PERGAMON

Progress in Biophysics & Molecular Biology 81 (2003) 81–115

Progress in Biophysics & Molecular Biology

www.elsevier.com/locate/pbiomolbio

Review

Membrane targeting

M. Cereijido*, R.G. Contreras, L. Shoshani, M.R. García-Villegas

Center for Research and Advanced Studies (CINVESTAV), Av. Instituto Politécnico Nacional 2508, San Pedro Zacatenco, México D.F. 07300, Mexico

Contents

*Corresponding author. Tel.: +5255-5747-3800x3364; fax: +5255-5747-9893.
E-mail address: cereijido@fisio.cinvestav.mx (M. Cereijido).

PII: S0079-6107(02)00047-0

1. Introduction

Once the basic mechanisms for coding and synthesizing proteins were understood, it became necessary to learn how they are targeted, incorporated and retained in the place of the cell where they function. To understand why this knowledge has now become so important, we will sketch the main experimental models of the cell developed after the Second World War (Fig. 1).

(1) *The biophysicist's cell.* the measurement of unidirectional fluxes and microelectrodes to study transporting mechanisms and the electrical potential across the cell membrane, led to depict the cell as a single compartment, filled with a well-stirred solution. Solutes were assumed to have chemical activities identical to their concentrations, and diffusion coefficients to be so much higher than membrane permeability, that the only restriction to their movement was thought to be due to the mechanisms of penetration into the cell.
(2) *Ling's cell*. In 1962 Gilbert N. Ling summarized the evidence indicating that macromolecules in the cytoplasm have enough net electric charges and hydrogen binding sites to insure that no water molecule nor solute would be free as it would be in a biophysicist's cell (Fig. 1, *upper right*).
(3) *The biologist's cell*. Electron microscopy demonstrated that the nucleus occupies a considerable space of the cell, and the rest of the cytoplasm is almost completely filled with structures such as mitochondria, cisternae of the endoplasmic reticulum, the Golgi apparatus, lysosomes, endosomes, microtubes, microfilaments, etc. (Fig. 1, *bottom left*).
(4) *The biochemist's cell*. After the Second World War most chemical studies of the cell started by mincing tissues with scissors and blenders, followed by a thorough homogenization in which most cell structures were destroyed. The introduction of cell fractionation and immunomicroscopy indicated that different protein species tended to be associated with a specific cell organelle. This demonstrated that the thousands of molecular species contained in the cell are secluded in separated chemical and physical compartments, and therefore do not interfere with each other.
(5) *The physiologist's cell*. The use of tracers indicated that most biological molecules are only transient dwellers of specific compartments, implying that they are continually arriving from

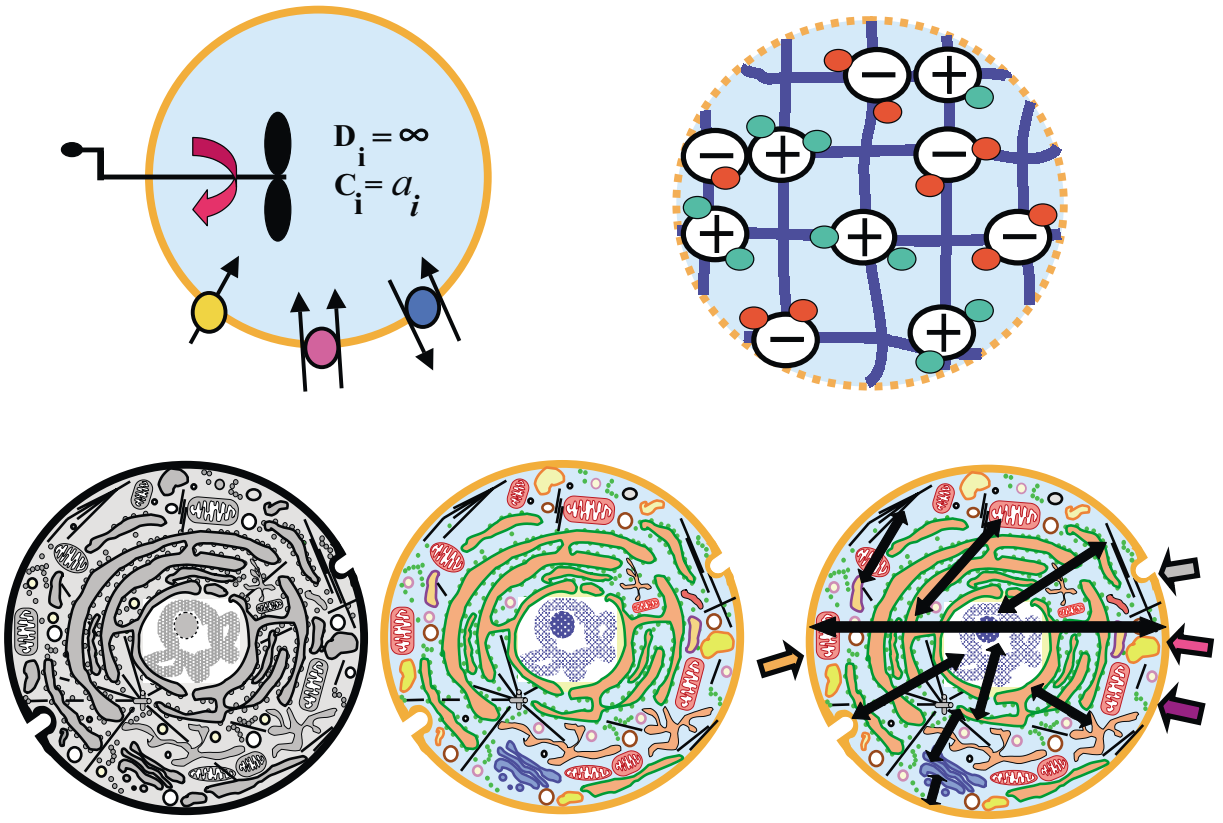

Fig. 1. Working models of the cell after the Second World War. *Upper left*: for biophysicists it was enough to assume that the only relevant feature of the cell is its plasma membrane containing specific translocating mechanisms. The cytoplasm was supposed to be a well-stirred solution where diffusion coefficients have an infinite value, activities were identical to concentrations, and the nucleus as well as other organelles were not taken into consideration. *Upper right*: Gilbert N. Ling disregarded the membrane as the seat of translocating mechanisms. He considered that the cytoplasm is an entangled mesh of macromolecules with enough fixed electric charges and hydrogen binding sites to insure that water and ions would not be entirely free. *Bottom left*: cell biologists observed that the cell contains densely packed organelles, such as the nucleus, endoplasmic reticulum, Golgi apparatus, lysosomes, endosomes, peroxisomes, mitochondria, microfilaments, microtubes and shuttling vesicles. *Bottom center*: biochemists demonstrated that different organelles have different molecular species, membranes have domains, and proteins have different life spans. *Bottom right*: physiologists study the mechanisms and signals that enable a given protein species to move through a labyrinth of organelles, and travel from the point of synthesis to very specific targets.

and departing to other cell compartments. Furthermore, the life of a cell is a cyclic process, in whose steps the expression of some molecular species changes, so that each species has to be synthesized, delivered, and retrieved from the specific compartments where it belongs. This led to the concept that ribosomes, microtubules, microfilaments, vesicles and the rest of the cellular structures are not "things", but the frozen image of vertiginous processes. Structures like microtubes and the Golgi apparatus are literally shattered and reassembled in each cell cycle. Furthermore, until a few decades ago, a worldview based on the concept of body and

soul, justified a sharp division into an *anatomy* that studied "structures" as opposed to a *physiology* that studied "pure functions". Today, the study of biology at cellular and molecular levels shows that functions comport a feverish change of the structure of molecules, channels, receptors and other ephemeral arrangements. In this sense, *protein targeting* is the set of signals and mechanisms that forces a given protein species to reach a highly specific site of the cell, and remain for a while as part of the structure while performing a strictly defined function.

1.1. The roles of protein targeting

The understanding of protein targeting would benefit from a brief description of a few co-involved phenomena:

(a) *Restrictions*: substances in a test tube have an enormous degree of freedom. In living systems instead, their fate is severely restricted in physical and chemical compartments, and molecules that run away from the compartment where they belong, are swiftly destroyed, so that substances cannot deviate from the constrained routes that constitute their characteristic metabolic pathways. Moreover, restrictions enormously increase the possibility of the remaining degrees of freedom, and are responsible in part for the remarkable efficiency of life processes (see Pattee, 1971). Differentiation, the execution of the information contained in the genome to produce an adipocyte or a neuron, a fly or an elephant, is a wonder of selective repression (Wolffe and Matzke, 1999). Targeting prevents a given molecular species from ending up in a multitude of "wrong" organelles, and permits it to reach the precise location where it works.

(b) *Economy*: The assembly of a given membrane structure, e.g. a multimeric ion channel, a hormone receptor, the budding site of a yeast cell, is far more efficient when its molecular components are delivered and restricted to the same point, than if they were spread at random after synthesis, and had to diffuse throughout the cell, and later on meet by chance by diffusing in the plane of the membrane.

(c) *Polarity*: in the last century and a half it became obvious that the function of some cells is entirely vectorial. Such was the case of neurons that integrate exquisite circuits, and epithelial cells that transport substances in the inward or the outward direction (Cereijido et al., 2001). In the last 20 years, though, it was realized that *all* cells—even single ones—have some degree of polarization (Cereijido et al., 1998, 2000; Shoshani and Contreras, 2001; Tkacz and Lampen, 1972).

Taken together, these considerations indicate that, for life to proceed, once a protein is synthesized, it has to be addressed to the point where it works. Upon arrival, proteins have to be recognized, regardless of whether they are "immigrants" that will be incorporated and retained to participate in the processes taking place in that particular compartment, or "messengers" that only convey a signal.

2. Toolbox

Understanding of the mechanisms involved in targeting and retention depends on some basic molecular concepts that we will review briefly in this section:

2.1. Configurations and functions

The shape adopted by a peptide and thereby its function, as well as its associations with other molecular species, depend primarily on its sequence of amino acids and a balance of intricate interactions between its thousands and thousands of atoms. This configuration is not static, but changes according to a multitude of circumstances, such as the type of solvents, presence of mobile ions, vicinity of other molecules, phosphorylations, etc. The complexities of these interactions can be understood in terms of affinities and repulsions that attract or separate the different segments of the peptide. This interrelationship between composition, configuration and function can be illustrated with the potassium channel called "Maxi-K". Each of its four monomers has a site with affinity for the other like-monomers that joins them forming a tetramer. The center of this tetramer is hydrophilic and forms a *pore* used by K^+ ions to cross the membrane. When this pore is in the "closed" configuration, a change in the electrical potential difference across the membrane can perturb the aminoacids bearing a net electric charge constituting the fourth transmembrane domain of each subunit[1] ("voltage gating"). The perturbation is transmitted throughout the peptide backbone of the protein, causing the aminoacids forming the pore (30–40 aminoacid residues away) to adopt the "open" configuration, and allowing K^+ ions to cross the membrane. In turn, the aminoacids at the NH_2 terminus of each monomer hide their hydrophobic regions from water, adopting the shape of a ball, that is tethered to the rest of the molecule by a chain of aminoacids. This ball has an affinity for the aminoacids forming the pore, but only has access to it when the pore opens ("activation"). In this moment the ball docks, clogs the pore, and blocks the passage of K^+ ions ("inactivation").

2.2. Ion selectivity

Actual calculation of the mind boggling interactions between the different atoms of the four subunits that bring about tetramerization, gating, activation and inactivation of the Maxi-K channel that we used above is very complicated, takes powerful computers and sophisticated methods, such as conformational space annealing (CSA) and algorithms (e.g. Empirical Conformational Energy Program for Peptides), and are still in the hands of a relatively few specialists (see Lee et al., 1998). However, since these concepts are essential to understand the processes of targeting and selective retention, we can grasp them by describing the basic ideas of ion selectivity put forward by Eisenman (1962). These authors proposed that, in order to exchange an absorbed ion I^+ for a mobile ion J^+ in the solution, ion I^+ has to detach itself from the fixed negative charge X^- and undergo hydration, and ion J^+ has to detach itself from water molecules and attach to charge site X^-. This involves a certain change in free energy, ΔG_{ij}, and consists essentially of two components: the difference in free energies of hydration of the ions I^+ and J^+, that is a well known quantity available in textbooks, and the difference of free energies of their interaction with the fixed anion X^-. The free energies of interaction between X^- and the cations is

[1] To picture the field strength of the sensed by a charged amino acid in the plasma membrane, imagine that at the electrical potential of 100 mV across the 50 Å thick lipid sheet of the membrane of a neuron, imposes an electric field of 2000 volts per centimeter. This field is roughly 200 times stronger than the field that moves whole proteins in a gel of electrophoresis.

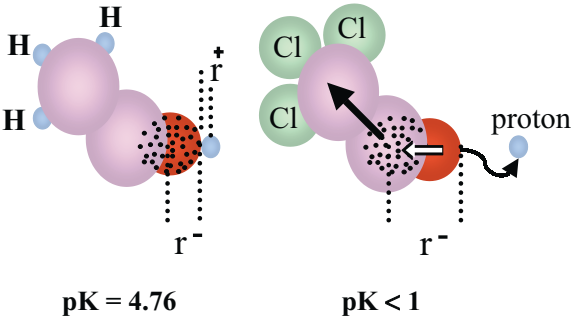

Fig. 2. Inductive effect. Molecules of acetic (*left*) and trichloroacetic acids (*right*), where dots represent the electronic cloud of the acid group, and r^- and r^+ the equivalent radiuses of the acid group and the proton respectively (not to scale). In the case of trichloroacetic acid, the pull of the electrons exerted by the three chloride atoms (*black arrow*) has increased r^- (*white arrow*), weakened the attraction between the acid and the proton, and increased its chances of breaking loose.

given in first approximation by the distances at which their electric charges can interact, i.e. their radii: Fig. 2 shows that according to Coulomb's Law, the larger the radius, the weaker the attraction between the fixed and the mobile charges. Therefore, one may take an anionic site X^- of radius r^- and calculate the ΔG_{ij} of exchange between K^+ (taken as reference) for another cation (say Na^+) (Fig. 3). Then repeat the calculation for the ΔG_{ij} between K^+ and some other cation (Cs^+, Rb^+, Li^+ or H^+), then do this all over for another value of r^-. This generates a series of isotherms like the ones depicted in Fig. 3. Each time two isotherms cross, the order of selectivity changes. These calculations can be also performed for a fixed cationic X^+ site that selects among mobile anions present in the solution. Likewise, it can be carried out for mobile divalent ions and for sites that do admit water, as well as for biological and non-biological systems. For a review of biological selectivity see Diamond and Wright (1969) and Cereijido and Rotunno (1970).

Of course, carboxyl and the amino groups of a protein do not actually have a spherical shape. Yet it is possible to calculate for them an *equivalent radius* (see Eisenman, 1962). Furthermore, the *force field* of these fixed ions belonging to a protein is not constant, but can vary according to the status of the whole molecule (vide infra). In addition, the processes summarized in this section are not only valid for substances with a *net* electric charge, but also for molecules that can establish hydrogen bonds at one or several points, so that these notions can be used to understand the interactions between the different segments of a protein, as well as the interactions of this protein with water, sugars and other components, including its relationship with other protein species in a scaffold.

2.3. Proteins

The name *protein*[2] appeared for the first time in 1838 in a paper by Gerrit Mulder. "*protein*" relates to Proteus, a minor sea god or "Old Man of the Sea" of Greek mythology, who knew the

[2] From the Greek πρωτειοξ, meaning "in the lead", or "holding first place".

