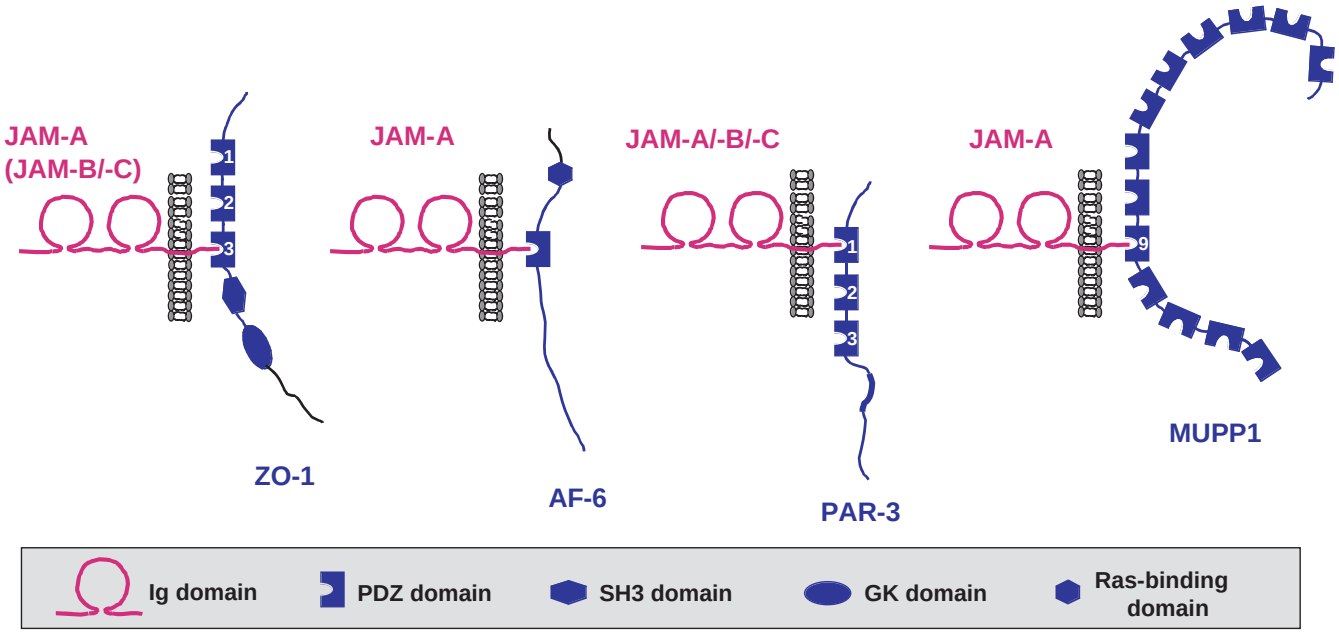

Fig. 3. PDZ-domain-containing proteins at TJs associated with JAMs. Four TJ-associated peripheral membrane proteins, ZO-1, AF-6, PAR-3 and MUPP1, directly bind to JAM-A. ZO-1 and PAR-3 associate also with JAM-B and JAM-C. In all cases, the associations are mediated through PDZ-domain-dependent interactions. Note that the PDZ domain(s) of ZO-1 interacting with JAM-B and JAM-C have not yet been determined.

vascular injury where the endothelial cell lining is denuded and deposited platelets serve as a surrogate surface for leukocyte binding (McEver, 2001). In both cases, JAM-A and JAM-C should help to recruit leukocytes and promote inflammatory processes. Because all heterophilic JAM ligands on leukocytes (LFA-1, Mac-1 and α4β1) have additional counter-receptors on endothelial cells (ICAM-1 and ICAM-2, ICAM-1, and VCAM-1, respectively), JAM-mediated interactions probably play a supporting role in these interactions. Given the complexity of the regulation of cell-cell interactions during lymphocyte homeostasis and inflammation, this is not surprising. JAM-mediated interactions might help to provide the high specificity required to guide the various leukocyte subsets to the appropriate sites of activation and effector function.

JAMs and the regulation of cell polarity

Intracellular ligands: scaffolding proteins and signalling complexes

The identification of binding partners for JAMs has provided some insight into their function(s) in the regulation of interactions involving leukocytes, platelets and endothelial cells during immune responses. However, until recently it was not clear why these molecules are localized at tight junctions and whether this localization relates to their role in supporting leukocyte-endothelial cell interactions.

