INTRODUCTION

A key role of epithelia and endothelia is the formation of diffusion barriers that allow the generation and maintenance of compartments with different compositions, a fundamental requirement for the physiological functioning of organs. Individual epithelial and endothelial cells are joined to each other by a specialized complex consisting of gap junctions, desmosomes, adherence junctions and the zonula occludens or tight junctions (Farquhar and Palade, 1963). Tight junctions encircle the cells at the apical end of the lateral membrane and, in freeze fracture replicas, show a particular net-like meshwork of fibrils (Staehelin, 1973). By restricting paracellular diffusion, tight junctions are essential for the barrier function of epithelia and endothelia. Nevertheless, they do not form absolute diffusion barriers but are semipermeable since they allow the selective passage of certain solutes but not others (Cereijido, 1991). Furthermore, paracellular permeability differs from epithelium to epithelium and can change due to physiological and pathological stimuli (Madara, 1988; Bentzel et al., 1991). Tight junctions are also crucial for the development of cell surface polarity since they form an intramembrane diffusion fence that restricts diffusion of lipids in the exoplasmic leaflet of the plasma membrane (Dragsten et al., 1981; van Meer and Simons, 1986).

The identification of tight junction proteins and their use as experimental tools is starting to help us to understand the functioning of this fascinating structure and suggest the involvement of tight junctions in thus far unexpected cellular processes. In this review, we will discuss the physiological functions of tight junctions in the light of the more recent findings and, if possible, will try to relate them to the molecular composition.

SUMMARY

Tight junctions are the most apical intercellular junctions of epithelial and endothelial cells and create a regulatable semipermeable diffusion barrier between individual cells. On a cellular level, they form an intramembrane diffusion fence that restricts the intermixing of apical and basolateral membrane components. In addition to these well defined functions, more recent evidence suggests that tight junctions are also involved in basic cellular processes like the regulation of cell growth and differentiation.

Key words: Zonula occludens, Paracellular permeability, Cell polarity, Differentiation

Molecular Composition

ZO-1 (zonula occludens-1) was the first tight junction-associated protein identified (Stevenson et al., 1986). It can also localize to other subcellular sites and is even expressed by cells that never form tight junctions (Howarth et al., 1992; Itoh et al., 1993; Gottardi et al., 1993). In epithelial cells, ZO-1 forms a heteromeric complex with ZO-2 and p130 (Gumbiner et al., 1991; Balda et al., 1993); ZO-2 appears to be expressed in epithelial cells only (Jesaitis and Goodenough, 1994). Molecular cloning of ZO-1 and ZO-2 revealed homologies to a Drosophila tumor suppressor, DlgA, and a component of postsynaptic densities, PSD-95 or SAP-90 (Tsukita et al., 1993; Willott et al., 1993; Woods and Bryant, 1993; Jesaitis and Goodenough, 1994). The homologous region includes a domain with similarities to yeast guanylate kinase, an SH3 domain, and three PDZ-domains (PSD-95/DlgA/ZO-1 homology domain). PDZ-domains have been found in many different proteins and most of them appear to be involved in the clustering and docking of other proteins to particular membrane subdomains (Fanning and Anderson, 1996). The C-terminal half of ZO-1 contains an actin-binding site and several alternatively spliced domains (Itoh et al., 1997; Anderson and Van Itallie, 1995). Expression of one of those alternatively spliced domains, the alpha-domain, has been shown to correlate with the plasticity of tight junctions and appears to be developmentally regulated (Balda and Anderson, 1993; Sheth et al., 1997).

Rather unexpected was the finding of small GTPases of the rab family (e.g. rab 13 and rab3B) that are associated with tight junctions (Zahraoui et al., 1994; Weber et al., 1994). Rabs are generally thought to participate in the control of vesicular transport steps. In fact, rab 13 localizes to vesicular structures in fibroblasts (Zahraoui et al., 1994) and rab8, which is...
involved in basolateral transport, partially co-localizes with ZO-1 in MDCK cells (Huber et al., 1993). The functions of rabs at tight junctions are not clear.