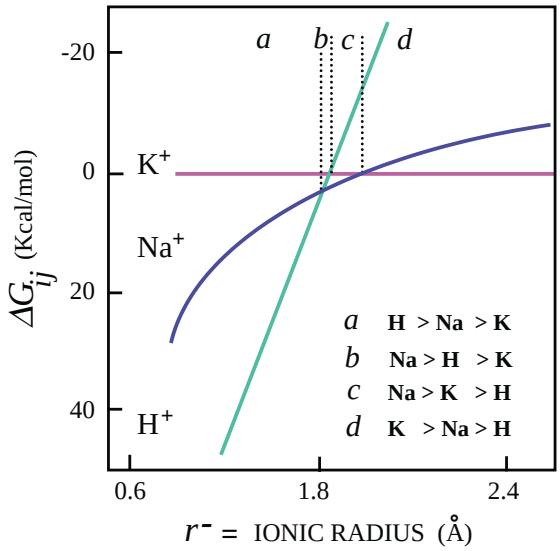

Fig. 3. Cation selectivity expressed in a logarithmic scale as ΔG_{ij}, as a function of the anionic strength represented by r^{-}, the equivalent ionic radius of the anion. Anionic strength decreases from left to right. The lower the value of an isotherm, the most preferred is the ion with respect to K^+. Each time isotherms cross each other, the order of selectivity changes (vertical dotted lines). Thus, order of selectivity *a* is $H^+ > Na^+ > K^+$, order *b* is $Na^+ > H^+ > K^+$, *c* is $Na^+ > K^+ > H^+$, and *d* is $K^+ > Na^+ > H^+$ (After Eisenman, 1962).

future but adopted various shapes to escape those that questioned him. Homer's odyssey relates that king Menelaus encountered Proteus on the island of Pharos off the coast of Egypt, held the god fast and forced him to answer. So to chemists of the 19th century *protein* was a suitable name for molecules whose shapes and other properties changed with the method of purification, ionic composition of the solvent, etc., in particular because, for a while, they thought that they were dealing with a single molecular species, regardless of the source (milk, egg, muscle, etc.). It took more than a century to "force" proteins to reveal the secret of their changing shapes and properties and, as we shall discuss below, this ability to change shapes and properties is crucial to understand targeting, selective retention, and thereby the fate of a protein.

2.4. The inductive effect

Fig. 2 depicts a molecule of acetic acid in which the dots represent the electronic cloud surrounding the oxygen of the carboxyl of acetic and trichloroacetic acids. When the hydrogen atoms on the methyl group are replaced by chlorine, the electrons of the molecule are "sucked" toward these chlorine atoms. The electron density at the carboxyl group is therefore reduced, with the consequence that the attraction between this group and the proton weakens. The fact that trichloroacetic acid has three chlorine atoms where the acetic acid has three hydrogens lower its attraction for protons at the other end of the molecule, reflected in their pK: 4.76 and less than 1 respectively. At the beginning of the 20th century G.N. Lewis used the concept of *induction* to characterize the change of reactivity of a chemical group produced by the introduction in the

molecule of another chemical group which, as in the case of acetic/trichloroacetic, may be located at a considerable distance from the first.

The peptide backbone of a protein can easily transmit the inductive effect throughout its molecule (Figs. 4 and 5). Methyl groups (CH_2) instead act as insulators, so the changes in reactivity of the chemical groups at the end of their amino acids will depend on their nature, and on how many CH_2 groups are interposed between the peptide backbone and the functional group. Gilbert N. Ling (1962) used these concepts to propose his "Association-Induction Hypothesis" to account for the changes in the assembly of proteins, very much as pins would change their associations according to the induction caused by a magnet. Since the groups of a protein sense the induction effects, they would change their reactivity accordingly, modify the selectivity for other groups, and change the shape and properties of the whole molecule (Fig. 5). Accordingly, the hydrogen and hydrophobic bonds that a protein molecule establishes within their different parts as well as their surroundings, arrange its structure into well known structures known as α-helices, β-sheets, loops, turns, fingers, cages, whose description falls beyond this article.

Of course these considerations on ion selectivity and its effects on the structure/function of proteins also hold when the mobile cation is H^+ (Fig. 4), and its consequences permit us to visualize the profound modifications that even small changes in pH have on the configuration and function of proteins.

In summary the chemical reactions taking place in a cell are compatible with life, because the thousands of different intervening protein species are secluded in chemical and physical compartments. This requires that, once a given type of protein is synthesized, it should be addressed and retained in a specific compartment. In this section we have briefly reviewed the basis of the affinity that a given part of the protein may have with another part of the same protein, and thereby impose on the whole molecule a configuration in space. It can also be used to

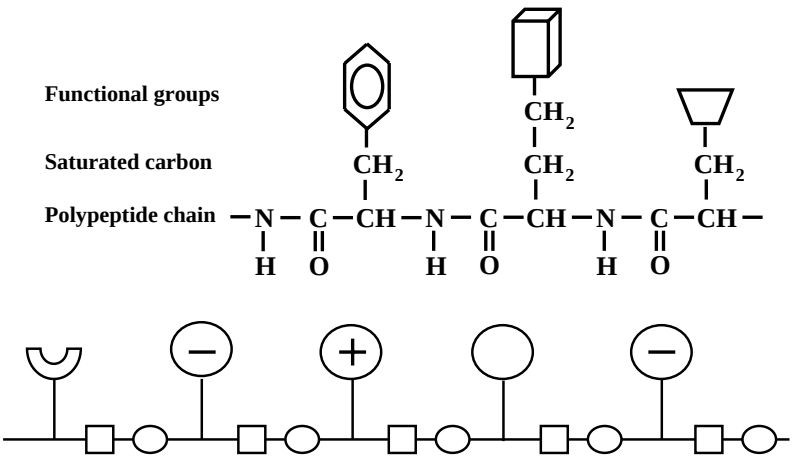

Fig. 4. *The basis of inductive effects in peptides*. *Above*: a peptide segment with the three relevant parts for the inductive effect: the polypeptide chain, that transmits easily and through the whole length of the molecule the inductive effect; saturated carbons that do not transmit the inductive effect easily, and the functional groups (represented by geometrical figures) of the different aminoacids forming the peptide, that interact with water, solute ions, etc. *Below*: simplified representation of a segment.

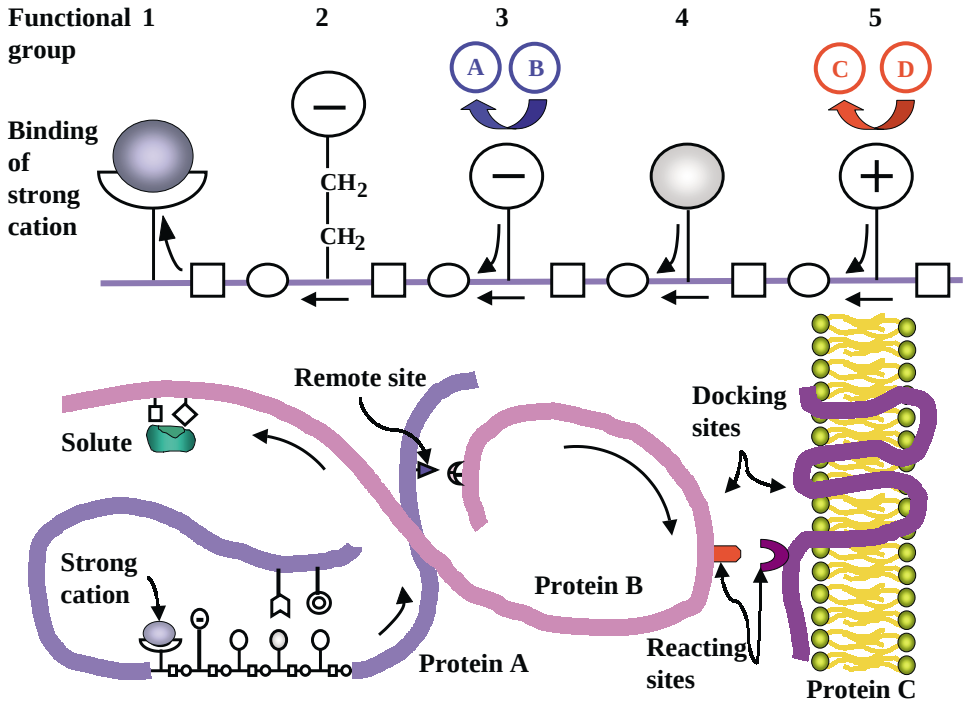

Fig. 5. Effects of association, induction and selectivity on the behavior of proteins. *Above*: a strong cation (e.g. Ca^{2+}, Mg^{2+}) binds to the first functional group on the left, thus pulling electrons from the rest of the molecule (*small black arrows*). This does not affect the selectivity of functional group 2, because it is insulated by two saturated carbons. It weakens the strength of the positive charge of group 3, with the consequent change in ion selectivity that detaches cation A^+ and binds cation B^+. Induction elicits no noticeable change in functional group 4 because it is neutral. Withdrawal of electrons toward the peptide backbone increases the ionic strength of cationic group 5, changing its selectivity of anion C^- for anion D^-. *Below*: The segment above is represented as a fragment of protein A. Binding of the strong cation perturbs selectivity at a remote site, that thereby develops an affinity for protein B, which in turns modifies the affinity of a couple of non-ionic sites at its other end. Binding of the strong cation to protein A may therefore result in the adsorption of a specific solute, that not necessarily bears a net electric charge. The ensemble may now generate an affinity for protein C at docking sites, and binding of these sites may allow suitable sites to react.

grasp the sudden and transient affinity of one of its parts for other molecules in the vicinity. This glimpse of selectivity and inductive effects may also help us to understand why two different proteins (A and B) may coexist in a free state, without interacting, until a change in pH, Ca^{2+} concentration, the arrival of a third protein, or the phosphorylation/dephosphorylation of one of them may induce association in a scaffold, or develop an attraction for a particular docking place in the membrane.

Targeting and retention are studied in an enormous variety of cells, animal species, genes and proteins. Fortunately, certain genes, signals and mechanisms are homologous in different model systems. The mass of information available precludes a thorough review, so we will only deal with paradigmatic cases. But even with this format, we are forced to restrict this article to the plasma membrane of mammalian cells, and only allude to other systems when these shed light on the former.

3. Signals and mechanisms

As mentioned above, a century ago, the evidence that neurons and epithelial cells function vectorially led to the conviction that cells must be structurally polarized. Yet only half a century later Koefoed-Johnson and Ussing (1958) put forward the first successful model of cell asymmetry to account for the electrical potential difference and Na^+ transport across the frog skin. It took another half a century, to reach the conclusion that, in fact, *all* cells have some degree of polarization. This gave to the field its present status: (a) most of the information available derives from studies of apical/basolateral polarity in transporting epithelial cells, as well as dendrites/axon polarity in neurons of higher organisms; (b) the signals and mechanisms found tend to be analogous in the different preparations, indicating that they were well conserved; (c) yet genes involved in polarity are detected in studies with cells of simpler organisms, such as yeast, *C. elegans* and *Drosophila*, where the role of the coded proteins is still poorly understood; (d) therefore, one of the most fertile approaches consists in detecting these genes in simple organism, look for their homologous in higher ones, and investigate the function of the different protein species in epithelial and neuronal cells. Thus, we have introduced a model system based on the use of cultured MDCK cells (epithelial) that afford a great proportion of the information discussed below (Cereijido et al., 1978a, b)[3].

3.1. Sorting organelles

Because signals in the molecule to be sorted are incorporated during translation (e.g. a dileucine motif) and maturation (e.g. glycosilation, addition of lipid chains), apical or basolateral sorting can be said to start much earlier than the trans-Golgi network (TGN). However it is in this structure where proteins to be addressed to a given pole are clearly sorted into different transporting vesicles (Rindler et al., 1984; Simons and Fuller, 1985).

From the TGN specific proteins follow two main routes, the direct or the indirect ones (Fig. 6). In the direct route sorted proteins are incorporated into vesicle that carry them right to the apical or to the basolateral surface (Rodriguez-Boulan et al., 1984; Caplan et al., 1986a). In the indirect one, proteins are sent first to one surface, are subsequently endocyted and delivered to early endosomes, where another type of vesicles shuttle them to their final destination (Hubbard et al., 1989; Casanova et al., 1991; Matter et al., 1994; Mostov et al., 2000).

3.2. Apical sorting

3.2.1. Signals

Apical targeting has been attributed to a number of different types of sorting signals, including O- or N-linked carbohydrates, specific protein transmembrane determinants in their lumenal or cytoplasmic segments, glycosylphosphatidylinositol (GPI) anchors, etc. (Rodriguez-Boulan and Gonzalez, 1999; Table 1). However, there are clear examples where N-glycans and GPI may not be sufficient to act as apical signals (Benting et al., 1999), and GPI behaves as a basolateral

[3] For a brief historical description see M. Cereijido, *Citation Classics*. Current Contents, November 13, 1988, 32 (46) Piladelphia, Penn.

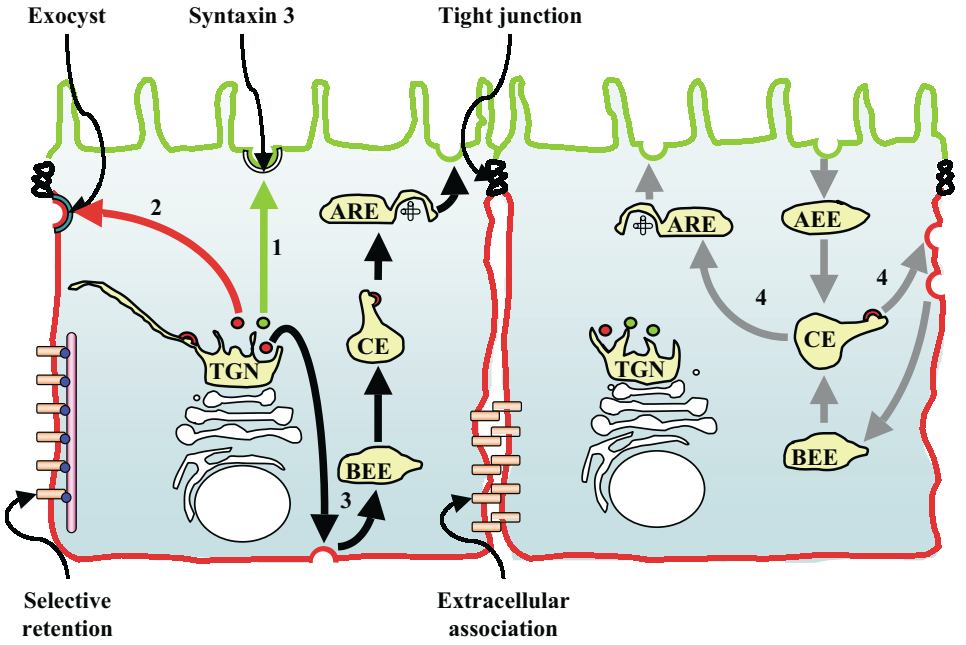

Fig. 6. Prototypical epithelial cells with apical (*green*) and basolateral (*red*) membrane domains separated by a tight junction. Newly synthesized membrane proteins are sorted in the Trans-Gogi Network (TGN) and targeted directly to the apical (1) or basolateral (2) membrane domain. Syntaxin 3 and anexin xiiib are involved in apical delivery, while the exocyst participates in basolateral targeting. The indirect route (3) starts by addressing all proteins to the basolateral membrane, endocyting them, and finally sending them to the apical domain through the basolateral early endosome (BEE), the common endosome (CE) and the apical recycling endosome (ARE). Transcytosis begins by an internalization from the basolateral membrane to the basolateral early endosome (BEE), or from the apical membrane to the apical early endosome (AEE). From the BEE or AEE proteins proceed towards the CE, and are transcytosed from this organelle to the apical or to the basolateral. The route to the apical involves another station: the ARE. Notice that some proteins are retained in their specific location thanks to their association with the cytoskeleton (*bottom left*) or through interactions with proteins in the neighboring cell (*bottom center*).

determinant (Zurzolo et al., 1993). Kundu et al. (1996), Dunbar et al. (2000) and Slimane et al. (2001) have found that the transmembrane domain of influenza virus neuroaminidase, H^+, K^+-ATPase and dipeptidyl-peptidase respectively contains apical signals; which are independent on whether the protein associates or not with rafts (vide infra).