Evidence for a role for JAM proteins in the formation of tight junctions came from the identification of PDZ-domain-containing proteins that associate with the cytoplasmic domain of JAM-A. PDZ domains are protein-protein interaction modules frequently present in multiple copies within a single protein. In most cases, PDZ domains recognize C-terminal motifs in their partner proteins (Harris and Lim, 2001), and all three JAMs have type II PDZ-domain-binding motifs at their C-termini (Songyang et al., 1997) (Fig. 1C). PDZ-domain-containing proteins often serve as scaffolding proteins used to build up larger protein complexes at distinct subcellular domains of the plasma membrane (Fanning and Anderson, 1999). The number of PDZ-domain-containing proteins identified at TJs is still increasing. These include the MAGUK (membrane-associated guanylate kinase) proteins zonula occludens (ZO)-1 (Stevenson et al., 1986), ZO-2 (Jesaitis and Goodenough, 1994), ZO-3 (Haskins et al., 1998) and protein associated with Lin-7 (Pals1) (Roh et al., 2002b). Members of a MAGUK subfamily of proteins characterized by an inverted orientation of the guanylate kinase (GK) and the PDZ domains – membrane-associated guanylate kinase with an inverted arrangement of protein-protein interaction domains (MAGI) proteins (Dobrosotskaya et al., 1997) – are also present. Confocal and electron microscopy has identified MAGI-1 (Ide et al., 1999) and MAGI-3 (Adamsky et al., 2003) at TJs. Besides these MAGUK proteins, the following PDZ domain-containing proteins are present at TJs: AF-6/afadin (Yamamoto et al., 1997), atypical PKC isotype-specific interacting protein (ASIP) partitioning-defective protein (PAR)-3 (Izumi et al., 1998), multi-PDZ-domain protein 1 (MUPP1) (Hamazaki et al., 2002) and the MUPP1 paralogue Pals1-associated TJ protein, also known as protein associated with TJs (PATJ) (Roh et al., 2002b; Lemmers et al., 2002).

JAM-associated PDZ proteins at tight junctions

Several proteins associate with JAM-A. Some of these, however, do not localize to TJs or might not be directly associated with JAM-A. Among these is cingulin, a peripheral membrane component at TJs; it is present in JAM-A immunoprecipitates (Bazzoni et al., 2000b) but might not directly associate with JAM-A. Similarly, JAM-A associates

with the MAGUK protein CASK/LIN-2 through a PDZ-domain-dependent interaction in epithelial cells (Martinez-Estrada et al., 2001), as well as with the $\alpha_V\beta_3$-integrin in endothelial cells (Naik et al., 2003). Both proteins, however, are not specifically enriched at TJs. Here, we focus on TJ components that directly associate with JAMs.

ZO-1

All three JAMs associate with ZO-1 in a PDZ-domain-dependent manner (Bazzoni et al., 2000b; Ebnet et al., 2000; Ebnet et al., 2003). ZO-1 contains three PDZ domains, and the association with JAM-A is mediated through PDZ domain 3 (Ebnet et al., 2000; Itoh et al., 2001). ZO-1 associates with several other integral membrane and cytosolic proteins at TJs, including occludin, claudins, ZO-2 and ZO-3 (Mitic and Anderson, 1998; Stevenson and Keon, 1998; Tsukita et al., 2001); it also binds directly to F-actin (Fanning et al., 1998). ZO-1 associates with most of these proteins through nonoverlapping domains; it associates with claudins, ZO-2 and JAM-A through PDZ domains 1, 2, and 3, respectively (Itoh et al., 1999; Fanning et al., 1998; Itoh et al., 2001); it associates with occludin through the GK domain (Fanning et al., 1998) and with F-actin through a 220-residue actin-binding region (ABR) in the C-terminal half of the molecule (Fanning et al., 2002). This raises the possibility that these molecules can bind to ZO-1 simultaneously and exist in a large protein complex. In this scenario the association of JAMs with ZO-1 would help to recruit or retain JAMs at the tight junctions.