Several other peripheral membrane proteins have been localized to tight junctions. The primary structure of most of these proteins has not yet been described (cingulin, 7H6, and BG9, and a 210 kDa protein from Xenopus: Citi et al., 1988; Chapman and Eddy, 1989; Zhong et al., 1993; Merzdorf and Goodenough, 1997). An interesting exception is symplekin, a peripheral membrane protein that not only associates with tight junctions but can also be found in the nucleus (Keon et al., 1996). Similar to ZO-1, symplekin is also expressed by cells that do not form tight junctions, where it appears to be only in the nucleus. Also ZO-1 can be in the nucleus but, unlike symplekin, only in growing and not in differentiated epithelial cells (Gottardi et al., 1996). This dual localization suggests that tight junctions might also be involved in the regulation of cell growth and differentiation.

Occludin is the only known transmembrane protein of tight junctions (Furuse et al., 1993). On the basis of its amino acid sequence, occludin was predicted to be a polytopic membrane protein with four transmembrane domains, N- and C-termini in the cytosol and, consequently, two extracellular loops. The predicted membrane topology is supported by several observations: the end of the C-terminal cytoplasmic domain can interact with ZO-1 (Furuse et al., 1994); both predicted extracellular loops become glycosylated if N-linked glycosylation sites are introduced (Matter and Balda, 1998); peptides corresponding to the extracellular loops interfere with occludin localization or function if added to the extracellular medium (Wong and Gumbiner, 1997; Van Itallie and Anderson, 1997) and an antibody raised against a part of the first extracellular loop has access to occludin in non-permeabilized cells (Van Itallie and Anderson, 1997). Molecular cloning of occludin from different species revealed that some regions of occludin are well conserved (i.e. the end of the C terminus) while other domains show a surprisingly poor conservation (Ando-Akatsuka et al., 1996). Divergent regions often possess a rather unusual amino acid composition that has the same characteristics in all analyzed species. This is particularly striking in the case of the extracellular loops. For example, in the first extracellular domain, which is 46 amino acids long in human occludin, there are 18 glycine and 11 tyrosine residues. In the corresponding domain of chicken occludin, which is 3 amino acids shorter, 16 are glycine residues and the number of tyrosines is the same as in the human protein. It is thus likely that the function of these extracellular domains depends on their overall physicochemical properties rather than on the linear amino acid sequence.

**BIOGENESIS OF TIGHT JUNCTIONS**

Classically, studies focusing on the biogenesis and assembly of tight junctions made use of filter-grown epithelial cells that form electrically tight monolayers upon the addition of calcium and, hence, were looking at the de novo assembly of tight junctions (Cereijido et al., 1978; González-Mariscal, 1991a). Such calcium-switch experiments represented the only way to study the biogenesis of tight junctions with an experimental system that was easy to manipulate; therefore, it was often used to study the regulation of junction assembly.

The assembly of tight junctions is tightly regulated by a network of signal transduction pathways that include G-proteins, phospholipase C, protein kinase C, and calmodulin (Balda et al., 1991; Denker et al., 1996; Rosson et al., 1997). The complexity of the signaling pathways involved is a reflection of the amount of required modifications of protein activities and cytoskeletal rearrangements that are required for the de novo assembly of the junctional complex. Assembly and disassembly of tight junctions in epithelial cells appear to be largely controlled by E-cadherin-mediated cell-cell adhesion (Behrens et al., 1985; Gumbiner and Simons, 1986). Dissociation of tight junctions upon the addition of anti-E-cadherin antibodies to MDCK monolayers can be overcome by cAMP (Behrens et al., 1985) or by the diacylglycerol analog diC8 (Balda et al., 1993). Moreover, diC8 can stimulate at least partial assembly of tight junctions at low extracellular calcium concentrations (Balda et al., 1993). Also the opposite process depends on protein kinases since calcium chelation induced disassembly of tight junctions can be blocked by protein kinase inhibitors (Citi, 1992; Citi et al., 1994).