3.2.2. *Lipid microdomains (rafts) mediated sorting*

The packing density of lipid molecules in a monolayer depends on whether their hydrocarbon chains are saturated, or have kinks due to unsaturation, and whether their electric charges repel or attract each other. Thus, as molecules of L-α-dipalmitoyl lecithin (DPL) in a monolayer approach each other in a film balance of the Langmuir type, the lateral pressure increases, but below a certain distance molecules arrange their polar groups so that positive and negative charges attract, and hydrocarbon chains approach so closely, that van der Waals forces[4] stick them in a tight

[4] Van der Waals force becomes meaningful at very close intermolecular proximity, because it decays with the sixth power of distance. For comparison, coulombic forces decrease with the second power of distance.

Table 1
Epithelial sorting signals

Signal	Protein	Location	References
Apical			
GPI-anchor	Decay accelerating factor	Membrane	Lisanti et al. (1989)
N-glycans	Gp80, Erythropoietin	Luminal	Scheiffele et al. (1995), Kitagawa et al. (1994)
O-glycans	P75 Neurotrophin receptor	Luminal	Yeaman et al. (1997)
Proteinaceous signals	Influenza virus hemaglutinin, influenza neuraminidase, H, K-ATPase	Membrane	Scheiffele et al. (1997), Kundu et al. (1996), Dunbar et al. (2000)
	Rhodopsin (C-terminal),	Cytoplasmic	Tai et al. (1999), Marzolo et al. (1997)
	Hepatitis virus antigen	Luminal	
Basolateral			
Tyrosine motifs YXXΦ[a]	Vesicular stomatitis virus G protein, influenza virus hemaglutinin (Tyr mutant), LDL receptor	Cytoplasmic	Thomas et al. (1993), Brewer and Roth (1991), Matter et al. (1992)
Dileucine motifs LL/IL	FcRII-B2	Cytoplasmic	Hunziker and Fumey (1994), Matter et al. (1994)
Other	N-CAM140 and 180 kDa forms, RET-PE2 antigen (EMMPRIM), Polymeric Ig Receptor	Cytoplasmic	Le Gall et al. (1995), Marmorstein et al. (1998), Casanova et al. (1991)

[a] YXXΦ, where X can be polar residues and Φ is a bulky hydrophobic residue.

packing (see Cereijido et al., 1969). This ordering is reflected in a decrease of entropy (Fernández et al., 1970). Furthermore, in mixed monolayers of membrane lipids with cholesterol the area may be smaller than the sum of the area of lipid plus the area of cholesterol due to the formation of complexes (Vanderheuvel, 1967). When only a fraction of the lipids of a leaflet can form complexes, they compact and segregate in microdomains. This situation seems to occur spontaneously in the lipid leaflets of some cell membranes (Edidin, 1997; Dietrich et al., 2002). Proteins that establish protein–lipid interactions either directly (Lisanti et al., 1989; Scheiffele et al., 1997), or indirectly by protein–protein or protein–carbohydrate interactions (Rodriguez-Boulan and Gonzalez, 1999), can be incorporated into lipid microdomains of the membranes of the TGN named "rafts", which are rich in glycosphingolipids and cholesterol. When these rafts pinch off from the TGN and are addressed to the apical domain, they deliver polarizedly the molecules they carry (Lisanti et al., 1989; Simons and Ikonen, 1997; Brown and London, 1998). However, in FRT cells rafts are sent to the basolateral domain (Zurzolo et al., 1993).

3.2.3. Caveoli

Caveoli are vesicles of 60–90 nm or flask-shaped invaginations of the plasma membrane that constitute a more stable form of rafts (Rothberg et al., 1992). They are membrane domains

involved in the trafficking and internalization of proteins that associate with glycolipid-rich regions of the membrane (Parton, 1996) and function in transmembrane signaling events (Lee, 2001). Quite frequently they are found in the basolateral membrane.

3.2.4. Apical non-raft

There are apical membrane proteins that are sorted directly to the apical membrane of MDCK cells without the participation of rafts. Such is the case of chimeras of dipeptidyl peptidase IV with GFP (Slimane et al., 2001), as well as alkaline phosphatase (Arreaza and Brown, 1995) and an enteropeptidase of the enterocyte brush border (Zheng and Sadler, 2002).

3.2.5. Specific proteins of the sorting machinery

VIP17/MAL is a membrane protein found in post-Golgi transport vesicles and the apical domain of MDCK cells. Its overexpression disturbes the morphology of the MDCK cells by increasing apical delivery and expanding the area of this domain (Cheong et al., 1999; Puertollano et al., 2001). Annexin XIIIb localizes to the apical plasma membrane, and is highly enriched in vesicles targeted to this domain (Fiedler et al., 1995; Lafont et al., 1998).

Vectorial transport to the apical membrane involves transport along microtubules with both dynein and kinesin as motor proteins, whereas the basolateral surface transport depends on kinesin alone (Lafont et al., 1994). Myosin-I has been implicated in apical transport of Golgi-derived vesicles in epithelial cells (Fath and Burgess, 1993). Kreitzer et al. (2000) demonstrated that kinesin and dynamin are required for different stages of post-Golgi transport.

Docking and fusion models involving soluble *N*-ethylmaimide-sensitive factor attachment receptors (SNAREs) are based on the existence of receptors both in the arriving vesicle (v-SNARE) and in the target membrane (t-SNARE) that recognize each other. This system was first described in cultured neurons (Sollner et al., 1993) and has syntaxin 3 (a t-SNARE) as one of its central components. Interestingly, this protein is also present in the apical membrane of epithelial cells (Delgrossi et al., 1997; Low et al., 1996). When syntaxin 3 is overexpressed in epithelial cells, it inhibits TGN-to-apical transport and transporting vesicles accumulate underneath the apical membrane (Low et al., 1998). These cells also contain a v-SNARE (TI-VAMP = tetanus toxin insensitive vesicle associated membrane protein) in the membrane of apically targeted vesicles (Galli et al., 1998).

3.3. Basolateral sorting

3.3.1. Signals

Targeting determinants for basolateral sorting are continuous amino-acid sequences in the cytoplasmic domains of membrane proteins, such as tyrosine-dependent or dileucine-dependent motifs, frequently followed by a cluster of acidic residues (Matter et al., 1990a, b; Matter, 2000; Matter and Mellman, 1994) (see table).

3.3.2. The basolateral machinery: clathrine coat mediated sorting

Tyrosine and dileucine-dependent motifs interact with adaptor proteins (AP) of the clathrin coat (Matter and Mellman, 1994) that constitute a family of four tetrameric complexes named

from 1 to 4. These AP complexes recruit clathrin into budding areas of the vesicle-donor membrane (Odorizzi et al., 1998; Dell'Angelica et al., 1999; Kirchhausen, 1999).

Subunit μ1B of clathrin adaptor complex AP-1 interacts with tyrosine-based sorting signals (Ohno et al., 1999). LLC-PK1 cells do not express μ1B, and therefore missort basolateral proteins with the tyrosine-based motif (Roush et al., 1998). Folsch et al. (1999) transfected the μ1B subunit into these cells, and observed that these now correctly sort to the basolateral membrane LDL and transfectin receptors. Simmen et al. (2002) have shown that AP-4 can bind different types of cytosolic signals known to mediate basolateral transport in epithelial cells. These authors demonstrated that when subunit μ4 is depleted, basolateral proteins are missorted to the apical membrane.

3.4. Hierarchy of signals

In the absence of specific sorting signals, transmembrane proteins accumulate in Golgi apparatus, suggesting that none of the routes to the cell surface is an efficient default pathway (Gut et al., 1998; Wittchen et al., 1999). Basolateral targeting determinants are generally dominant over apical sorting ones.

Newly synthesized Na^+, K^+-ATPase is directly addressed to the basolateral membrane domain in MDCK cells (Caplan et al., 1986a; Gottardi and Caplan, 1993). This targeting seems to be determined by the impossibility of this enzyme to board the glycosphingolipid (GSL)-rich rafts that assemble in the Golgi complex, and form vesicles that carry proteins towards the apical domain. This exclusion may be overcome by endowing the Na^+, K^+-ATPase with a sequence signal from the 4th transmembrane segment of the α-subunit of H^+, K^+-ATPase (TM4), that suffices to readdress the Na^+, K^+-ATPase towards the apical domain. Yet, there are non-gastric H^+, K^+-ATPases that are addressed towards the apical domain, in spite of lacking the TM4 signal. Dunbar and Caplan (2001) convincingly suggest that these differences in the polarized expression of ATPases can be explained as follows: (i) gastric H^+, K^+-ATPase has the TM4 apical addressing signal already formed; (ii) non-gastric H^+, K^+-ATPases lack the TM4 signal, but may form it through a particular twist of the molecule that would join two separated segments and thereby complete the signal sequence; (iii) also Na^+, K^+-ATPase lacks a TM4-signal but, at variance with non-gastric H^+, K^+-ATPases, it is unable to form one by reconfiguring the α-subunit in space; therefore only the insertion of a TM4-signal in this ATPase by molecular engineering achieves its apical expression (Dunbar et al., 2000; Dunbar and Caplan, 2000). Once Na^+, K^+-ATPase arrives to the basolateral membrane it interacts with ankyrin, and becomes anchored to the cytoskeleton that stabilizes the enzyme in this position (Hammerton et al., 1991).

Three different mechanisms have been proposed to explain the expression of Na^+, K^+-ATPase at the lateral membrane of epithelial cells (Fig. 7). The *first* relies on sorting events in the secretory pathway that segregate newly synthesized proteins into transport vesicles destined for the lateral domain (Caplan et al., 1986b; Contreras et al., 1989; Gottardi and Caplan, 1993; Zurzolo and Rodriguez-Boulan, 1993). The *second* relies on selective stabilization of Na^+, K^+-ATPase molecules upon arrival to the lateral domain, whether or not the protein was previously sorted in the secretory pathway. This model was based on the following series of observations: (a) the Na^+, K^+-ATPase co-localized with sites of polarized spectrin/ankyrin assembly at the cytoplasmic face

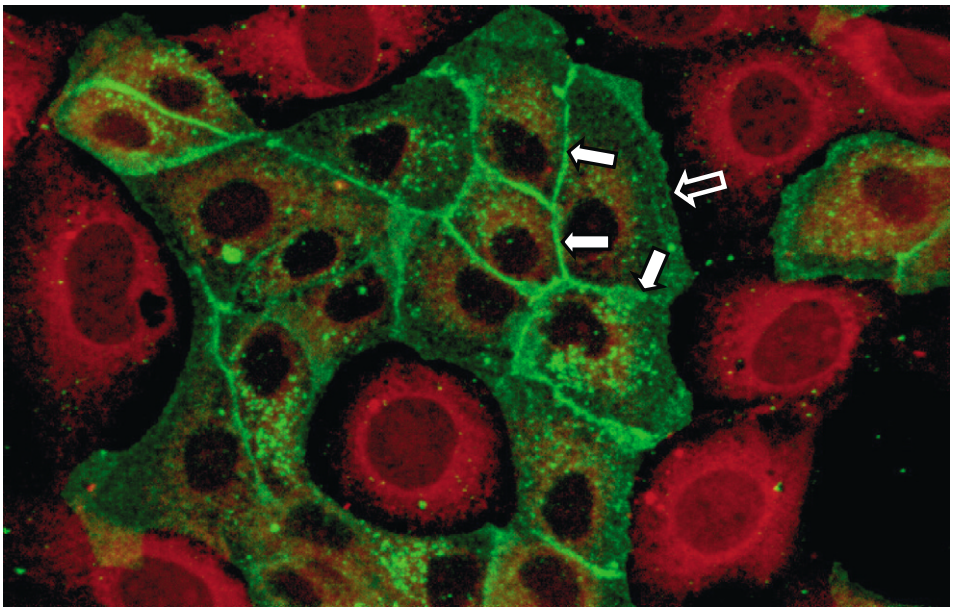

Fig. 7. Monolayer of co-culture of COS cells (monkey, red) labeled with CMTMR, and MDCK cells (dog, dark). The monolayer was stained with a first antibody against the β subunit of the Na^+, K^+-ATPase of MDCK cells, followed by a secondary FITC labeled antibody (green). MDCK cells express the Na^+, K^+-ATPase β subunit in homologous (dark/dark, white arrows) but not in the hetorologous borders (dark/red, open arrow). The fact that the expression of this enzyme at a given domain of the plasma membrane depends on the neighboring cell, suggests that Na^+, K^+-ATPase may be anchored by β–β interaction. This image was obtained by confocal laser scanning microscopy.

of the plasma membrane (Nelson and Veshnock, 1986; McNeill et al., 1990); (b) Na^+, K^+-ATPase was shown to physically interact with ankyrin in vitro (Nelson and Veshnock, 1987); and (c) lateral accumulation of Na^+, K^+-ATPase was observed in an MDCK cell line that did not sort this protein in the secretory pathway (Hammerton et al., 1991; Mays et al., 1995). The *third* mechanism to account for the polarized distribution of Na^+, K^+-ATPase stems instead from the following series of observations: (a) MDCK cells cultured in low Ca^{2+} establish no tight junctions and have the Na^+, K^+-ATPase distributed at random on the plasma membrane but, upon addition of this ion, cell–cell contacts are established, intracellular enzyme is incorporated to the plasma membrane, and concentrates in the lateral domain of adjacent cells (Contreras et al., 1989). (b) MDCK cells (dog) co-cultured with LLC-PK_1 (pig), or Ma104 cells (monkey) only express Na^+, K^+-ATPase in homotypic MDCK/MDCK borders, but not in heterotypic ones (MDCK/LLC-PK_1 or MDCK/Ma104) (Contreras et al., 1995) (Fig. 7). (c) The β subunit has the typical structure of an attaching molecule (a short cytoplasmic tail, a single transmembrane domain, and a long and highly glycosilated extracellular domain) (Gloor et al., 1990). On this basis, we have suggested that Na^+, K^+-ATPase is retained at the lateral membrane, because it depends on the anchorage provided by specific interaction between the β subunits in neighboring cells (Cereijido et al., 2000). Furthermore, the β-subunit/β-subunit interaction must have a considerable specificity, as an MDCK expresses its Na^+, K^+-ATPase at a given border provided

the neighboring cell is another MDCK cell, but not if it is an LLC-PK_1 or an Ma104 one (Cereijido, 1992; Cereijido et al., 1998, 2000; Contreras et al., 1995, 2002). Of course, the mechanism based on a specific interaction between the β subunits does not preclude that the enzyme had been sorted before arriving at the lateral membrane, nor that it becomes immobilized in this position through a cytoskeleton scaffold at the lateral but not at other membrane domains where this structure also exist but does not immobilizes Na^+, K^+-ATPase. An additional observation that remains to be integrated into this picture is that, in spite of the fact that before the addition of Ca^{2+} the MDCK cell has a considerable intracellular pool of Na^+, K^+-ATPase, the polarized expression of this enzyme depends on the synthesis of another type of a yet unidentified protein (Contreras et al., 1989).

4. Docking and retention

Several membrane-associated structures and mechanisms were suggested to perform this function: (1) *Targeting patches* for docking and fusion of vesicles carrying apical or basolateral proteins. (2) *A membrane skeleton* helping to direct the retention and accumulation of specific proteins in different membrane domains. (3) *Anchorage to extracellular components* that retain and accumulate proteins in specific membrane domains. (4) *Tight junctions*, that constitute a physical barrier that prevents the intermixing of membrane proteins as well as lipids in the apical and basolateral domains.