AF-6

JAM-A directly associates with AF-6/afadin in a PDZ-domain-dependent manner (Ebnet et al., 2000). AF-6 also associates with ZO-1 and is a target for the small GTPases Ras and Rap-1 (Yamamoto et al., 1997; Boettner et al., 2000). Interestingly, ZO-1 and Ras compete for the same binding site in AF-6, which indicates that activated Ras might disrupt the ZO-1-AF-6 complex and thus perturb cell-cell contacts (Yamamoto et al., 1997). The functional relevance of the interaction with JAM-A is not clear. AF-6 might recruit JAM-A to cell contact sites. This is supported by the observation that, when microinjected into epithelial cells, JAM-A localizes to cell-cell contacts only at sites where AF-6 is present. Interestingly, the presence of ZO-1 at cell contacts in these cells is not sufficient for JAM-A recruitment, which suggests that AF-6 and ZO-1 have different roles in JAM-A localization (Ebnet et al., 2000). More recent evidence indicates that JAM-A can be recruited to nectin-2α-based cell-cell contacts through both ZO-1 and AF-6, ZO-1 serving as linker molecule between AF-6 and JAM-A (Fukuhara et al., 2002). Because AF-6 and JAM-A are present at early sites of cell-cell contact formation, so-called primordial spot-like adherens junctions or puncta (Asakura et al., 1999; Ebnet et al., 2001), the association between AF-6 and JAM-A might be relevant at early steps of cell contact formation rather than in fully polarized cells that have well-developed TJs.

MUPP1

MUPP1 contains 13 PDZ domains (Ullmer et al., 1998), is localized to TJs and directly associates with claudin-1 and JAM-A in a PDZ-domain-dependent manner (Hamazaki et al., 2002). Its function at TJs is unclear. The MUPP1 paralogue PATJ contains ten PDZ domains and also localizes to TJs (Roh et al., 2002b; Lemmers et al., 2002). PATJ forms a complex with Pals1 and the integral membrane proteins CRB1 or CRB3 (Roh et al., 2002b; Makarova et al., 2003); this complex has been conserved through evolution and regulates the polarization of epithelial cells in *Drosophila melanogaster* (Tepass et al., 2001). Interestingly, like MUPP1, PATJ associates with claudin-1. It is recruited to TJs by its association with ZO-3 (Roh et al., 2002a). Thus, the claudin- and JAM-associated protein complex, which contains ZO-1, ZO-2, ZO-3 and AF-6, may be linked to the CRB1- and CRB3-associated protein complex (Pals1, PATJ, ZO-3) through ZO-3 (Fig. 6). MUPP1 might have a function similar to that of PATJ and, by way of its 13 PDZ domains, serve as a scaffolding protein that links different protein complexes and helps to organize higher-order protein complexes.

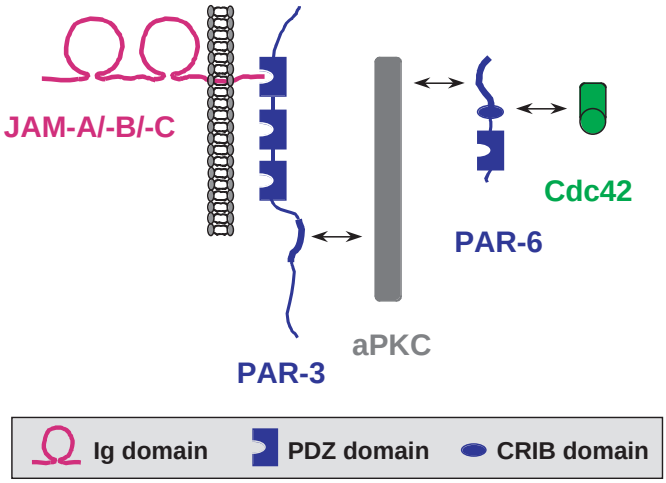

Fig. 4. The PAR-3–aPKC–PAR-6 complex is associated with JAMs through PAR-3. PAR-3 binds directly to JAM-A, JAM-B and JAM-C through its first PDZ domain. Atypical PKC (aPKC) links PAR-3 with PAR-6. The binding of active Cdc42 to the CRIB domain of PAR-6 results in aPKC activation, possibly through a Cdc42-induced conformational change of PAR-6. A direct association between PAR-3 and PAR-6 in vitro, mediated through the PDZ domain 1 of PAR-3 and the PDZ domain of PAR-6, has also been reported (Lin et al., 2000).