More recently, it has been demonstrated that E-cadherin null mouse blastocysts have morphologically normal tight junctions, as seen by transmission electron microscopy of Epon sections, that contain occludin but not ZO-1 (Ohsugi et al., 1997). It is not clear though whether this means that maternally derived E-cadherin is sufficient to stimulate partial assembly of intercellular junctions or, alternatively, that non-ligated E-cadherin negatively influences assembly of tight junctions in the above described experiments.

The discovery of the transmembrane protein occludin resulted in a fascinating tool to analyze the integration of newly synthesized components into existing tight junctions. The potential usefulness of chicken occludin to study tight junctions in mammalian epithelial cells was demonstrated by transient and stable expression experiments that showed that chicken occludin is efficiently sorted to tight junctions in bovine, human, and canine epithelial cells (Furuse et al., 1994; Balda et al., 1996a; McCarthy et al., 1996). The evolutionary conservation of the targeting mechanism that is responsible for the transport of occludin to tight junctions is also supported by expression of chicken occludin in Xenopus embryos (Chen et al., 1997).

The C-terminal cytoplasmic domain of occludin is important for targeting to tight junctions in transiently transfected cells (Furuse et al., 1994). Nevertheless, occludin lacking the entire C-terminal cytoplasmic domain becomes at least partially integrated into tight junctions of stably transfected MDCK cells as well as microinjected Xenopus embryos (Balda et al., 1996a; Chen et al., 1997). In MDCK cells, the intracellular pool of mutant occludin localizes to the Golgi complex while only comparably small amounts of transfected full length occludin can be detected there suggesting that the C-terminal domain is required for efficient exit from the Golgi apparatus. Since transfected occludin oligomerizes with endogenous occludin in both expression systems, transfected mutant occludin appears to be dragged to tight junctions by endogenous occludin (Chen et al., 1997; Matter and Balda, 1998).

In polarized epithelial cells, tight junctions form a physical
and morphological barrier between the apical and the basolateral plasma membrane domains; hence, biogenesis of tight junctions could involve either one or both of the two cell surface domains. The C-terminal domain of occludin is sufficient to mediate basolateral targeting of a reporter protein indicating that it contains a basolateral sorting signal (Matter and Balda, 1998). An involvement of the basolateral membrane in the transport of occludin to tight junctions is also supported by the basolateral accumulation of occludin molecules with mutated extracellular loops but that possess the entire C-terminal cytoplasmic domain. Together with the observation of low amounts of occludin in the lateral membrane of chicken intestinal epithelial cells (Sakakibara et al., 1997) and lateral intramembrane strands under certain experimental conditions (Bentzel et al., 1991; Polak-Charcon, 1991), these experiments suggest that the biogenesis of tight junctions occurs from the basolateral membrane.

Once in the basolateral membrane, accumulation of occludin in tight junctions appears to involve the extracellular domains. This is supported by the finding that addition of a peptide corresponding to the second extracellular domain of occludin to A6 cells results in the disappearance of occludin from the junction (Wong and Gumbiner, 1997). Complete disappearance from tight junctions of mature monolayers is a very slow process suggesting that the peptide inhibits integration of newly synthesized occludin into tight junctions rather than enhancing the turnover of already integrated occludin. Because of the targeting experiments described above, one would predict that the effect of the peptide should be polar (i.e. occurring only if added basolaterally). An involvement of the extracellular domains in the accumulation of occludin in tight junctions is also suggested by the partial lateral accumulation of occludin molecules with deletions in the extracellular loops (M. S. Balda, C. Flores, M. Cereijido and K. Matter, unpublished). How the extracellular domains mediate accumulation of the protein in the junction is not clear. The simplest explanation would be that the extracellular loops directly mediate an intercellular homo- or heterotypic interaction. Occludin is part of an intercellular complex since the distribution of occludin in one cell can affect the distribution of occludin in the neighboring cell (Balda et al., 1996a). Furthermore, peptides representing sequences from the first extracellular loops inhibit occludin-mediated aggregation of transfected fibroblasts (Van Itallie and Anderson, 1997).