4.1. Targeting sites

4.1.1. SNAREs and SNAPs

Once a cell–cell contact forms, it immediately attracts and promotes the assembly of arriving vesicles, forming patches that specify and enhance the efficiency of vesicular trafficking to the correct surface domain, and prevent docking and fusion to the incorrect membrane domain.

Fusion of intracellular membranes in eukaryotic cells involves several protein families including SNAREs, Rab proteins, and Sec1/Munc-18 related proteins (Jahn and Sudhof, 1999). SNAREs form a superfamily of small and mostly membrane-anchored proteins that share a common motif of about 60 amino acids (SNARE motif). SNAREs reversibly assemble into tightly packed helical bundles. Assembly is thought to pull the fusing membranes closely together, thus inducing fusion. SM-proteins bind to certain types of SNAREs and prevent the formation of core complexes (Jahn and Sudhof, 1999). A basic tenet of the SNARE hypothesis is that the minimal machinery for membrane fusion is a cognate set of v-SNAREs (VAMP-1 and VAMP-2) and t-SNAREs (Syntaxin and SNAP-25) on opposing membranes. SNAREs seem to clamp proteins and prevent them from forming spontaneous assemblies.

Rab proteins are GTPases that undergo highly regulated GTP-GDP cycles (sec4p, rab8, rab10 and rab13). While in their GTP form, they interact with specific effector proteins. Rab proteins appear to function in the initial membrane contact connecting the fusing membranes, but are not involved in the fusion reaction itself. Interestingly, rab8 and rab13 are enriched on the plasma membrane in the apical junctional complex, which includes the tight junctions and the adherens junctions (Huber et al., 1993). The restricted distribution of similar proteins, such as rab3b,

depends on cell–cell contact (Weber et al., 1994; Zahraoui et al., 1994). Rab proteins by themselves cannot mark a site on the plasma membrane for vesicle docking, since before the arrival of transport vesicles they are not restricted to sites of exocytosis on the plasma membrane.

4.1.2. The exocyst

Yeasts do not secrete nor bud at random everywhere in the plasma membrane, but at sites located very precisely (Tkacz and Lampen, 1972; Farkas et al., 1974; Field and Schekman, 1980). Mutants affecting these processes led to the identification of a series of genes involved in secretion and budding, such as Sec3p, Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, Exo84p. SEC3, SEC5, SEC10, EXO70, etc. These proteins form a complex that is called *exocyst* (TerBush et al., 1996; Guo et al., 1999a), that is required for the docking of exocytic vesicles, and is present throughout the life cycle of the yeast (Novick et al., 1980; Finger and Novick, 1998).

Work in this field progressively identifies more components of the exocyst, detects that different members play special roles, and finds connections of the group with other cellular structures and processes. Thus the exocyst was identified as an effector for small GTPases, including Sec4, Rho1, Rho3, Cdc42 and RalA (Lipschutz and Mostov, 2002). Sec10p and Sec15p appear to form a subcomplex that acts as a bridge between the rab GTPase Sec4p on the vesicle and the rest of the exocyst (Guo et al., 1999b). Cdc42 in its GTP-bound form interacts directly with Sec3p and coordinates the vesicle docking machinery and the actin cytoskeleton (Zhang et al., 2001).

Likewise, in mammalian cells, proteins arriving at the membrane do not insert at random anywhere in the apical or in the basolateral membrane, but in well-defined sites of these membrane domains. Thus Louvard (1980) found that when antigens belonging to the apical membrane of MDCK cells are endocyted, they reappear minutes later at the region of the tight junction, and diffuse thereafter all over the apical domain. Vega-Salas et al. (1988) found that in cells incubated in Ca^{2+}-free media apical markers are contained in intercellular compartments and, upon addition of this ion, these compartments fuse to the plasma membrane at a place where the tight junction is simultaneously being formed. Therefore it comes as no surprise that mammalian cells have counterparts of the yeast exocyst complex, such as hSec10p (Guo et al., 1997) and Sec6/8 (Grindstaff et al., 1998). Interestingly, upon initiation of calcium-dependent cell–cell adhesion, approximately 70% of this complex is rapidly recruited to sites of cell–cell contact (Grindstaff et al., 1998). Sec6 is another component of the exocyst complex, that appears to function in synapse formation and synaptic plasticity (Chin et al., 2000). Ral GTPases regulate targeting of basolateral proteins in epithelial cells, secretagogue-dependent exocytosis in neuroendocrine cells, and assembly of exocyst complexes (Moskalenko et al., 2002). To target secretory vesicles to specific domains of the plasma membrane the exocyst complex depends on its association with microtubules (Vega and Hsu, 2001). Activated RalA may act as an effector for integrating exocytosis to the cytoskeleton (Brymora et al., 2001; Sugihara et al., 2002).

4.2. Membrane skeleton

Spatial cues, such as Ca-activated cell–cell contacts (Balda et al., 1991, 1993; Cereijido et al., 2000) trigger the assembly of submembrane patches of cytoskeletal components, that act like platforms for vesicles targeted from remote cellular origins, like the TGN, the basolateral

membrane and the nucleus (Drubin and Nelson, 1996; Gonzalez-Mariscal et al., 1999; Islas et al., 2002). Usually, the affinity of one of the molecules for a given protein species is increased (or decreased) not only by the interaction with a third protein molecule, but also by phosphorylation, combination with Ca^{2+}, K^+ or Na^+, changes in local pH, etc. (Fig. 5), it is well established that the actin cytoskeleton organizes the microvilli at the apical domain and that this organization is regulated by the actin-binding proteins fimbrin and villin. Disruption of microtubules and microfilaments with drugs like colchicine, nocodazole and cytochalasin results in missorting or reduction of the degree of polarization of HA proteins of influenza virus, but does not interfere with the delivery of G protein from stomatitis virus to the basolateral surface (Rindler et al., 1987).

Formation of actin cytoskeleton precedes the assembly of a fodrin-based membrane skeleton at sites of cell adhesion (Nelson and Veshnock, 1986). Spectrin forms a submembrane protein scaffold that consist primarily of $\alpha_2\beta_2$ spectrin tetramer crosslinked into a hexagonal lattice by their interaction with actin filaments. The major attachment between spectrin and the plasma membrane occurs through the protein ankyrin, which functions as an adapter linking the β subunit of spectrin to the cytoplasmic domains of integral membrane proteins (Platt et al., 1993). Fodrin (spectrin) assembles with actin, protein 4.1, aduccin, and some others to form a protein membrane skeleton. This skeleton is associated with the cadherin/catenin complex, probably through binding of either actin or fodrin to α-catenin. Ankyrin, on the other hand, binds to integral membrane proteins, including Na^+, K^+-ATPase and Cl^-/HCO_3^- exchanger (Bennett, 1990) and the cell adhesion molecule neuroglian (Dubreuil et al., 1996).

The sequence of the ankyrin binding site of the Na^+, K^+-ATPase is conserved in *Drosophila* (Zhang et al., 1998), and the Na^+, K^+-ATPase co-distributes with ankyrin and spectrin in polarized fly cells (Baumann et al., 1994; Dubreuil et al., 1997). Yet, despite these conserved features, Na^+, K^+-ATPase polarity was not detectably altered in epithelial cells from null spectrin mutants (Lee et al., 1993, 1997). These results led to the conclusion that basolateral accumulation of the Na^+, K^+-ATPase in *Drosophila* epithelia did not require a stabilizing interaction with the spectrin membrane skeleton. Furthermore recent genetic evidence does not support this role of spectrin (Thomas, 2001).

Protein retention is not exclusive to the basolateral domain. Thus Ezrin is a linker between the cytoskeleton of the apical microvilli and membrane receptors like Cd44, ICAM-1 and ICAM-2 (Bonilha et al., 1999). Ezrin can also interact with protein EBP-50, one of whose PDZ domains immobilizes CFTR, the cystic fibrosis receptor (Bretscher, 1999).

4.3. The protein–protein attaching motive PDZ

The name PDZ derives from the first three proteins in which these domains were identified: PSD-95, DLG and ZO-1. It is also referred to as DHR (Discs Large homology repeat) or GLGF repeats (after the highly conserved 4-residue domains GLGF sequence within the domain). It is one of the most common modular domains for protein-interaction. Its primary function is to recognize specific $\sim$5-residue motifs that occur at the C-terminus of target proteins or structurally related internal motifs. Like many other protein–protein interaction domains, it is relatively small ($<$90 residues), has N- and C-termini close to one another in the folded structure and folds into a compact globular arrangement, consisting of six β-strands (βA-βF) and

two α-helices (αA and αB). These domains are highly modular and could easily have been integrated into existing proteins without significant structural disruption through the course of evolution.

PDZ domains were found in receptors (Yamada et al., 1999), ion channels (Nehring et al., 2000), ion pumps (Kim et al., 1998), and cell–cell junction proteins (Buchert et al., 1999). Proteins containing PDZ-domain play a central role in specifying or maintaining polarized membrane domains. For example, in *C. elegans*, a complex of three PDZ proteins, LIN-2, LIN-7 and LIN-10, localizes the LET-23 receptor to the basolateral domain of the epithelial vulval precursors, and LIN-10 is also required to target the glutamate receptor to the postsynaptic elements of motor neurons (Kaech et al., 1998; Setou et al., 2000). PDZ-domain proteins also participate in apical membrane targeting: the *Drosophila* Discs Lost protein, which contains four PDZ domains, is required for the apical localization of the transmembrane protein Crumbs and the maintenance of epithelial polarity (Bhat et al., 1999). But, at least in the case of the GABA transporter, the PDZ interaction is involved in retention of the protein at the basolateral surface, but not in its targeting to that surface (Perego et al., 1999). In contrast to the numerous examples in higher eukaryotes, no PDZ proteins have been implicated in membrane polarization in yeast, suggesting that this family of proteins has evolved specifically in relationship with the maintenance of multiple membrane domains.

4.4. Extracellular associations

Interactions of Ca^{2+}-independent cell adhesion molecules with ligands on adjacent cells could also account for other type of cell polarity. Plasma membrane proteins in non-polarized cells such as fibroblasts are randomly distributed. Nevertheless, expression of exogenous adhesion proteins in fibroblasts results in accumulation of these proteins in the contacting plasma membrane domain (Nagafuchi et al., 1987; McNeill et al., 1990; Matter et al., 1990a, b; Mostov, 1994; Van Itallie and Anderson, 1997). Interestingly, the expression of endothelial cell adhesion molecule-1 (PECAM-1/CD31) at cell–cell contacts in endothelia, shows this localization even when transfected into COS or 3T3 cells, suggesting that recruitment of PECAM-1 is an intrinsic property of the molecule. Sun et al. (2000) studied the localization to cell–cell contacts of mutants and chimeric constructs transfected in non-PECAM-expressing cells, and found that only constructs that support PECAM-1 mediated adhesion localize to cell–cell borders.

5. Genes involved in polarity

Since *polarity* was first noticed as a greatly differentiated trait of neurons and epithelial cells of higher organisms, and *targeting* arose as a conceivable mechanism to explain the distribution of specific proteins in eukariotic cells stuffed full with organelles (Fig. 1), it took a long time to realize that polarity and targeting are also attributes of single, ancient and simpler cells. Today, when polarity and targeting are recognized as a fundamental characteristics of all cells, and information is found to be coded in well conserved genes, the use of simpler organisms offers the clear advantage of performing genetic studies to detect them.

Unlike yeast, however, complex eukaryotic cells can manifest multiple axes of polarity, suggesting that additional mechanisms have evolved to generate more elaborate patterns. Thus, Rho-family GTPases initially process and reinforce polarity cues by remodeling cortical actin, and these local asymmetries are subsequently conveyed to the microtubules, membrane and secretory pathway to generate the final pattern.

5.1. Polarity in Saccharomyces cerevisiae

In the yeast *S. cerevisiae*, Cdc42 activation in response to positional cues and cell cycle signals is a crucial factor in the switch from isotropic to polarized growth that occurs when the cyclin-dependent protein kinase Cdc28 is activated by G1 cyclins (Adams et al., 1990; Ayscough et al., 1997; Chant, 1999). In budding yeast spatial markers left by previous cell divisions stimulate the local activation of the Ras-related Rsr1/Bud1 GTPase, which recruits and activates Cdc42 via interaction with the guanidine nucleotide exchange factor Cdc24 (Park et al., 1999). Polarized growth in yeast is mediated by a series of steps involving cortical landmarks, Rho GTPases, and a polarized actin cytoskeleton. Secretion is targeted to the bud or mating projection, allowing selective growth in that area (Drubin and Nelson, 1996). In haploid cells exposed to mating pheromone, the protein Far1 interacts with Cdc24 and recruits Cdc42 to the tip of the mating projections (Butty et al., 1998). The activated GTP-bound form of Cdc42 interacts with several proteins that are presumed to be effectors that transduce its signal to bring about polarization of the actin cytoskeleton (Bi et al., 2000). Actin cables are proposed to serve as tracks for vesicle, organelle, and mRNA transport, whereas cortical actin patches are important for endocytosis (Pruyne and Bretscher, 2000a, b). Drees et al. (2001) used the two-hybrid screening approach to analyze the interaction of 54 proteins required for cell polarity development in budding yeast included Cdc42 and other Rho-type GTPases, their regulators and effectors, actin cytoskeleton-associated proteins, septin-associated proteins, and proteins involved in secretion. Besides these 54 proteins, they identified another 20 previously uncharacterized ones involved in polarized growth. The protein–protein interaction map that they describe shows a highly complex process of cell polarity development involving cortical cues, signaling proteins, the cytoskeleton, and the secretory apparatus, each of which is itself characterized by considerable complexity. More experimental work will be required to elucidate the mechanisms that underlie the cell polarity development due to the vast number of proteins involved.

5.2. Drosophila genes involved in epithelial cell polarity

Drosophila genes involved in the establishment of the ectoderm and midgut epithelia during embryogenesis, epithelial polarity of imaginal discs, and epithelial development of the follicle cells during oogenesis, shed light on the polarity of epithelia in mammals.

In simple epithelial monolayers, adherens junctions are organized into an adhesive belt-like structure around the circumference of the cell called the zonula adherens (ZA). The molecular components of adherens junctions are very similar between vertebrates and invertebrates, with cadherins and catenins being the heart of an adhesive interaction that connects actin belts of adjacent cells with each other. Although the ZA forms the most apical junction in *Drosophila* epithelia, the ZA do not appear to be always required for the maintenance of membrane

asymmetry (Wodarz et al., 1995). Septate junction (SJ) in invertebrate, and TJ in mammals, share some overlapping function in epithelia, which are the control of paracellular transport, and fence to lateral diffusion of membrane lipids and membrane proteins at the border between the apical and the basolateral domains. However, TJs are not required for this function in all polarized cells types, the separation of membrane domains can be maintained in the absence of any junction (Müller and Hausen, 1995; Winckler and Mellman, 1999). Many of the molecular components of vertebrate TJs are conserved in Drosophila, although TJs have not been found by ultrastructural criteria. Desmosomes are absent from invertebrate epithelia; in addition, cytoplasmic intermediate filaments, like cytokeratins that anchor in desmosomes, have so far not been found in *Drosophila*.

The coincidence of generation of apical-basal polarity with cellularization (de novo formation of a polarized epithelium) in *Drosophila* embryo suggests that mutations affecting either process will also affect the other. Genome-wide genetic screens have identified seven loci that are required zygotically for these processes (Merrill et al., 1988; Wieschaus and Sweeton, 1988). Three of these genes, *nullo*, *bottleneck* (*bnk*), *and serendipity* α (*sry* α) are required for the formation of the highly organized contractile actin-based network that drives cytokinesis during cellularization (Schejter et al., 1992).