PAR-3

Recently, we and others identified PAR-3 as a TJ-associated protein that interacts with JAM-A (Ebnet et al., 2001; Itoh et al., 2001). PAR (*par*titioning defective) proteins were originally identified by genetic analyses as proteins whose mutation leads to defects in early asymmetric cell divisions (Kemphues, 2000). Vertebrate PAR-3 (also known as ASIP) (Izumi et al., 1998) localizes to the TJs of epithelial cells and forms a complex with atypical protein kinase C (aPKC) and PAR-6 (Joberty et al., 2000; Johansson et al., 2000; Lin et al., 2000; Qiu et al., 2000; Suzuki et al., 2001) (Fig. 4). This complex has been highly conserved through evolution. In hypodermal epithelia of *Caenorhabditis elegans* and ectodermal epithelia of *Drosophila*, it localizes to the subapical

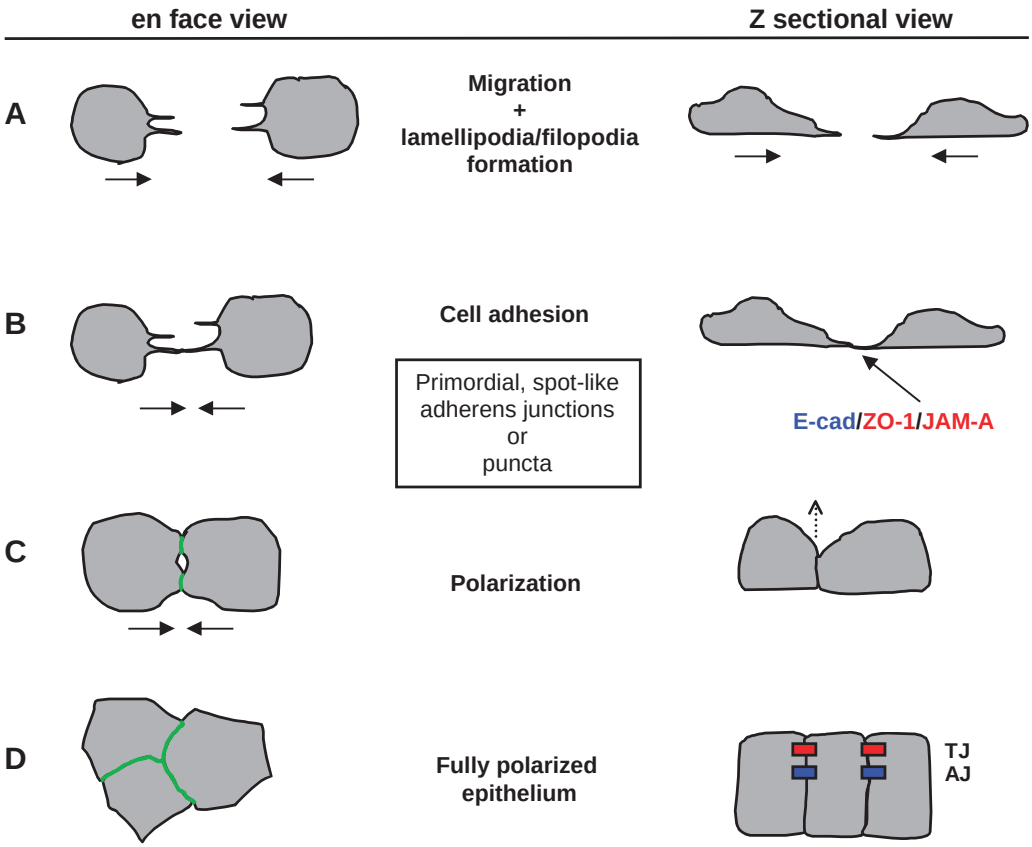

Fig. 5. Stages of cell-cell contact formation. (A) Migrating cells without cell contact protrude lamellipodia and filopodia at the leading edge of the cell. (B) Initial cell contacts at the tips of thin cellular protrusions result in the formation of primordial, spot-like adherens junctions or puncta, which are positive for E-cadherin, ZO-1 and JAM-A. (C) On further maturation of cell-cell contacts, the spot-like junctions fuse into a linear pattern; the cells start to polarize and proteins specifically enriched at adherens junctions (E-cadherin) or TJs (ZO-1, JAM-A) start to segregate; (D) In fully polarized cells, proteins specific for AJs and TJs are completely segregated, and adherens junctions and TJs can be distinguished at a structural level. The figure is based on previous studies (Yonemura et al., 1995; Adams et al., 1996; Ando-Akatsuka et al., 1999).

zone (also called the marginal zone), which lies apical to the adherens junctions at the site where TJs lie in vertebrate epithelial cells (Ohno, 2001; Knust and Bossinger, 2002).