Since it has been suggested that phosphorylation of occludin is involved in the formation of tight junctions (Sakakibara et al., 1997), it could also be that extracellular interactions somehow regulate phosphorylation of the intracellular domains, which might activate interactions with the submembrane cytoskeleton. This is an attractive model since there are many experiments in the literature that imply an involvement of protein kinases in the regulation of both tight junction assembly and disassembly (Anderson and Van Itallie, 1995). Since the C-terminal domain of occludin contains a ZO-1-binding site, it has been suggested that ZO-1 is involved in targeting of occludin to tight junctions (Furuse et al., 1994). The two observations, however, that occludin can localize to tight junctions in the absence of detectable amounts of junctional ZO-1 (Ohsugi et al., 1997) and that ZO-1 exhibits a normal distribution in the absence of junctional occludin (Wong and Gumbiner, 1997) suggest that targeting of the two proteins can occur independently from one another. Whether ZO-1 associates with tight junctions or with adherens junctions is therefore more likely to be determined by other binding partners like, for instance, ZO-2 and p130 (Gumbiner et al., 1991; Balda et al., 1993; Jesaitis and Goodenough, 1994).

TIGHT JUNCTIONS AND SELECTIVE PARACELLULAR PERMEABILITY

Morphological studies with electron dense macromolecules demonstrated that tracers can freely diffuse along the paracellular pathway until the level of tight junctions where they are blocked indicating that tight junctions are fundamental for formation of epithelial and endothelial barriers by restricting diffusion along the paracellular pathway (Cereijido, 1991). The use of hydrophilic tracers of different sizes suggested that the paracellular pathway is not blocked by an absolute diffusion barrier but by a semipermeable one that involves pores with radii of 30-40 Å (Lindemann and Solomon, 1962; van Os et al., 1974). This is supported by experiments with microelectrodes that showed that the paracellular pathway has a higher conductivity than the transcellular route in Necturus gallbladder and cultured epithelial monolayers (Frömter, 1972; Cereijido et al., 1980; Reuss, 1991). Since the ion selectivity of the paracellular route is altered by acidification or the addition of polyvalent cations, it was proposed that these relatively large pores contain dipoles with negative sides oriented towards the aqueous phase (Diamond and Wright, 1969; Cereijido et al., 1978; Reuss, 1991). Even though the transepithelial electrical resistance of different epithelia can vary from just a few to several thousand Ωcm², also so called ‘leaky’ epithelia (i.e. epithelia with a low transepithelial electrical resistance) exhibit selective transport of water and solutes (Diamond and Wright, 1969; Cereijido, 1991).

Initial freeze-fracture analysis of different tissues revealed an exponential relationship between the transepithelial electrical resistance and the number of intramembrane strands (Claude and Goodenough, 1973). This led to the proposal that selective diffusion across tight junctions is mediated by fluctuating aqueous channels embedded into the intramembrane strands (Claude, 1978; Cereijido et al., 1989; Gumbiner, 1993). Today we know many exceptions to this rule indicating that the number of intramembrane strands can at least not be the only parameter that determines the junctional resistance (González-Mariscal, 1991b). The tight junctions of high and low resistance MDCK cells, for example, do not exhibit any evident morphological differences but the transepithelial electrical resistance can differ by two orders of magnitude (Stevenson et al., 1988; González-Mariscal et al., 1989).