At least four proteins containing PDZ protein interaction domains are expressed in the blastoderm during cellularization: Bazooka (BAZ), Canoe (Cno), Polychaetoid (Pyd), and Disc Lost (Dlt) Pyd represents a Drosophila homologue of the TJ-associated protein ZO-1 and physically interacts with Cno. AF-6, the human homologue of Cno, also interacts with ZO-1 and localizes to the submembraneous actin-cytoskeleton associated with TJs. Another four genes, *bazooka* (*baz*), *crumbs* (*crb*), *shotgun* (*shg*) and *stardust* (*sdt*) seem to be specifically involved in the formation or the maintenance of the surface epithelium.

The maternal expression of two genes is involved in the epithelia generation, DE-cadherin, the product of the *shg* locus, and Arm, the fly homologue of β-catenin and product of the *armadillo* gene. It has been shown that Arm is required for the generation of the ZA in the early embryo (Cox et al., 1996; Muller and Wieschaus, 1996).

Adjacent imaginal disc cells exhibit a fully elaborated junctional complex with an apical ZA and a more basal SJ. In addition to Arm and phosphotyrosyl proteins, the product of the *expanded* (*ex*) locus, as well as Dmerlin and Dmoesin are concentrated in the ZA (McCartney and Fehon, 1996). However, their localization appears to be not tightly restricted to the ZA and extends further basal (in the case of Ex) or further apical (in the case of Dmoesin). Ex protein is a member of the large superfamily of band 4.1 proteins. Coracle (Cor), another member of the Protein 4.1 family, is a molecular component of the SJs in the imaginal disc (Fehon et al., 1994). SJs have also been shown to contain large amounts of Disc Large (Dlg) protein and Neurexin (Nrx) (Woods and Bryant, 1991; Baumgartner et al., 1996). The lateral plasma membrane of the columnar wing disc cells is marked by the transmembrane proteins FasIII and Neuroglian, which show only partly overlapping distributions (Woods et al., 1996). FasIII is present at high levels at the SJ and at lower levels on the remaining lateral membrane domains. In contrast, Neuroglian is present on the entire lateral membrane domain, including the region apical to the SJ. The Crb protein is also expressed in imaginal discs (Tepass et al., 1990). However, its absence does not result in defects in the wing disc epithelium (Müller, 2000).

The functions of some of the mentioned molecular components of the SJs have been investigated. Nrx and Cor are key molecules that are important for the function of SJs.

In homozygous mutant *cor* clones, the characteristic ultrastructure of SJs between the cells is lost and paracellular transport is no longer restricted (Lamb et al., 1998). Cor is required for the localization of Nrx to the plasma membrane. Similarly, Nrx has been shown to be required for Cor localization and the maintenance of transepithelial barrier function of glial cells at the blood brain/barrier (Baumgartner et al., 1996). Despite defects in SJs, *cor* mutant disc epithelia maintain normal membrane asymmetry and do not exhibit any changes in the structure of the ZA nor in the cytoskeleton (Lamb et al., 1998). These results illustrate that the SJs are required for the gate function, i.e., the restriction of paracellular transport. On the other hand, SJs are not required for maintenance of apicobasal membrane polarity or the maintenance of adherens junctions.

In summary, genes involved in the epithelial polarity development in Drosophila could be separated in defined cellular functions: (1) genes for cell adhesion proteins: *armadillo* (*arm*), *dachsous* (*ds*), and *shotgun* (*shg*); (2) genes for proteins complex assembly: *bazzoka* (*baz*) and *crumbs* (*crb*) and probably *stardust* (*sdt*) (Hong et al., 2001; Bachmann et al., 2001); (3) genes involved in cell signaling *brainniac* (*brn*), *egghead* (*egh*) and *Notch* (*N*); (4) genes for cytoskeleton proteins: *canoe* (*cno*), *coracle* (*cor*), *expanded* (*ex*), *karst* (*kst*) and α-*Spectrin*; (5) tumor suppressor genes: *discs large* (*dlg*), *Dmerlin*, *fat*, *l(2)giant larva* (*lgl*) and *warts* (*wts*); and (6) genes for components of septate junctions *Neurexin* (*nrx*), and *scribble* (*scrib*) (Müller, 2000).

5.3. *Caenorhabditis elegans par genes and their role in mammalian epithelial cell polarity*

The past years have revealed that homologs of the *C. elegans par* (*par*titioning defective) genes are also essential for establishing polarity in *Drosophila* and vertebrate cells. There is growing evidence that the proteins encoded by these genes interact with key regulators of both the actin and the microtubule cytoskeletons.

In the nematode worm *C. elegans*, the zygote acquires polarity along the future anterior-posterior axis, after sperm entry. Goldstein and Hird (1996) showed that the zygote, anterior-posterior polarity is determined by the point where the sperm enters the oocyte. Although they showed that the sperm provides the crucial polarizing signal, the nature of the signal remained a mystery. Polarization of the zygote does not require the male pronucleus, suggesting the hypothesis that the sperm centrosome might be responsible for the polarizing activity of the sperm, (Sadler and Shakes, 2000). The centrosome will organize the mitotic spindle, it has been proposed that the contact of spindle microtubules with the cortex may promote localization of protein PAR-2, a posterior cortical marker.

Before the first division of the zygote, distinct anterior–posterior cortical domains are established characterized by the non-overlapping localization of PAR-3, PAR-6 and PKC-3 to the anterior cortex and PAR-1 and PAR-2 to the posterior cortex (Rose and Kemphues, 1998). The products of *par* genes are not simply markers of the distinct cortical domains they are also required for establishment of these domains. One general feature of *par* mutants is the loss of asymmetry in the first cell division.

The PAR proteins control the asymmetric spindle position determining the pulling force that positions the spindle poles before first cell division. The net pulling force acting on the posterior spindle pole is higher than that acting on the anterior spindle pole (Grill et al., 2001).

In addition to the *par* genes, several others genes involved in controlling zygote polarity have recently been identified. Some of the proteins encoded by these genes interact directly with PAR proteins. This is the case for PKC-3, an atypical protein kinase C (aPKC) isoform that binds to PAR-3 (Tabuse et al., 1998). The small GTPase Cdc42 binds to PAR-6 (Gotta et al., 2001) and NMY-2, a non-miscle myosin II heavy chain, binds to PAR-1 (Guo and Kemphues, 1996).

In the case of mammalian epithelial cells that are highly polarized and have distinct apical and basolateral plasma membrane domain, the spatial cues that lead to the initial polarization are cell–cell contacts and cell-extracellular matrix (ECM) contacts. Cell–cell contacts are mainly mediated by transmembrane adhesion molecules of the cadherin superfamily, whereas cell-ECM contacts are mediated by transmembrane receptors of the integrin family (Giancotti and Ruoslahti, 1999; Tepass et al., 2000).

Before the fully polarized epithelial phenotype is established, cadherin-containing cell contact, i.e. called adherent junctions, are scattered along the lateral plasma membrane, tight junctions have not yet formed, and several of its components colocalize with adherens junction components along the lateral membrane. Later on, adherent junctions coalesce in the apicolateral region of the plasma membrane and are assembled to form the zonula adherens. At the same time components of the adherens and tight junctions sort out, and assembly of the tight junction begins (Fleming et al., 2000).

So, little is known about the mechanisms responsible for separating adherens junction and tight junction components during establishment of the fully polarized fenotype of epithelial cells. Several pieces of evidence have shown that a protein complex consisting of mammalian homologs of PAR-3, PAR-6, Cdc42 and an aPKC is crucial to this process. ASIP/PAR-3, the mammalian PAR-3 homolog, was first identified as a binding partner of PKCs ζ and λ, the two mammalian aPKC isoforms (Izumi et al., 1998). Both aPKCs and ASIP/PAR-3 colocalize at the epithelial tight junction together with the tight junction marker ZO-1 (Izumi et al., 1998). PAR-6 and the small GTPase Cdc42 are also associated with ASIP/PAR-3 and aPKCs in mammalian epithelial cells (Qiu et al., 2000; Noda et al., 2001). Over expression of dominan-negative form of aPKC λ resulted in mislocalization of ASIP/PAR-3 and ZO-1 and the transepithelial electrical resistance broke down, indication that formation of functional tight junctions was impaired (Suzuki et al., 2001).

It has been reported that an association of ASIP/PAR-3 with JAM-1, a transmembrane adhesion molecule localized to the tight junction (Ebnet et al., 2001). JAM-1 binds through its PDZ binding domain to the first PDZ domain of ASIP/PAR-3 and JAM-1 can also bind to the third PDZ domain of ZO-1. As JAM-1 molecules can dimerize and tend to form large macromolecular clusters in the membrane, JAM-1 may link the PAR-3/PAR-6/aPKC/Cdc42 complex to the integral tight junction components ZO-1 and claudin.

The kinase activity of aPKC seems to be suppressed when aPKC is associated with PAR-6. This suppression can be overcome by binding of activated GTP-bound Cdc42 to the CRIB domain of PAR-6 (Yamanaka et al., 2001). Interestingly, cadherin-mediated cell–cell adhesion can activate Cdc42, which provides a possible explanation for the close connection between cadherin-mediated cell adhesion and tight junction assembly. One can speculate that PAR-3, PAR-6 and aPKC form the core of a protein complex that acts as a scaffold to which additional proteins can be recruited. At least in mammalian epithelial cells, ASIP/PAR-3 can be anchored in the cortex by binding to

the transmembrane protein JAM-1. The main effector molecule in the PAR-3/PAR-6/aPKC/Cdc42 complex may be the aPKC, because it is a protein kinase that can modulate the function of its targets by phosphorilation. The kinase activity of aPKCs appears to be regulated by PAR-6 together with Cdc42 (Wodarz, 2001).

6. External cues

Polarity is triggered by cell-substrate and cell–cell contacts as well as by hormones and a variety of factors that differ in nature (Cereijido et al., 1998; Wodarz, 2001).

6.1. Ca^{2+} activated cell–cell contacts

Tight junctions can be opened and resealed by the removal and the restoration of Ca^{2+} to the bathing media (Cereijido et al., 1978a, b, 1981; Meza et al., 1980; Martinez-Palomo et al., 1980; Citi, 1992). Cells plated at sub-confluence in media with normal Ca^{2+} concentrations, or at confluence but in low Ca^{2+}, do not develop TJs (Gonzalez-Mariscal et al., 1985) nor polarize membrane components like Na^+, K^+-ATPases (Contreras et al., 1989) and voltage dependent ion channels (Talavera et al., 1995). When cells are incubated at confluence, the addition of this ion restores TJs and apical/basolateral polarity. Ca^{2+} elicits this effect by acting on the cell–cell attaching molecule E-cadherin (Gumbiner and Simons, 1986; Yap et al., 1997; Gumbiner et al., 1988; Gumbiner, 2000; Mora et al., 2002; Buchert et al., 1999). Ca^{2+} is needed on the extracellular side, as a La^{3+} blockade of Ca^{2+} penetration into the cytoplasm fails to prevent the triggering effect (Contreras et al., 1992; Gonzalez-Mariscal et al., 1990). Ca^{2+} activates a cascade of intracellular reactions that includes protein kinase C (PKC), phospholipase C (PLC) and calmodulin (CaM) (Balda et al., 1991, 1993; Gonzalez-Mariscal et al., 1990; Ponce et al., 1991a, b; Ponce and Cereijido, 1991; Bilder and Perrimon, 2000; Braga, 1999).

6.2. Interactions with extracellular matrix components (ECM)

Integrins mediated cell adhesion to ECM is particularly important for organizing the apico-basal axis of epithelial cell polarity. Apical orientation towards the outside of epithelial cells grown in suspension and forming clumps, is inverted by the addition of collagen (Barriere et al., 1988; Chambard et al., 1981). Thus the orientation of apical and basolateral membrane domain depends on the biological compartments separated by the epithelium. Interestingly, intercalated cells of the collecting tubule exist in a spectrum of types. While the α-form secretes acid by an apical H^+-ATPase and a basolateral anion exchanger which is an alternatively spliced form of the red cell band 3 (kAE1), the β-form secretes HCO_3^- on the opposite membrane (Al Awqati et al., 2000). A protein, termed *hensin*, member of the scavenger receptor family, was deposited in the extracellular matrix of high-density cells that reversed the polarity of the transporters. Hensin also induces the expression of the microvillar protein villin, and caused the appearance of the apical terminal web proteins, cytokeratin 19 and actin, all of which led to the development of an exuberant microvillar structure (Hikita et al., 1999). In addition, hensin causes β cells to assume a columnar shape. These studies demonstrate that, at least in vitro, the conversion of polarity in the

intercalated cell, represents a terminal differentiation, and that hensin is the first protein in a new pathway that mediates this process (Al Awqati et al., 2000; Hikita et al., 1999; Crepaldi et al., 1994).

7. Pathologies derived from the alteration of polarity

As the intricacies of sorting and retention machineries are unraveled, their failure is associated to a rapidly growing number of clinical manifestations. We will just mention a few of these pathologies as examples. (1) In the familial hypercholesterolemia (FH)-Turku a mutation of glycine 823 residue of the LDL receptor affects the signal required for its basolateral targeting; the receptor is mistargeted to the apical surface, resulting in reduced endocytosis of LDL hypercholesterolemia (Koivisto et al., 2001). (2) In the intestinal congenital sucrase-isomaltase deficiency, a point mutation of glutamine by arginine at residue 117 of sucrase-isomaltase, affects the access of the O-glycans to a putative sorting element (Spodsberg et al., 2001). (3) In the polycystic kidney disease, polycystin-1, a component of desmosomal junctions of epithelial cells involved in tubulogenesis is altered, leading to cystic transformation (Bukanov et al., 2002). In keeping with the ideas discussed in Fig. 7, in polycystic kidney disease the Na^+, K^+-ATPase is not expressed on the lateral, but on the apical domain, due to an aberrant expression of the β_2 subunit (Wilson et al., 2000). (4) The Zellweger syndrome, neonatal adrenoleukodystrophy, infantile Refsum's disease, and classical rhizomelic chondrodysplasia punctata are lethal genetic disorders caused by defects in peroxisome biogenesis. Slawecki et al. (1995) identified three distinct defects that affect the delivery of the peroxisomal protein of these patients.

8. Concluding remarks

To appreciate the progress made in the field of targeting in the last decade, one only needs to remember that not more than 10 years ago it appeared conceivable that a single sorting signal (say to the apical domain) would suffice to explain polarity, as the basolateral domain would be reached by default. Furthermore, it was tacitly expected that different cell types would use the same sorting motif. On the contrary, today we have abundant evidence that there is no universal signal nor single mechanism, as the requirement of different protein types in a given domain of the same cell can be different, and require separate modulations along the cell cycle, in response to functional needs, hormonal challenge, etc. This poses a formidable problem to a reviewer, because at this moment the field is too vast to enumerate even a small proportion of the findings made, and too heterogeneous to order the available information according to emerging patterns. This situation where there is a considerable amount of information but comparatively little knowledge constitutes a perpetual challenge for research, in particular because any of the signals and mechanisms discussed may certainly misfunction and provoke diseases. That was also our justification for restricting this review to the underlying phenomena that we grouped in "Toolbox", and to the paradigmatic examples we have chosen.

Acknowledgements

We wish to acknowledge the efficient and pleasant help of Elizabeth del Oso, and the economic support of the National Research Council of México (CONACYT).

References

Adams, A.E.M., Johnson, D.I., Longnecker, R.M., Sloat, B.F., Pringle, J.R., 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. 111, 131–142.