Dominant-negative mutants of all three components of the PAR-3–aPKC–PAR-6 complex structurally alter TJs, causing mislocalization of several TJ-associated proteins. They also alter TJ function – for example, they decrease transepithelial electrical resistance (TER), increase the paracellular permeability and induce a loss of membrane polarity (Suzuki et al., 2001; Yamanaka et al., 2001; Gao et al., 2002; Nagai-Tamai et al., 2002). Importantly, these effects are observed only in cells that are in the process of developing cell polarity. Overexpressing the dominant-negative mutants in fully polarized cells has no effect on the structure or function of TJs (Suzuki et al., 2001; Yamanaka et al., 2001; Nagai-Tamai et al., 2002). These observations strongly suggest that the PAR-3–aPKC–PAR-6 complex plays a central role in the formation of TJs. Because TJs are important for the generation of membrane polarity – they prevent the diffusion of lipids and membrane proteins and thus separate the membrane into an apical and a basolateral compartment (Dragsten et al., 1981) – the PAR-3–aPKC–PAR-6 complex is of general importance for cell polarity.

The interaction between JAM-A and PAR-3 is mediated through the first of three PAR-3 PDZ domains (Ebnet et al., 2001; Itoh et al., 2001) (Fig. 3). Ectopic expression of JAM-A in CHO cells results in the recruitment of PAR-3 to cell-cell contacts, and the overexpression of a dominant-negative mutant of JAM results in a redistribution of PAR-3 away from cell-cell contacts (Ebnet et al., 2001). Both findings indicate that JAM-A can actively recruit endogenous PAR-3. The localization of both JAM-A and PAR-3 to TJs of fully polarized epithelial cells suggests that JAM-A anchors the PAR-3–aPKC–PAR-6 complex to TJs. However, given the predicted role of the PAR-3–aPKC–PAR-6 complex in polarization, the JAM-A–PAR-3 association might also be important during this process.

Polarization is thought to require the formation of adherens junctions before tight junctions are formed (Mitic and Anderson, 1998). The initial cell contacts between two epithelial cells appear as E-cadherin-positive spots at the tips of lamellipodia and filopodia, the so-called primordial, spot-like adherens junctions or puncta (Yonemura et al., 1995; Adams et al., 1996) (Fig. 5). These spots also contain ZO-1 but lack occludin (Ando-Akatsuka et al., 1999). Interestingly, JAM-A colocalizes with E-cadherin and ZO-1 in spot-like adherens junctions (Ebnet et al., 2001; Suzuki et al., 2002); other TJ-associated proteins, such as occludin, claudin-1, PAR-3 and aPKC, appear later. Membrane-bound JAM-A in spot-like adherens junctions might thus serve as a docking structure for the PAR-3–aPKC–PAR-6 complex. It is conceivable that the E-cadherin-mediated activation of Cdc42 or Rac1 (Kim et al., 2000; Nakagawa et al., 2001) leads to the activation of aPKC through Cdc42/Rac1 binding to PAR-6 (Yamanaka et al., 2001). Activated aPKC might then promote the polarization process, which results in the formation of the TJ. In such a scenario, E-cadherin together with JAM-A at primordial cell contacts might represent a positional cue instructing the cell where to localize and subsequently activate the polarization-promoting protein complexes.