Occludin appears to be directly involved in the formation of the paracellular barrier. Stable expression of chicken occludin in MDCK cells increases transepithelial electrical resistance by 40 to 400%, depending on the expression system used (Balda et al., 1996a; McCarthy et al., 1996). There is a direct relationship between the amount of expressed occludin and transepithelial electrical resistance indicating that the differences from one expression system to another are due to the achieved expression levels. The amount of endogenously
expressed occludin correlates also with the tightness in endothelia (Hirase et al., 1997) and transepithelial electrical resistance drops when occludin is depleted from tight junctions (Wong and Gumbiner, 1997). Occludin cannot be the only component of the paracellular barrier, however, since a continuous junctional ring of occludin is not required for electrically tight MDCK monolayers (Balda et al., 1996a). That the presence of occludin in tight junctions is required for an electrically tight barrier but that its distribution within the junction can be discontinuous without a loss of this function would be compatible with a model in which occludin is a regulatory component of the paracellular diffusion barrier.

MDCK cells stably expressing chicken occludin exhibit not only higher transepithelial electrical resistance but also higher selective paracellular permeability (Balda et al., 1996a; McCarthy et al., 1996). This phenotype is even stronger in MDCK cells expressing occludin mutants that lack the C-terminal domain independently of whether they are continuously or discontinuously distributed along the junction (Balda et al., 1996a; M. S. Balda and K. Matter, unpublished). Importantly, induction of higher levels of paracellular permeability by the expression of wild-type and mutant occludin does not affect the size and ion selectivity of the paracellular pathway suggesting that occludin is involved in selective paracellular permeability. This is also supported by experiments in which chicken occludin mutants with deletions in the extracellular loops were found to drastically inhibit selective paracellular permeability in transfected MDCK cells (M. S. Balda, C. Flores, M. Cereijido and K. Matter, unpublished).

In microinjected Xenopus embryos, C-terminally truncated, but not wild-type, chicken occludin was reported to affect the integrity of tight junctions as detected by biotinylation at 10°C of membranes along intercellular spaces (Chen et al., 1997). Since paracellular permeability is temperature dependent (González-Mariscal et al., 1984), it is not clear whether this result is due to increased selective paracellular flux or to effects on the kinetics of tight junction assembly.

Whether occludin directly mediates selective paracellular flux or is rather a regulator of selective paracellular permeability is not known. Since the C-terminal domain of occludin appears to be important for the regulation of this activity (Balda et al., 1996a) and binds to ZO-1 in vitro (Furuse et al., 1994), this interaction could be involved in this process. Since both proteins ZO-1 and occludin are phosphoproteins (Anderson et al., 1988; Sakakibara et al., 1997), it could be that this interaction and, thereby, paracellular permeability are regulated by phosphorylation.

TIGHT JUNCTIONS AND THE RESTRICTION OF APICAL/BASOLATERAL LIPID DIFFUSION

Tight junctions are also involved in the maintenance of the distinct distribution of lipids in the outer leaflets of the apical and basolateral plasma membrane domains (Dragsten et al., 1981; van Meer and Simons, 1986). If fluorescent lipids or lipid probes are inserted into the outer leaflet of the plasma membrane, they stay in the cell surface domain into which they have been inserted if they are not able to flip-flop into the inner membrane leaflet. This fence function of tight junctions is not directly related to the paracellular barrier function since 10 minutes of ATP-depletion are sufficient to decrease transepithelial electrical resistance by 95% but not to affect the fence function or the ultrastructural appearance of tight junctions (Mandel et al., 1993). Breakdown of the lipid diffusion fence at longer times of ATP depletion correlates with the fragmentation of the intramembrane strands suggesting that they might be directly responsible for the fence function (Bacallao et al., 1994).