Al Awqati, Q., Vijayakumar, S., Takito, J., Hikita, C., Yan, L., Wiederholt, T., 2000. Phenotypic plasticity and terminal differentiation of the intercalated cell: the hensin pathway. Exp. Nephrol. 8, 66–71.

Arreaza, G., Brown, D.A., 1995. Sorting and intracellular trafficking of a glycosylphosphatidylinositol-anchored protein and two hybrid transmembrane proteins with the same ectodomain in Madin-Darby canine kidney epithelial cells. J. Biol. Chem. 270, 23641–23647.

Ayscough, K.R., Stryker, J., Pokala, N., Sanders, M., Crews, P., Drubin, D.G., 1997. High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. J. Cell Biol. 137, 399–416.

Bachmann, A., Schneider, M., Theilenberg, E., Grawe, F., Knust, E., 2001. Drosophila Stardust is a partner of Crumbs in the control of epithelial cell polarity. Nature 414 (6864), 638–643.

Balda, M.S., Gonzalez-Mariscal, L., Contreras, R.G., Macias-Silva, M., Torres-Marquez, M.E., Garcia-Sainz, J.A., Cereijido, M., 1991. Assembly and sealing of tight junctions: possible participation of G-proteins, phospholipase C, protein kinase C and calmodulin. J. Membr. Biol. 122, 193–202.

Balda, M.S., Gonzalez-Mariscal, L., Matter, K., Cereijido, M., Anderson, J.M., 1993. Assembly of the tight junction: the role of diacylglycerol. J. Cell Biol. 123, 293–302.

Barriere, H., Chambard, M., Selzner, J.P., Mauchamp, J., Gabrion, J., 1988. Polarity reversal of inside-out thyroid follicles cultured within collagen gel: structure of the junctions assessed by freeze-fracture and lanthanum permeability. Biol. Cell 62, 133–144.

Baumann, O., Lautenschlager, B., Takeyasu, K., 1994. Immunolocalization of Na,K-ATPase in blowfly photoreceptor cells. Cell Tissue Res. 275, 225–234.

Baumgartner, S., Littleton, J.T., Broadie, K., Bhat, M.A., Harbecke, R., Lengyel, J.A., Chiquet-Ehrismann, R., Prokop, A., Bellen, H.J., 1996. A Drosophila neurexin is required for septate junction and blood-nerve barrier formation and function. Cell 87, 1059–1068.

Bennett, V., 1990. Spectrin: a structural mediator between diverse plasma membrane proteins and the cytoplasm. Curr. Opin. Cell Biol. 2, 51–56.

Benting, J.H., Rietveld, A.G., Simons, K., 1999. N-Glycans mediate the apical sorting of a GPI-anchored, raft-associated protein in Madin-Darby canine kidney cells. J. Cell Biol. 146, 313–320.

Bhat, M.A., Izaddoost, S., Lu, Y., Cho, K.O., Choi, K.W., Bellen, H.J., 1999. Discs Lost, a novel multi-PDZ domain protein, establishes and maintains epithelial polarity. Cell 96, 833–845.

Bi, E., Chiavetta, B.J., Chen, H., Chen, G.-C., Chan, C.S.M., Pringle, J.R., 2000. Identification of novel, evolutionarily conserved Cdc42p-interacting proteins and of redundant pathways linking Cdc24p and Cdc42p to actin polarization in yeast. Mol. Biol. Cell 11, 773–793.

Bilder, D., Perrimon, N., 2000. Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature 403, 676–680.

Bonilha, V.L., Finnemann, S.C., Rodriguez-Boulan, E., 1999. Ezrin promotes morphogenesis of apical microvilli and basal infoldings in retinal pigment epithelium. J. Cell Biol. 147, 1533–1548.

Braga, V.M., 1999. Small GTPases and regulation of cadherin dependent cell–cell adhesion. Mol. Pathol. 52, 197–202.

Bretscher, A., 1999. Regulation of cortical structure by the ezrin-radixin-moesin protein family. Curr. Opin. Cell Biol. 11, 109–116.

Brewer, C.B., Roth, M.G., 1991. A single amino acid change in the cytoplasmic domain alter the polarized delivery of influenza virus hemagglutnin. J. Cell Biol. 114 (3), 413–421.

Brown, D.A., London, E., 1998. Functions of lipid rafts in biological membranes. Annu. Rev. Cell Dev. Biol. 14, 111–136.

Brymora, A., Valova, V.A., Larsen, M.R., Roufogalis, B.D., Robinson, P.J., 2001. The brain exocyst complex interacts with RalA in a GTP-dependent manner: identification of a novel mammalian sec3 gene and a second sec15 gene. J. Biol. Chem. 276, 29792–29797.

Buchert, M., Schneider, S., Meskenaite, V., Adams, M.T., Canaani, E., Baechi, T., Moelling, K., Hovens, C.M., 1999. The junction-associated protein AF-6 interacts and clusters with specific Eph receptor tyrosine kinases at specialized sites of cell–cell contact in the brain. J. Cell Biol. 144, 361–371.

Bukanov, O., Husson, H., Dackowski, W.R., Lawrence, B.D., Clow, P.A., Roberts, B.L., Klinger, K.W., Ibraghimov-Beskrovnaya, O., 2002. Functional polycystin-1 expression is developmentally regulated during epithelial morphogenesis in vitro: downregulation and loss of membrane localization during cystogenesis. Hum. Mol. Genet. 11, 923–936.

Butty, A.-C., Pryciak, P.M., Huang, L.S., Herskowitz, I., Peter, M., 1998. The role of Far1p in linking the heterotrimeric G protein to polarity establishment proteins during yeast mating. Science 282, 1511–1516.

Caplan, M.J., Anderson, H.C., Palade, G.E., Jamieson, J.D., 1986a. Intracellular sorting and polarized cell surface delivery of (Na^+, K^+)ATPase, an endogenous component of MDCK cell basolateral plasma membranes. Cell 46, 623–631.

Caplan, M.J., Palade, G.E., Jamieson, J.D., 1986b. Newly synthesized Na+, K+-ATPase alpha-subunit has no cytosolic intermediate in MDCK cells. J. Biol. Chem. 261, 2860–2865.

Casanova, J.E., Mishumi, Y., Ikehara, Y., Hubbard, A.L., Mostov, K.E., 1991. Direct apical sorting of rat liver dipeptidylpeptidase IV expressed in Madin-Darby canine kidney cells. J. Biol. Chem. 266, 24428–24432.

Cereijido, M., 1992. On the evolution on the ideas of the tight junction. In: Cereijido, M. (Ed.), Tight Junctions. CRC Press, Boca Raton, pp. 1–13.

Cereijido, M., Rotunno, C.A., 1970. Introduction to the Study of Biological Membranes. Gordon and Breach, New York.

Cereijido, M., Fernández, M., Rotunno, C., Cereijido, M., 1969. The interactions of L-α-dipalmitoyl lecithin monolayers with Na^+, K^+ or Li^+, and its possible role in membrane phenomena. Biochim. Biophys. Acta 183, 98–109.

Cereijido, M., Robbins, E.S., Dolan, W.J., Rotunno, C.A., Sabatini, D.D., 1978a. Polarized monolayers formed by epithelial cells on a permeable and translucent support. J. Cell Biol. 77, 853–880.

Cereijido, M., Rotunno, C.A., Robbins, E.S., Sabatini, D.D., 1978b. Polarized epithelia membranes produced in vitro. In: Hoffman, J. (Ed.), Membrane Transport Processes. Raven, New York, pp. 433–461.

Cereijido, M., Meza, I., Martinez-Palomo, A., 1981. Occluding junctions in cultured epithelial monolayers. Am. J. Physiol. 240, C96–C102.

Cereijido, M., Valdes, J., Shoshani, L., Contreras, R.G., 1998. Role of tight junctions in establishing and maintaining cell polarity. Annu. Rev. Physiol. 60, 161–177.

Cereijido, M., Shoshani, L., Contreras, R.G., 2000. Molecular physiology and pathophysiology of tight junctions I. Biogenesis of tight junctions and epithelial polarity. Am. J. Physiol. 279, G477–G482.

Cereijido, M., Shoshani, L., Contreras, R.G., 2001. Polarized distribution of Na^+, K^+-ATPase and active transport across epithelia. J. Membr. Biol. 184, 299–304.

Chambard, M., Gabrion, J., Mauchamp, J., 1981. Influence of collagen gel on the orientation of epithelial cell polarity: follicle formation from isolated thyroid cells and from preformed monolayers. J. Cell Biol. 91, 157–166.

Chant, J., 1999. Cell polarity in yeast. Ann. Rev. Cell Dev. Biol. 15, 365–391.

Cheong, K.H., Zacchetti, D., Schneeberger, E.E., Simons, K., 1999. VIP17/MAL, a lipid raft-associated protein, is involved in apical transport in MDCK cells. Proc. Natl. Acad. Sci. USA 96, 6241–6248.

Chin, L.S., Weigel, C., Li, L., 2000. Transcriptional regulation of gene expression of sec6, a component of mammalian exocyst complex at the synapse. Brain Res. Mol. Brain Res. 79, 127–137.

Citi, S., 1992. Protein kinase inhibitors prevent junction dissociation induced by low extracellular calcium in MDCK epithelial cells. J. Cell Biol. 117, 169–178.

Contreras, R.G., Avila, G., Gutierrez, C., Bolivar, J.J., Gonzalez-Mariscal, L., Darzon, A., Beaty, G., Rodriguez-Boulan, E., Cereijido, M., 1989. Repolarization of Na^+-K^+ pumps during establishment of epithelial monolayers. Am. J. Physiol. 257, C896–C905.

Contreras, R.G., Miller, J.H., Zamora, M., Gonzalez-Mariscal, L., Cereijido, M., 1992. Interaction of calcium with plasma membrane of epithelial (MDCK) cells during junction formation. Am. J. Physiol. 263, C313–C318.

Contreras, R.G., Lazaro, A., Bolivar, J.J., Flores-Maldonado, C., Sanchez, S.H., Gonzalez-Mariscal, L., Garcia-Villegas, M.R., Valdes, J., Cereijido, M., 1995. A novel type of cell–cell cooperation between epithelial cells. J. Membr. Biol. 145, 305–310.

Contreras, R.G., Shoshani, L., Flores-Maldonado, C., Lazaro, A., Monroy, A.O., Roldan, M.L., Fiorentino, R., Cereijido, M., 2002. E-cadherin and tight junction between epithelial cells of different animal species. Pflügers. Archiv. 444, 467–475.

Cox, R.T., Kirkpatrick, C., Peifer, M., 1996. Armadillo is required for adherens junction assembly, cell polarity, and morphogenesis during Drosophila embryogenesis. J. Cell Biol. 134, 133–148.

Crepaldi, T., Pollack, A.L., Prat, M., Zborek, A., Mostov, K., Comoglio, P.M., 1994. Targeting of the SF/HGF receptor to the basolateral domain of polarized epithelial cells. J. Cell Biol. 125, 313–320.

Delgrossi, M.H., Breuza, L., Mirre, C., Chavrier, P., Le Bivic, A., 1997. Human syntaxin 3 is localized apically in human intestinal cells. J. Cell Sci. 110, 2207–2214.

Dell'Angelica, E.C., Mullins, C., Bonifacino, J.S., 1999. AP-4, a novel complex related to clathrin adaptors. J. Biol. Chem. 274, 7278–7285.

Diamond, J.M., Wright, E., 1969. Biological membranes: the physical basis of ion and non-electrolyte selectivity. Annu. Rev. Physiol. 31, 581–646.

Dietrich, C., Yang, B., Fujiwara, T., Kusumi, A., Jacobson, K., 2002. Relationship of lipid rafts to transient confinement zones detected by single particle tracking. Biophys. J. 82 (1 Part 1), 274–284.

Drees, B.L., Sundin, B., Brazeau, E., Caviston, J.P., Chen, G.-C., Guo, W., Kozminski, K.G., Lau, M.W., Moskow, J.J., Tong, A., Schenkman, L.R., McKenzie III, A., Brennwald, P., Longtine, M., Bi, E., Chan, C., Novick, P., Boone, C., Pringle, J.R., Davis, T.N., Fields, S., Drubin, D.G., 2001. A protein interaction map for cell polarity development. J. Cell Biol. 154, 549–571.

Drubin, D.G., Nelson, W.J., 1996. Origins of cell polarity. Cell 84, 335–344.

Dubreuil, R.R., MacVicar, G., Dissanayake, S., Liu, C., Homer, D., Hortsch, M., 1996. Neuroglian-mediated cell adhesion induces assembly of the membrane skeleton at cell contact sites. J. Cell. Biol. 133, 647–655.

Dubreuil, R.R., Maddux, P.B., Grushko, T.A., MacVicar, G.R., 1997. Segregation of two spectrin isoforms: polarized membrane-binding sites direct polarized membrane skeleton assembly. Mol. Biol. Cell 8, 1933–1942.

Dunbar, L.A., Caplan, M.J., 2000. The cell biology of ion pumps: sorting and regulation. Eur. J. Cell Biol. 79, 557–563.

Dunbar, L.A., Caplan, M.J., 2001. Ion pumps in polarized cells: sorting and regulation of the Na^+, K^+- and H^+, K^+-ATPases. J. Biol. Chem. 276, 29617–29620.

Dunbar, L.A., Aronson, P., Caplan, M.J., 2000. A transmembrane segment determines the steady-state localization of an ion-transporting adenosine triphosphatase. J. Cell Biol. 148, 769–778.

Ebnet, K., Suzuki, A., Horikoshi, Y., Hirose, T., Meyer Zu Brickwedde, M.K., Ohno, S., Vestweber, D., 2001. The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). EMBO J. 20, 3738–3748.

Edidin, M., 1997. Lipid microdomains in cell surface membranes. Curr. Opin. Struct. Biol. 7, 528–532.

Eisenman, G., 1962. Cation selective glass electrodes and their mode of operation. Biophys. J. 2 (part 2), 259–323.

Farkas, V., Kovarik, J., Kosinova, A., Bauer, S., 1974. Autoradiographic study of mannan incorporation into the growing cell walls of *Saccharomyces cerevisiae*. J. Bacteriol. 117, 265–269.

Fath, K.R., Burgess, D.R., 1993. Golgi-derived vesicles from developing epithelial cells bind actin filaments and possess myosin-I as a cytoplasmically oriented peripheral membrane protein. J. Cell Biol. 120, 117–127.

Fehon, R.G., Dawson, I.A., Artavanis-Tsakonas, S., 1994. A Drosophila homologue of membrane-skeleton protein 4.1 is associated with septate junctions and is encoded by the coracle gene. Development 120, 545–557.

Fernández, M.S., vilallonga, F., Rotunno, C.A., Cereijido, M., 1970. Discrimination of Na^+ and K^+ by monolayers of lipids from epithelial cells. Biochim. Biophys. Acta 203, 586–589.

Fiedler, K., Lafont, F., Parton, R.G., Simons, K., 1995. Annexin XIIIb: a novel epithelial specific annexin is implicated in vesicular traffic to the apical plasma membrane. J. Cell Biol. 128, 1043–1053.

Field, C., Schekman, R., 1980. Localized secretion of acid phosphatase reflects the pattern of cell surface growth in *Saccharomyces cerevisiae*. J. Cell Biol. 86, 123–128.

Finger, F.P., Novick, P., 1998. Spatial regulation of exocytosis: lessons from yeast. J. Cell Biol. 142, 609–612.

Fleming, T.P., Papenbrock, T., Fesenko, I., Hausen, P., Sheth, B., 2000. Assembly of tight junctions during early vertebrate development. Semin. Cell Dev. Biol. 11, 291–299.

Folsch, H., Ohno, H., Bonifacino, J.S., Mellman, I., 1999. A novel clathrin adaptor complex mediates basolateral targeting in polarized epithelial cells. Cell 99, 189–198.