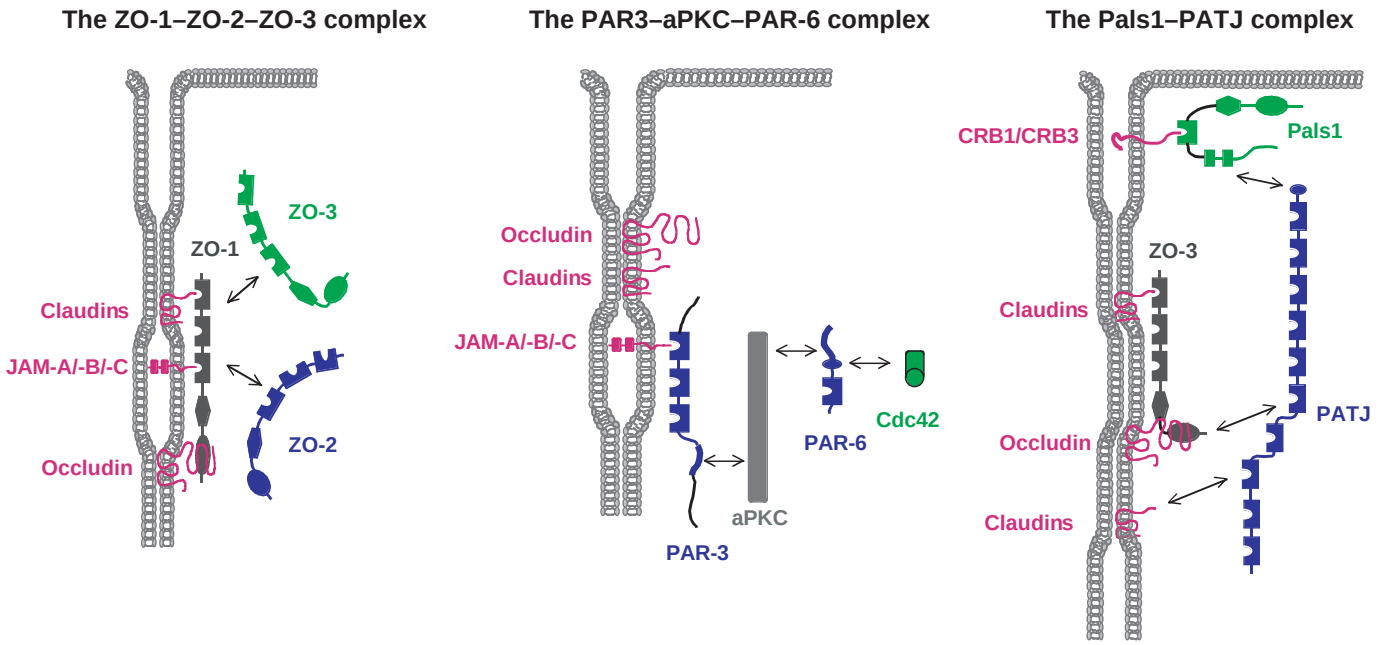

Fig. 6. Multiprotein-complexes at TJs. Three major multiprotein complexes are located at TJs. The ZO-1–ZO-2–ZO-3 complex is associated with occludin, claudins and JAMs through multiple interactions. ZO-1 directly associates with all three integral membrane proteins at TJs, i.e. occludin, claudins and JAMs. ZO-2 and ZO-3 directly associate with ZO-1. Both ZO-2 and ZO-3 can also be directly targeted to the plasma membrane by a direct association with occludin and claudins. The PAR-3–aPKC–PAR-6 complex is targeted to the plasma membrane through the interaction of PAR-3 with JAMs. The active form of Cdc42 can associate with the complex by binding to PAR-6, leading to the activation of aPKC. The Pals1-PATJ complex is associated with the membrane through Pals1 binding to CRB1 and CRB3. PATJ associates with Pals1 through its N-terminal MAGUK recruitment (MRE) domain that binds to the L27N domain of Pals1. It also associates with ZO-3 and claudin-1 through PDZ domains. CRB1 and CRB3, similar to the *Drosophila* orthologue Crumbs, are both localized at the apical surface of epithelial cells but are concentrated at TJs. These three protein complexes might exist independently, but they could also be physically linked. For instance, the Pals1-PATJ complex could be linked to the ZO-1–ZO-2–ZO-3-complex through PATJ binding to ZO-3. In addition, Pals-1 can directly associate with PAR-6 providing a link between the Pals1-PATJ and the PAR-3–aPKC–PAR-6 complexes. Genetic evidence from studies in *Drosophila* suggests that these protein complexes are also functionally linked in the regulation of epithelial cell polarization (Tanentzapf and Tepass, 2003; Bilder et al., 2003).

A general role for JAMs in TJ formation and cell polarity?

The identification of JAM-A as a binding partner for PAR-3 strongly suggests that JAM-A functions in TJ formation. These findings also provide a molecular basis for the earlier observations that anti-JAM-A antibodies, when applied during Ca^{2+}-switch-induced junction formation, inhibit the redistribution of JAM-A and occludin to cell contact sites, as well as the reformation of TER but not the redistribution of ZO-1 and E-cadherin (Liu et al., 2000; Liang et al., 2000). Blocking homophilic JAM-A binding probably interferes with the stable localization of JAM-A to early sites of cell contact, which might result in inefficient recruitment of the PAR-3–aPKC–PAR-6 complex. The antibody blocking studies, therefore, nicely correlate with the studies involving dominant-negative mutants of aPKC and PAR-6 (Suzuki et al., 2002; Yamanaka et al., 2001). Blocking either of the two systems – JAM-A localization to cell-cell contacts or the generation of a functional PAR-3–aPKC–PAR-6 complex – seems to arrest cell contact formation at the stage of immature adherens junctions.