In contrast, disruption of the continuous junctional distribution of endogenous and transfected occludin in MDCK cells causes a breakdown of the intramembrane diffusion fence but not fragmentation of the intramembrane strands (Balda et al., 1996a). This indicates that occludin is involved in the restriction of lipid diffusion and that the presence of intramembrane strands is not sufficient for a functional intramembrane fence. Since occludin localizes to the strands (Fujimoto, 1995; Furuse et al., 1996), this may mean that only strands containing occludin are functional as a diffusion fence suggesting that occludin somehow influences the functional and/or structural properties of the intramembrane strands.

TIGHT JUNCTION MORPHOLOGY AND CELL-CELL INTERACTION

The ultrastructural appearance of tight junctions has fascinated biologists for decades. In freeze-fracture replicas, tight junctions appear as a netlike meshwork of fibrils (Staehelin, 1973), while in thin sections they can be seen as zones of closely opposed neighboring plasma membranes (Farquhar and Palade, 1963). Depending on the sample preparation, very close focal contacts can be observed where the neighboring plasma membranes appear to be partially fused (Pinto da Silva and Kachar, 1982). These very close contacts are thought to represent the intramembrane strands seen by freeze-fracture or, more precisely, two opposing intramembrane strands that are slightly shifted relative to each other.

The biochemical composition of these strands is still unclear. Because of the morphological appearance of the contact sites within tight junctions, it had been proposed that they represent hemifusions and the strands inverted micelles (Kachar and Reese, 1982; Wegener and Gall, 1996). The opposing and today most widely accepted model is that the strands represent rows of intramembrane proteins that form the cell-cell contacts (Gumbiner, 1993). Since the exoplastic leaflets of neighboring cells are continuous in a model that predicts hemifusions, lipids were studied to test whether they would be able diffuse from one cell to the other. Glycolipids as well as fluorescently headgroup-labeled phosphatidylethanolamine are unable to diffuse from one cell to another in MDCK monolayers (van Meer et al., 1991). Opposite results were recently reported using a short chain fluorescent lipid (Wegener and Gall, 1996). Due to the physical properties of such short chain lipids, however, interpretation of this result is difficult. Nevertheless, that most lipids do not diffuse from one cell to another does not automatically exclude an involvement of unconventional lipid structures in the architecture of tight junctions since diffusion from one cell to another could be restricted by the physicochemical properties of a particular lipid and/or properties of the junction.
While the actual composition of the intramembrane strands is still unclear, occludin is clearly part of them (Fujimoto, 1995; Furuse et al., 1996). Since discontinuities in the junctional distribution of occludin are not paralleled by similar interruptions in the intramembrane strands, occludin cannot be their main structural element (Balda et al., 1996a). The role of occludin appears to lie rather in the formation or regulation of these strands since overexpression of occludin leads to a slight increase in the average number of strands (McCarthy et al., 1996; Balda et al., 1996a). This was only observed in MDCK cells expressing wild-type chicken occludin but not in cells expressing a version with an N-terminal epitope indicating that the N terminus of occludin might be involved in this function. The importance of N-terminal interactions is supported by the finding that both termini of the protein need to be inactivated (i.e. blocked or deleted) for occludin to become discontinuously distributed (M. S. Balda, C. Flores, M. Cereijido and K. Matter, unpublished).

The protein model of tight junctions predicts transmembrane proteins that form the intramembrane strands and make a tight contact between neighboring cells and even in a model based on hemifusions one would need proteins to stabilize such an energetically unstable lipid structure. Occludin is clearly part of such intercellular complexes (Balda et al., 1996a) and can even mediate aggregation of transfected fibroblasts in a calcium-independent manner (Van Itallie and Anderson, 1997). Disruption of the continuous junctional distribution of occludin, however, does not cause morphological changes of the junction as seen by transmission electron microscopy of Epon sections as well as freeze-fracture replicas (Balda et al., 1996a) and depletion of occludin from tight junctions of A6 cells does not result in gross morphological changes as observed by scanning electron microscopy of the apical surface of the monolayer (Wong and Gumbiner, 1997). These observations suggest that occludin-mediated cell-cell interactions are not critical for tight junction morphology.