Galli, T., Zahraoui, A., Vaidyanathan, V.V., Raposo, G., Tian, J.M., Karin, M., Niemann, H., Louvard, D., 1998. A novel tetanus neurotoxin-insensitive vesicle-associated membrane protein in SNARE complexes of the apical plasma membrane of epithelial cells. Mol. Biol. Cell 9, 1437–1448.

Giancotti, F.G., Ruoslahti, E., 1999. Integrin signaling. Science 285, 1028–1032.

Gloor, S., Antonicek, H., Sweadner, K.J., Pagliusi, S., Frank, R., Moos, M., Schachner, M., 1990. The adhesion molecule on glia (AMOG) is a homologue of the beta subunit of the Na,K-ATPase. J. Cell Biol. 110, 165–174.

Goldstein, B., Hird, S.N., 1996. Specification of the anteroposterior axis in Caenorhabditis elegans. Development 122, 1467–1474.

Gonzalez-Mariscal, L., Chavez, D.R., Cereijido, M., 1985. Tight junction formation in cultured epithelial cells (MDCK). J. Membr. Biol. 86, 113–125.

Gonzalez-Mariscal, L., Contreras, R.G., Bolivar, J.J., Ponce, A., Chavez, D.R., Cereijido, M., 1990. Role of calcium in tight junction formation between epithelial cells. Am. J. Physiol. 259, C978–C986.

Gonzalez-Mariscal, L., Islas, S., Contreras, R.G., Garcia-Villegas, M.R., Betanzos, A., Vega, J., Diaz-Quinonez, A., Martin-Orozco, N., Ortiz-Navarrete, V., Cereijido, M., Valdes, J., 1999. Molecular characterization of the tight junction protein ZO-1 in MDCK cells. Exp. Cell Res. 248, 97–109.

Gotta, M., Abraham, M.C., Ahringer, J., 2001. CDC-42 controls early cell polarity and spindle orientation in *C. elegans*. Curr. Biol. 11, 482–488.

Gottardi, C.J., Caplan, M.J., 1993. Molecular requirements for the cell-surface expression of multisubunit ion-transporting ATPases. Identification of protein domains that participate in Na,K-ATPase and H,K-ATPase subunit assembly. J. Biol. Chem. 268, 14342–14347.

Grill, S.W., Gonczy, P., Stelzer, E.H., Hyman, A.A., 2001. Polarity controls forces governing asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. Nature 409, 630–633.

Grindstaff, K.K., Yeaman, C., Anandasabapathy, N., Hsu, S.C., Rodriguez-Boulan, E., Scheller, R.H., Nelson, W.J., 1998. Sec6/8 complex is recruited to cell–cell contacts and specifies transport vesicle delivery to the basal–lateral membrane in epithelial cells. Cell 93, 731–740.

Gumbiner, B.M., 2000. Regulation of cadherin adhesive activity. J. Cell Biol. 148, 399–404.

Gumbiner, B., Simons, K., 1986. A functional assay for proteins involved in establishing an epithelial occluding barrier: identification of a uvomorulin-like polypeptide. J. Cell Biol. 102, 457–468.

Gumbiner, B., Stevenson, B., Grimaldi, A., 1988. The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J. Cell Biol. 107, 1575–1587.

Guo, S., Kemphues, K.J., 1996. A non-muscle myosin required for embryonic polarity in *Caenorhabditis elegans*. Nature 382, 455–458.

Guo, W., Roth, D., Gatti, E., De Camilli, P., Novick, P., 1997. Identification and characterization of homologues of the Exocyst component Sec10p. FEBS Lett. 404, 135–139.

Guo, W., Grant, A., Novick, P., 1999a. Exo84p is an exocyst protein essential for secretion. J. Biol. Chem. 274, 23558–23564.

Guo, W., Roth, D., Walch-Solimena, C., Novick, P., 1999b. The exocyst is an effector for Sec4p, targeting secretory vesicles to sites of exocytosis. EMBO J. 18, 1071–1080.

Gut, A., Kappeler, F., Hyka, N., Balda, M.S., Hauri, H.P., Matter, K., 1998. Carbohydrate-mediated Golgi to cell surface transport and apical targeting of membrane proteins. EMBO J. 17, 1919–1929.

Hammerton, R.W., Krzeminski, K.A., Mays, R.W., Ryan, T.A., Wollner, D.A., Nelson, W, 1991. Mechanism for regulating cell surface distribution of Na^+, K(+)-ATPase in polarized epithelial cells. Science 254, 847–850.

Hikita, C., Takito, J., Vijayakumar, S., Al Awqati, Q., 1999. Only multimeric hensin located in the extracellular matrix can induce apical endocytosis and reverse the polarity of intercalated cells. J. Biol. Chem. 274, 17671–17676.

Hong, Y., Stronach, B., Perrimon, N., Jan, L.Y., Jan, Y.N., 2001. Drosophila Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts. Nature 414, 634–638.

Hubbard, A.L., Stieger, B., Bartles, J.R., 1989. Biogenesis of endogenous plasma membrane proteins in epithelial cells. Annu. Rev. Physiol. 51, 755–770.

Huber, L.A., Pimplikar, S., Parton, R.G., Virta, H., Zerial, M., Simons, K., 1993. Rab8, a small GTPase involved in vesicular traffic between the TGN and the basolateral plasma membrane. J. Cell Biol. 123, 35–45.

Hunziker, W., Fumey, C., 1994. A di-leucine motif mediates endocytosis and basolateral sorting of macrophage IgG Fc receptors in MDCK cells. EMBO J. 13, 2963–2967.

Islas, S., Vega, J., Ponce, L., Gonzalez-Mariscal, L., 2002. Nuclear localization of the tight junction protein ZO-2 in epithelial cells. Exp. Cell Res. 274, 138–148.

Izumi, Y., Hirose, T., Tamai, Y., Hirai, S., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kemphues, K.J., Ohno, S., 1998. An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. J. Cell Biol. 143, 95–106.

Jahn, R., Sudhof, T.C., 1999. Membrane fusion and exocytosis. Annu. Rev. Biochem. 68, 863–911.

Kaech, S.M., Whitfield, C.W., Kim, S.K., 1998. The LIN-2/LIN-7/LIN-10 complex mediates basolateral membrane localization of the C. elegans EGF receptor LET-23 in vulval epithelial cells. Cell 94, 761–771.

Kim, E., DeMarco, S.J., Marfatia, S.M., Chishti, A.H., Sheng, M., Strehler, E.E., 1998. Plasma membrane Ca^{2+} ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. J. Biol. Chem. 273, 1591–1595.

Kirchhausen, T., 1999. Adaptors for clathrin-mediated traffic. Annu. Rev. Cell Dev. Biol. 15, 705–731.

Kitagawa, Y., Sano, Y., Ueda, M., Higashio, K., Narita, H., Okano, M., Matsumoto, S., Sasaki, R., 1994. N-glycosylation of erythropoietin is critical for apical secretion by Madin-Darby canine kidney cells. Exp. Cell. Res. 213 (2), 449–457.

Koefoed-Johnson, V., Ussing, H., 1958. The nature of frog skin potential. Acta Physiol. Scand. 42, 298–308.

Koivisto, U.M., Hubbard, A.L., Mellman, I., 2001. A novel cellular phenotype for familial hypercholesterolemia due to a defect in polarized targeting of LDL receptor. Cell 105, 575–585.

Kreitzer, G., Marmorstein, A., Okamoto, P., Vallee, R., Rodriguez-Boulan, E., 2000. Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat. Cell Biol. 2, 125–127.

Kundu, A., Avalos, R.T., Sanderson, C.M., Nayak, D.P., 1996. Transmembrane domain of influenza virus neuraminidase, a type II protein, possesses an apical sorting signal in polarized MDCK cells. J. Virol. 70, 6508–6515.

Lafont, F., Burkhardt, J.K., Simons, K., 1994. Involvement of microtubule motors in basolateral and apical transport in kidney cells. Nature 372, 801–803.

Lafont, F., Lecat, S., Verkade, P., Simons, K., 1998. Annexin XIIIb associates with lipid microdomains to function in apical delivery. J. Cell Biol. 142, 1413–1427.

Lamb, R.S., Ward, R.E., Schweizer, L., Fehon, R.G., 1998. *Drosophila coracle*, a member of the protein 4.1 superfamily, has essential structural functions in the septate junctions and developmental functions in embryonic and adult epithelial cells. Mol. Biol. Cell 9, 3505–3519.

Lee, A., 2001. Membrane structure. Curr. Biol. 11, R811–R814.

Lee, J.K., Coyne, R.S., Dubreuil, R.R., Goldstein, L.S., Branton, D., 1993. Cell shape and interaction defects in alpha-spectrin mutants of Drosophila melanogaster. J. Cell Biol. 123, 1797–1809.

Lee, J.K., Brandin, E., Branton, D., Goldstein, L.S., 1997. Alpha-Spectrin is required for ovarian follicle monolayer integrity in Drosophila melanogaster. Dev. 124, 353–362.

Lee, J., Scheraga, H.A., Rackovsky, S., 1998. Conformational analyses of the 20-residue membrane-bound portion of melittin by conformational space annealing. Biopolymers 2, 103–116.

Le Gall, A.H., Yeaman, C., Muesch, A., Rodriguez-Boulan, E., 1995. Epithelial cell polarity: new perspectives. Semin. Nephrol. 15, 272–284.

Ling, G.N., 1962. A Physical Theory of the Living State: the Association-Induction Hypothesis. Blaisdell Publ. Co., Waltham, MA.

Lipschutz, J.H., Mostov, K.E., 2002. Exocytosis: the many masters of the exocyst. Curr. Biol. 12, R212–R214.

Lisanti, M.P., Caras, I.W., Davitz, M.A., Rodriguez-Boulan, E., 1989. A glycophospholipid membrane anchor acts as an apical targeting signal in polarized epithelial cells. J. Cell Biol. 109, 2145–2156.

Louvard, D., 1980. Apical membrane aminopeptidase appears at site of cell–cell contact in cultured kidney epithelial cells. Proc. Natl. Acad. Sci. USA 77, 4132–4136.

Low, S.H., Chapin, S.J., Weimbs, T., Komuves, L.G., Bennett, M.K., Mostov, K.E., 1996. Differential localization of syntaxin isoforms in polarized Madin-Darby canine kidney cells. Mol. Biol. Cell 7, 2007–2018.

Low, S.H., Chapin, S.J., Wimmer, C., Whiteheart, S.W., Komuves, L.G., Mostov, K.E., Weimbs, T., 1998. The SNARE machinery is involved in apical plasma membrane trafficking in MDCK cells. J. Cell Biol. 141, 1503–1513.

Marmorstein, A.D., Gan, Y.C., Bonilha, V.L., Finnemann, S.C., Csaky, K.G., Rodriguez-Boulan, E., 1998. Apical polarity of N-CAM and EMMPRIN in retinal pigment epithelium resulting from suppression of basolateral signal recognition. J. Cell Biol. 142, 697–710.

Martinez-Palomo, A., Meza, I., Beaty, G., Cereijido, M., 1980. Experimental modulation of occluding junctions in a cultured transporting epithelium. J. Cell Biol. 87, 736–745.

Marzolo, M.P., Bull, P., Gonzalez, A., 1997. Apical sorting of Hepatitis B surface antigen (HbsAg) is independent of N-glycosylation and glycosylphosphatidylinositol-anchored protein segretation. Proc. Natl. Acad. Sci. USA 94, 1834–1839.

Matter, K., 2000. Epithelial polarity: sorting out the sorters. Curr. Biol. 10, R39–R42.

Matter, K., Mellman, I., 1994. Mechanisms of cell polarity: sorting and transport in epithelial cells. Curr. Opin. Cell Biol. 6, 545–554.

Matter, K., Brauchbar, M., Bucher, K., Hauri, H.P., 1990a. Sorting of endogenous plasma membrane proteins occurs from two sites in cultured human intestinal epithelial cells (Caco-2). Cell 60, 429–437.

Matter, K., Bucher, K., Hauri, H.P., 1990b. Microtubule perturbation retards both the direct and the indirect apical pathway but does not affect sorting of plasma membrane proteins in intestinal epithelial cells (Caco-2). EMBO J. 9, 3163–3170.

Matter, K., Hunziker, W., Mellman, I., 1992. Basolateral sorting of LDL receptor in MDCK cells: The cytoplasmic domain contains two tyrosine-dependent targeting determinants. Cell 71, 741–753.

Matter, K., Yamamoto, E.M., Mellman, I., 1994. Structural requirements and sequence motifs for polarized sorting and endocytosis of LDL and Fc receptors in MDCK cells. J. Cell Biol. 126, 991–1004.

Mays, R.W., Siemers, K.A., Fritz, B.A., Lowe, A.W., van Meer, G., Nelson, W.J., 1995. Hierarchy of mechanisms involved in generating Na/K-ATPase polarity in MDCK epithelial cells. J. Cell Biol. 130, 1105–1115.

McCartney, B.M., Fehon, R.G., 1996. Distinct cellular and subcellular patterns of expression imply distinct functions for the Drosophila homologues of moesin and the neurofibromatosis 2 tumor suppressor, merlin. J. Cell Biol. 133, 843–852.

McNeill, H., Ozawa, M., Kemler, R., Nelson, W.J., 1990. Novel function of the cell adhesion molecule uvomorulin as an inducer of cell surface polarity. Cell 62, 309–316.

Merrill, P.T., Sweeton, D., Wieschaus, E., 1988. Requirements for autosomal gene activity during precellular stages of *Drosophila* melanogaster. Development 104, 495–509.

Meza, I., Ibarra, G., Sabanero, M., Martinez-Palomo, A., Cereijido, M., 1980. Occluding junctions and cytoskeletal components in a cultured transporting epithelium. J. Cell Biol. 87 (3 Part 1), 746–754.

Mora, R., Rodriguez-Boulan, E., Palese, P., Garcia-Sastre, A., 2002. Apical budding of a recombinant influenza A virus expressing a hemagglutinin protein with a basolateral localization signal. J. Virol. 76, 3544–3553.

Moskalenko, S., Henry, D.O., Rosse, C., Mirey, G., Camonis, J.H., White, M.A., 2002. The exocyst is a Ral effector complex. Nat. Cell Biol. 4, 66–72.

Mostov, K.E., 1994. Transepithelial transport of immunoglobulins. Annu. Rev. Immunol. 12, 63–84.

Mostov, K.E., Verges, M., Altschuler, Y., 2000. Membrane traffic in polarized epithelial cells. Curr. Opin. Cell Biol. 12, 483–490.

Müller, H.A., 2000. Genetic control of epithelial cell polarity: lessons from *Drosophila*. Dev. Dyn. 218, 52–67.

Müller, H.A., Hausen, P., 1995. Epithelial cell polarity in early *Xenopus* development. Dev. Dyn. 2002, 405–420.

Muller, H.A., Wieschaus, E., 1996. Armadillo, bazooka, and stardust are critical for early stages in formation of the zonula adherens and maintenance of the polarized blastoderm epithelium in Drosophila. J. Cell Biol. 134, 149–163.

Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K., Takeichi, M., 1987. Transformation of cell adhesion properties by exogenously introduced E- cadherin cDNA. Nature 329, 341–343.

Nehring, R.B., Wischmeyer, E., Doring, F., Veh, R.W., Sheng, M., Karschin, A., 2000. Neuronal inwardly rectifying K(+) channels differentially couple to PDZ proteins of the PSD-95/SAP90 family. J. Neurosci. 20, 156–162.

Nelson, W.J., Veshnock, P.J., 1986. Dynamics of membrane-skeleton (fodrin) organization during development of polarity in madin-darby canine kidney epithelial cells. J. Cell Biol. 103, 1751–1765.