In contrast to JAM-A, JAM-B and JAM-C are largely restricted to endothelial cells. Like JAM-A, however, both associate with PAR-3 in a PDZ-domain-dependent manner through the first PDZ domain of PAR-3, and PAR-3 is actively recruited to JAM-C-based cell-cell contact sites in CHO cells (Ebnet et al., 2003). Also, PAR-3 is localized at cell-cell contacts of cultured endothelial cells and is expressed by various endothelia in vivo (Ebnet et al., 2003). Therefore, it is likely that JAM-B and JAM-C have functions in endothelial cells similar to those of JAM-A in epithelial cells. A striking observation, however, is the specificity of PAR-3 for JAMs. PAR-3 associates with JAM-A, JAM-B and JAM-C but not with occludin, claudin-1, claudin-4, claudin-5, ESAM or CAR (Ebnet et al., 2001; Ebnet et al., 2003). This unique selectivity of PAR-3 suggests a specific function for JAM-A, JAM-B and JAM-C in TJ formation in vertebrate epithelial and endothelial cells.

Conclusions and perspectives

The identification of extracellular ligands and intracellular proteins associating with JAMs has highlighted dual functions for JAMs. In the immune system, they might help to orchestrate the recruitment of leukocytes to sites of inflammation and wound repair. In polarized epithelial and endothelial cells, they might help to organize the formation of TJs and membrane polarity. These two functions might eventually turn out to be related.

Several aspects of JAM biology need to be addressed. First, it will be important to know whether JAMs are part of a signal

transduction machinery. All three JAMs contain tyrosine as well as serine/threonine residues in their cytoplasmic tails, which are putative targets for phosphorylation. Indeed, JAM-A is serine phosphorylated on platelet activation (Ozaki et al., 2000), and JAM-C is serine phosphorylated in transfected CHO cells (Ebnet et al., 2003). Given that both JAM-A and aPKC are present in PAR-3 immunprecipitates (Ebnet et al., 2001) it is possible that JAMs serve as substrates for aPKC. Additional observations further support a signalling role for JAMs, albeit through distinct mechanisms. JAM-A serves as a receptor for reovirus, and the activation of nuclear factor (NF)-κB on reovirus binding to HeLa cells requires the presence of JAM-A (Barton et al., 2001). Also, JAM-A contributes to basic fibroblast growth factor (bFGF)-induced mitogen-activated protein kinase activation and endothelial cell tube formation (Naik et al., 2003), which suggests that it has a signalling role during angiogenesis. Second, despite the evolutionary conservation of the PAR-3–aPKC–PAR-6 complex, structural orthologues of JAMs have not yet been identified in invertebrates. It will be important to understand how the PAR-3–aPKC–PAR-6 complex is targeted to the subapical region of hypodermal epithelial cells in *C. elegans* and epithelial cells in *Drosophila*. It is possible that it is indirectly anchored to the membrane. In vertebrate epithelial cells, PAR-6 can interact with Pals-1 (Hurd et al., 2003), which associates with the integral membrane proteins CRB1 and CRB3 (Roh et al., 2002b; Roh et al., 2003) (see also Fig. 6). Third, given that cell polarity is of general importance in a wide variety of tissues and biological processes, it will be important to identify cell types other than epithelial/endothelial cells which display a polarized morphology and analyse whether JAMs and the PAR-3–aPKC–PAR-6 complex are involved. In this respect, it is interesting to note that the adherens junctions-associated Ig-superfamily members nectins mediate cell-cell contacts at puncta adhaerentia in synapses and heterotypic cell-cell contacts between spermatids and sertoli cells (Takai and Nakanishi, 2003). Finally, the mechanisms underlying the formation of TJs from primordial, spot-like adherens junctions are only beginning to be understood. The next important step will be to identify downstream targets of the PAR-3-/PAR-6-associated aPKC. Elucidating these aspects of JAM function will have important consequences for our understanding of biological processes such as cell migration, cell differentiation and tissue formation.

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