TIGHT JUNCTIONS AND THE REGULATION OF CELL GROWTH AND DIFFERENTIATION

In addition to adhesion, cell-cell junctions are thought to be involved in the regulation of cell growth and differentiation in vertebrates as well as invertebrates (reviewed by Takeichi, 1995; Kirkpatrick and Peifer, 1995; Huber et al., 1996; Gumbiner, 1997; Barth et al., 1997; Han, 1997). Mainly adherens junctions have been implicated in signaling to the nucleus. Classical cadherins are connected to the actin-cytoskeleton by a cytoplasmic complex that includes the catenins. β-Catenin or armadillo, its functional homologue in Drosophila, are key players in cell-cell adhesion as well as in the Wnt/Wingless signal transduction pathway. Wnt/Wingless signaling causes inactivation of a serine/threonine kinase and, subsequently, hypophosphorylation of β-catenin. In association with a member of the LEF-1/TCF transcription factor family, β-catenin then activates transcription of specific genes.

Several recent reports suggest that tight junctions might also play a role in the regulation of cell growth and differentiation. Molecular cloning of ZO-1 and ZO-2 revealed a homology to Disc large A (Dlg A), a Drosophila tumor suppressor that localizes at the septate junction (Tsukita et al., 1993; Willott et al., 1993; Woods and Bryant, 1993; Jesaitis and Goodenough, 1994). The members of this new protein family, which are generally part of the submembrane cytoskeleton, appear to be involved in the organization of membrane subdomains and often localize at cell-cell junctions. Their homologous regions include one or three PDZ domains, an SH3 domain, and a domain homologous to guanylate kinase (Fanning and Anderson, 1996). One of the best characterized examples is lin-2, a family member involved in the cell fate signal transduction pathway of vulval differentiation in Caenorhabditis elegans (Kim, 1995; Simske et al., 1996). Human Dlg, another family member that localizes to the lateral membrane of epithelial cells, has been shown to interact with adenomatous polyposis coli (APC) protein through one of its PDZ domains (Matsumine et al., 1996).

ZO-1 possesses several domains that could be involved in protein-protein interactions but the functions of most identified interacting proteins are not known. Nevertheless, ZO-1 associates with the catenin complex in the absence of tight junctions like, for instance, in MDCK cells grown in low calcium medium (Rajasekaran et al., 1996). This interaction is mediated by the N-terminal half of ZO-1 and appears to occur via α-catenin, not β-catenin (Itoh et al., 1997). ZO-1 also interacts with the ras target AF-6 (Yamamoto et al., 1997) and binds with its SH3 domain to a serine kinase that phosphorylates ZO-1 in vitro (Balda et al., 1996b). Since the SH3 domain of Drosophila Dlg A is crucial for its function as a tumor suppressor (Woods et al., 1996), this kinase could be a regulator of such a function mediated by ZO-1.

A fraction of ZO-1 accumulates in the nucleus in growing epithelial cells suggesting that it might be directly involved in nuclear processes (Gottardi et al., 1996). This dual localization at tight junctions as well as in the nucleus has also been observed for other proteins. Symplekin localizes at the tight junction and in the nucleus in confluent cells (Keon et al., 1996) while antigen 2.1, another new peripheral tight junction protein, co-localizes with ZO-1 at the tight junction as well as in the nucleus in growing MDCK cells (M. S. Balda and K. Matter; unpublished observations). While no functions are known for these proteins, these observations support the idea of tight junction components being involved in the regulation of nuclear processes.

While these data suggest a role for tight junctions in the regulation of cell growth and differentiation, it is difficult to speculate on the signaling pathway(s) in which ZO-1 or other tight junction proteins are involved. Furthermore, it is not known what kinds of stimuli are received at tight junctions, what the receiving molecules in the membrane are, and how such signals are transduced to the submembrane protein complexes and, ultimately, to the nucleus.

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