Nelson, W.J., Veshnock, P.J., 1987. Ankyrin binding to ($Na^+ + K^+$) ATPase and implications for the organization of membrane domains in polarized cells. Nature 328 (6130), 533–536.

Noda, Y., Takeya, R., Ohno, S., Naito, S., Ito, T., Sumimoto, H., 2001. Human homologues of the *Caenorhabditis elegans* cell polarity protein PAR6 as an adaptor that links the small GTPases Rac and Cdc42 to atypical protein kinase C. Genes Cells 6, 107–119.

Novick, P., Field, C., Schekman, R., 1980. Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell 21, 205–215.

Odorizzi, G., Cowles, C.R., Emr, S.D., 1998. The AP-3 complex a coat of many colours. Trends Cell Biol. 8 (7), 282–288.

Ohno, H., Tomemori, T., Nakatsu, F., Okazaki, Y., Aguilar, R.C., Foelsch, H., Mellman, I., Saito, T., Shirasawa, T., Bonifacino, J.S., 1999. Mu1B, a novel adaptor medium chain expressed in polarized epithelial cells. FEBS Lett. 449, 215–220.

Park, H.-O., Sanson, A., Herskowitz, I., 1999. Localization of Bud2p, a GTP-ase-activating protein necessary for programming cell polarity in yeast to the presumptive bud site. Genes Dev. 13, 1912–1917.

Parton, R.G., 1996. Caveolae and caveolins. Curr. Opin. Cell Biol. 8, 542–548.

Pattee, H.H., 1971. Physical theories of biological coordination. Quat. Rev. Biophys. 4, 255–276.

Perego, C., Vanoni, C., Villa, A., Longhi, R., Kaech, S.M., Frohli, E., Hajnal, A., Kim, S.K., Pietrini, G., 1999. Pdz-mediated interactions retain the epithelial gaba transporter on the basolateral surface of polarized epithelial cells. EMBO J. 18, 2384–2393.

Platt, O.S., Lux, S.E., Falcone, J.F., 1993. A highly conserved region of human erythrocyte ankyrin contains the capacity to bind spectrin. J. Biol. Chem. 268, 24421–24426.

Ponce, A., Cereijido, M., 1991. Polarized distribution of cation channels in epithelia. Cell. Physiol. Biochem. 1, 13–23.

Ponce, A., Bolivar, J.J., Vega, J., Cereijido, M., 1991a. Synthesis of plasma membrane and potassium channels in epithelia. Cell. Physiol. Biochem. 1, 195–204.

Ponce, A., Contreras, R.G., Cereijido, M., 1991b. Polarized distribution of chloride channels in epithelial cells (MDCK). Cell. Physiol. Biochem. 1, 160–169.

Pruyne, D., Bretscher, A., 2000a. Polarization of cell growth in yeast. I. Establishment and maintenance of polarity states. J. Cell Sci. 113, 365–375.

Pruyne, D., Bretscher, A., 2000b. Polarization of cell growth in yeast. II. The role of the cortical actin cytoskeleton. J. Cell Sci. 113, 571–585.

Puertollano, R., Martínez-Menarguez, J.A., Batista, A., Ballesta, J., Alonso, M.A., 2001. An intact dilysine-like motif in the carboxyl terminus of MAL is required for normal apical transport of the influenza virus hemagglutinin cargo protein in epithelial Madin-Darby canine kidney cells. Mol. Biol. Cell 12, 1869–1883.

Qiu, R.G., Abo, A., Steven Martin, G., 2000. A human homolog of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. Curr. Biol. 10, 697–707.

Rindler, M.J., Ivanov, I.E., Plesken, H., Rodriguez-Boulan, E., Sabatini, D.D., 1984. Viral glycoproteins destined for apical or basolateral plasma membrane domains traverse the same Golgi apparatus during their intracellular transport in doubly infected Madin-Darby canine kidney cells. J. Cell Biol. 98, 1304–1319.

Rindler, M.J., Ivanov, I.E., Sabatini, D.D., 1987. Microtubule-acting drugs lead to the nonpolarized delivery of the influenza hemagglutinin to the cell surface of polarized Madin-Darby canine kidney cells. J. Cell Biol. 104, 231–241.

Rodriguez-Boulan, E., Gonzalez, A., 1999. Glycans in post-Golgi apical targeting: sorting signals or structural props? Trends Cell Biol. 9, 291–294.

Rodriguez-Boulan, E., Paskiet, K.T., Salas, P.J., Bard, E., 1984. Intracellular transport of influenza virus hemagglutinin to the apical surface of Madin-Darby canine kidney cells. J. Cell Biol. 98, 308–319.

Rose, L.S., Kemphues, K., 1998. The let-99 gene is required for proper spindle orientation during cleavage of the C. elegans embryo. Development 125, 1337–1346.

Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.S., Glenney, J.R., Anderson, R.G., 1992. Caveolin, a protein component of caveolae membrane coats. Cell 68 (4), 673–682.

Roush, D.L., Gottardi, C.J., Naim, H.Y., Roth, M.G., Caplan, M.J., 1998. Tyrosine-based membrane protein sorting signals are differentially interpreted by polarized Madin-Darby canine kidney and LLC-PK1 epithelial cells. J. Biol. Chem. 273, 26862–26869.

Sadler, P.L., Shakes, D.C., 2000. Anucleate Caenorhabditis elegans sperm can crawl, fertilize oocytes and direct anterior-posterior polarization of the 1-cell embryo. Development 127, 355–366.

Scheiffele, P., Peranen, J., Simons, K., 1995. N-glycans as apical sorting signals in epithelial cells. Nature 378, 96–98.

Scheiffele, P., Roth, M.G., Simons, K., 1997. Interaction of influenza virus haemagglutinin with sphingolipid-cholesterol membrane domains via its transmembrane domain. EMBO J. 16, 5501–5508.

Schejter, E.D., Rose, L.S., Postner, M.A., Wieschaus, E., 1992. Role of the zygotic genome in the restructuring of the actin cytoskeleton at the cycle-14 transition during *Drosophila* embryogenesis. Cold Spring Harb Symp. Quant. Biol. 57, 653–659.

Setou, M., Nakagawa, T., Seog, D.H., Hirokawa, N., 2000. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. Science 288, 1796–1802.

Shoshani, L., Contreras, R.G., 2001. Biogenesis of epithelial polarity and tight junctions. In: Cereijido, M., Anderson, J. (Eds.), Tight Junctions, 2nd Edition. CRC Press, Boca Raton, pp. 165–197.

Simmen, T., Honing, S., Icking, A., Tikkanen, R., Hunziker, W., 2002. AP-4 binds basolateral signals and participates in basolateral sorting in epithelial MDCK cells. Nat. Cell Biol. 4, 154–159.

Simons, K., Fuller, S.D., 1985. Cell surface polarity in epithelia. Annu. Rev. Cell Biol. 1, 243–288.

Simons, K., Ikonen, E., 1997. Functional rafts in cell membranes. Nature 387, 569–572.

Slawecki, M.L., Dodt, G., Steinberg, S., Moser, A.B., Moser, H.W., Gould, S.J., 1995. Identification of three distinct peroxisomal protein import defects in patients with peroxisome biogenesis disorders. J. Cell Sci. 108 (Part 5), 1817–1829.

Slimane, T.A., Lenoir, C., Bello, V., Delaunay, J.L., Goding, J.W., Chwetzoff, S., Maurice, M., Fransen, J.A., Trugnan, G., 2001. The cytoplasmic/transmembrane domain of dipeptidyl peptidase IV, a type II glycoprotein, contains an apical targeting signal that does not specifically interact with lipid rafts. Exp. Cell Res. 270, 45–55.

Sollner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., Rothman, J.E., 1993. SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324.

Spodsberg, N., Alfalah, M., Naim, H.Y., 2001. Characteristics and structural requirements of apical sorting of the rat growth hormone through the O-glycosylated stalk region of intestinal sucrase-isomaltase. J. Biol. Chem. 276, 46597–46604.

Sugihara, K., Asano, S., Tanaka, K., Iwamatsu, A., Okawa, K., Ohta, Y., 2002. The exocyst complex binds the small GTPase RalA to mediate filopodia formation. Nat. Cell Biol. 4, 73–78.

Sun, J., Paddock, C., Shubert, J., Zhang, H.B., Amin, K., Newman, P.J., Albelda, S.M., 2000. Contributions of the extracellular and cytoplasmic domains of platelet-endothelial cell adhesion molecule-1 (PECAM-1/CD31) in regulating cell–cell localization. J. Cell Sci. 113 (Pt 8), 1459–1469.

Suzuki, A., Yamanaka, T., Hirose, T., Manabe, N., Mizuno, K., Shimizu, M., Akimoto, K., Izumi, Y., Ohnishi, T., Ohno, S., 2001. Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. J. Cell Biol. 152, 1183–1196.

Tabuse, Y., Izumi, Y., Piano, F., Kemphues, K.J., Miwa, J., Ohno, S., 1998. Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. Development 125, 3607–3614.

Tai, A.W., Chuang, J.Z., Bode, C., Wolfrum, U., Sung, C.H., 1999. Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1. Cell 97, 877–887.

Talavera, D., Ponce, A., Fiorentino, R., Gonzalez-Mariscal, L., Contreras, R.G., Sanchez, S.H., Garcia-Villegas, M.R., Valdes, J., Cereijido, M., 1995. Expression of potassium channels in epithelial cells depends on calcium-activated cell–cell contacts. J. Membr. Biol. 143, 219–226.

Tepass, U., Theres, C., Knust, E., 1990. Crumbs encodes an EGF-like protein expressed on apical tumor suppressor, merlin. J. Cell. Biol. 133, 843–852.

Tepass, U., Truong, K., Godt, D., Ikura, M., Peifer, M., 2000. Cadherins in embryonic and neural morphogenesis. Nature reviews. Mol. Cell Biol. 1, 91–100.

TerBush, D.R., Maurice, T., Roth, D., Novick, P., 1996. The exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. EMBO J. 15, 6483–6494.

Thomas, G.H., 2001. Spectrin: the ghost in the machine. Bio Essays 23, 152–160.

Thomas, D.C., Brewer, C.B., Roth, M.G., 1993. Vesicular stomatitis virus glycoprotein contains a dominant cytoplasmic basolateral sorting signal critically dependent upon a tyrosine. J. Biol. Chem. 268, 3313–3320.

Tkacz, J.S., Lampen, J.O., 1972. Wall replication in *Saccharomyces* species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. J. Gen. Microbiol. 72, 243–247.

Vanderheuvel, F.A., 1967. Structural role of water in lipoprotein systems. Protoplasma 63 (1), 188–193.

Van Itallie, C.M., Anderson, J.M., 1997. Occludin confers adhesiveness when expressed in fibroblasts. J. Cell Sci. 110 (Part 9), 1113–1121.

Vega, I.E., Hsu, S.C., 2001. The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. J. Neurosci. 21, 3839–3848.

Vega-Salas, D.E., Salas, P.J., Rodriguez-Boulan, E., 1988. Exocytosis of vacuolar apical compartment (VAC): a cell–cell contact controlled mechanism for the establishment of the apical plasma membrane domain in epithelial cells. J. Cell Biol. 107, 1717–1728.

Weber, E., Berta, G., Tousson, A., St John, P., Green, M.W., Gopalokrishnan, U., Jilling, T., Sorscher, E.J., Elton, T.S., Abrahamson, D.R., 1994. Expression and polarized targeting of a rab3 isoform in epithelial cells. J. Cell Biol. 125, 583–594.

Wieschaus, E., Sweeton, D., 1988. Requirements for X-linked zygotic gene activity during cellularization of early *Drosophila* embryos. Development 104, 483–493.

Wilson, P.D., Devuyst, O., Li, X., Gatti, L., Falkenstein, D., Robinson, S., Fambrough, D., Burrow, C.R., 2000. Apical plasma membrane mispolarization of NaK-ATPase in polycystic kidney disease epithelia is associated with aberrant expression of the beta2 isoform. Am. J. Pathol. 156, 253–268.

Winckler, B., Mellman, I., 1999. Neuronal polarity: controlling the sorting and diffusion of membrane components. Neuron 23, 637–640.

Wittchen, E.S., Haskins, J., Stevenson, B.R., 1999. Protein interactions at the tight junction. Actin has multiple binding partners, and ZO-1 forms independent complexes with ZO-2 and ZO-3. J. Biol. Chem. 274, 35179–35185.

Wodarz, A., 2001. Cell polarity: no need to reinvent the wheel. Curr. Biol. 11, R975–R978.

Wodarz, A., Hinz, U., Engelbert, M., Knust, E., 1995. Expression of *crumbs* confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. Cell 82, 67–76.

Wolffe, A.P., Matzke, M.A., 1999. Epigenetics: regulation through repression. Science 286, 481–486.

Woods, D.F., Bryant, P.J., 1991. The discs-large tumor suppressor gene of Drosophila encodes a guanylate kinase homolog localized at septate junctions. Cell 66, 451–464.

Woods, D.F., Hough, C., Peel, D., Callaini, G., Bryant, P.J., 1996. Dlg protein is required for junction structure, cell polarity, and proliferation control in Drosophila epithelia. J. Cell Biol. 134, 1469–1482.

Yamada, Y., Chochi, Y., Ko, J.A., Sobue, K., Inui, M., 1999. Activation of channel activity of the NMDA receptor-PSD-95 complex by guanylate kinase-associated protein (GKAP). FEBS Lett. 458, 295–298.

Yamanaka, T., Horikoshi, Y., Suzuki, A., Sugiyama, Y., Kitamura, K., Maniwa, R., Nagai, Y., Yamashita, A., Hirose, T., Ishikawa, H., Ohno, S., 2001. PAR-6 regulates aPKC activity in a novel way and mediates cell–cell contact-induced formation of the epithelial junctional complex. Genes Cells 6, 721–731.

Yap, A.S., Brieher, W.M., Gumbiner, B.M., 1997. Molecular and functional analysis of cadherin-based adherens junctions. Annu. Rev. Cell Dev. Biol. 13, 119–146.

Yeaman, C., Le Gall, A.H., Baldwin, A.N., Monlauzeur, L., Le Bivic, A., Rodriguez-Boulan, E., 1997. The O-glycosylated stalk domain is required for apical sorting of neurotrophin receptors in polarized MDCK cells. J. Cell Biol. 139, 929–940.

Zahraoui, A., Joberty, G., Arpin, M., Fontaine, J.J., Hellio, R., Tavitian, A., Louvard, D., 1994. A small rab GTPase is distributed in cytoplasmic vesicles in non-polarized cells but colocalizes with the tight junction marker ZO-1 in polarized epithelial cells. J. Cell Biol. 124, 101–115.

Zhang, Z., Devarajan, P., Dorfman, A.L., Morrow, J.S., 1998. Structure of the ankyrin-binding domain of alpha-Na,K-ATPase. J. Biol. Chem. 273, 18681–18684.

Zhang, X., Bi, E., Novick, P., Du, L., Kozminski, K.G., Lipschutz, J.H., Guo, W., 2001. Cdc42 interacts with the exocyst and regulates polarized secretion. J. Biol. Chem. 276, 46745–46750.

Zheng, X., Sadler, J.E., 2002. Mucin-like domain of enteropeptidase directs apical targeting in Madin- Darby canine kidney cells. J. Biol. Chem. 277, 6858–6863.

Zurzolo, C., Rodriguez-Boulan, E., 1993. Delivery of Na(+),K(+)-ATPase in polarized epithelial cells. Science 260, 550–552.

Zurzolo, C., Lisanti, M.P., Caras, I.W., Nitsch, L., Rodriguez-Boulan, E., 1993. Glycosylphosphatidylinositol-anchored proteins are preferentially targeted to the basolateral surface in Fischer rat thyroid epithelial cells. J. Cell Biol. 121, 1031–1039.